(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 456 346 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
15.02.2012 Bulletin 2012/07

(21) Application number: **02714955.8**

(22) Date of filing: **20.02.2002**

(51) Int Cl.:
***C12N 15/63** (2006.01)* ***C12N 15/85** (2006.01)*
***C12N 15/12** (2006.01)* ***C12N 15/62** (2006.01)*
***C07K 14/705** (2006.01)* ***C07K 14/72** (2006.01)*
***A01H 5/00** (2006.01)* ***C12Q 1/68** (2006.01)*
***G01N 33/68** (2006.01)*

(86) International application number:
PCT/US2002/005235

(87) International publication number:
WO 2002/066613 (29.08.2002 Gazette 2002/35)

(54) NOVEL ECDYSONE RECEPTOR/INVERTEBRATE RETINOID X RECEPTOR-BASED INDUCIBLE GENE EXPRESSION SYSTEM

NEUES INDUZIERBARES GENEXPRESSIONSSYSTEM AUF ECDYSONREZEPTOR/ INVERTEBRATEN-RETINOID-X-REZEPTOR-BASIS

NOUVEAU SYSTEME D'EXPRESSION GENETIQUE INDUCTIBLE BASE SUR LE RECEPTEUR D'ECDYSONE/RECEPTEUR DE RETINOIDE X INVERTEBRE

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

(30) Priority: **20.02.2001 US 269799 P**
31.05.2001 US 294814 P

(43) Date of publication of application:
15.09.2004 Bulletin 2004/38

(73) Proprietor: **Intrexon Corporation**
Blacksburg, VA 24060 (US)

(72) Inventors:
- **PALLI, Subba, Reddy**
  Lexington,KY 40513 (US)
- **KAPITSKAYA, Marianna Zinovjevna**
  North Wales, PA 19454 (US)

(74) Representative: **Harding, Charles Thomas**
D Young & Co LLP
120 Holborn
London
EC1N 2DY (GB)

(56) References cited:
EP-A- 0 965 644 **WO-A-01/02436**
WO-A-01/62780 **WO-A-96/27673**
WO-A2-01/36447 **WO-A2-01/70816**

- **CLAYTON G M ET AL: "The structure of the ultraspiracle ligand-binding domain reveals a nuclear receptor locked in an inactive conformation" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 98, no. 4, 13 February 2001 (2001-02-13), pages 1549-1554, XP002216496 ISSN: 0027-8424**
- **PALMER M J ET AL: "Characterization of EcR and RXR homologues in the ixodid tick, Amblyomma americanum (L.)" AMERICAN ZOOLOGIST, vol. 39, no. 4, September 1999 (1999-09), pages 747-757, XP008070257 ISSN: 0003-1569**
- **HAYWARD D C ET AL: "The sequence of Locusta RXR, homologous to Drosophila Ultraspiracle, and its evolutionary implications." DEVELOPMENT GENES AND EVOLUTION. SEP 1999, vol. 209, no. 9, September 1999 (1999-09), pages 564-571, XP002403943 ISSN: 0949-944X**
- **SHEA C ET AL: "An rxr/usp homolog from the parasitic nematode, Dirofilaria immitis" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, ELSEVIER, AMSTERDAM, NL, vol. 324, 7 January 2004 (2004-01-07), pages 171-182, XP004482485 ISSN: 0378-1119**

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Printed by Jouve, 75001 PARIS (FR)

(Cont. next page)

- BONNETON F ET AL: "RAPID DIVERGENCE OF THE ECDYSONE RECEPTOR IN DIPTERA AND LEPIDOPTERA SUGGESTS COEVOLUTION BETWEEN ECR AND USP-RXR" MOLECULAR BIOLOGY AND EVOLUTION, THE UNIVERSITY OF CHICAGO PRESS, US, vol. 20, no. 4, April 2003 (2003-04), pages 541-553, XP008028857 ISSN: 0737-4038
- HAYWARD D C ET AL: "The structure of the USP/RXR of Xenos pecki indicates that Strepsiptera are not closely related to Diptera." DEVELOPMENT GENES AND EVOLUTION. APR 2005, vol. 215, no. 4, April 2005 (2005-04), pages 213-219, XP002403944 ISSN: 0949-944X
- MORADPOUR ET AL.: 'Independent regulation of two separate gene activities in a continuous human cell line' BIOL. CHEM. vol. 379, 1998, pages 1189 - 1191, XP001070604

Description

FIELD OF THE INVENTION

[0001] This invention relates to the field of biotechnology or genetic engineering. Specifically, this invention relates to the field of gene expression. More specifically, this invention relates to a novel ecdysone receptor/invertebrate retinoid X receptor-based inducible gene expression system and methods of modulating the expression of a gene within a host cell using this inducible gene expression system.

BACKGROUND OF THE INVENTION

[0002] Various citations of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

[0003] In the field of genetic engineering, precise control of gene expression is a valuable tool for studying, manipulating, and controlling development and other physiological processes. Gene expression is a complex biological process involving a number of specific protein-protein interactions. In order for gene expression to be triggered, such that it produces the RNA necessary as the first step in protein synthesis, a transcriptional activator must be brought into proximity of a promoter that controls gene transcription. Typically, the transcriptional activator itself is associated with a protein that has at least one DNA binding domain that binds to DNA binding sites present in the promoter regions of genes. Thus, for gene expression to occur, a protein comprising a DNA binding domain and a transactivation domain located at an appropriate distance from the DNA binding domain must be brought into the correct position in the promoter region of the gene.

[0004] The traditional transgenic approach utilizes a cell-type specific promoter to drive the expression of a designed transgene. A DNA construct containing the transgene is first incorporated into a host genome. When triggered by a transcriptional activator, expression of the transgene occurs in a given cell type.

[0005] Another means to regulate expression of foreign genes in cells is through inducible promoters. Examples of the use of such inducible promoters include the PR1-a promoter, prokaryotic repressor-operator systems, immunosuppressive-immunophilin systems, and higher eukaryotic transcription activation systems such as steroid hormone receptor systems and are described below.

[0006] The PR1-a promoter from tobacco is induced during the systemic acquired resistance response following pathogen attack. The use of PR1-a may be limited because it often responds to endogenous materials and external factors such as pathogens, UV-B radiation, and pollutants. Gene regulation systems based on promoters induced by heat shock, interferon and heavy metals have been described (Wum et al., 1986, Proc. Natl. Acad. Sci. USA 83: 5414-5418; Arnheiter et al., 1990, Cell 62: 51-61; Filmus et al., 1992, Nucleic Acids Research 20: 27550-27560). However, these systems have limitations due to their effect on expression of non-target genes. These systems are also leaky.

[0007] Prokaryotic repressor-operator systems utilize bacterial repressor proteins and the unique operator DNA sequences to which they bind. Both the tetracycline ("Tet") and lactose ("Lac") repressor-operator systems from the bacterium *Escherichia coli* have been used in plants and animals to control gene expression. In the Tet system, tetracycline binds to the TetR repressor protein, resulting in a conformational change that releases the repressor protein from the operator which as a result allows transcription to occur. In the Lac system, a lac operon is activated in response to the presence of lactose, or synthetic analogs such as isopropyl-b-D-thiogalactoside. Unfortunately, the use of such systems is restricted by unstable chemistry of the ligands, *i.e.* tetracycline and lactose, their toxicity, their natural presence, or the relatively high levels required for induction or repression. For similar reasons, utility of such systems in animals is limited.

[0008] Immunosuppressive molecules such as FK506, rapamycin and cyclosporine A can bind to immunophilins FKBP12, cyclophilin, *etc.* Using this information, a general strategy has been devised to bring together any two proteins simply by placing FK506 on each of the two proteins or by placing FK506 on one and cyclosporine A on another one. A synthetic homodimer of FK506 (FK1012) or a compound resulted from fusion of FK506-cyclosporine (FKCsA) can then be used to induce dimerization of these molecules (Spencer et al., 1993, Science 262 :1019-24; Belshaw et al., 1996, Proc Natl Acad Sci USA 93:4604-7). Gal4 DNA binding domain fused to FKBP12 and VP16 activator domain fused to cyclophilin, and FKCsA compound were used to show heterodimerization and activation of a reporter gene under the control of a promoter containing Gal4 binding sites. Unfortunately, this system includes immunosuppressants that can have unwanted side effects and therefore, limits its use for various mammalian gene switch applications.

[0009] Higher eukaryotic transcription activation systems such as steroid hormone receptor systems have also been employed. Steroid hormone receptors are members of the nuclear receptor superfamily and are found in vertebrate and invertebrate cells. Unfortunately, use of steroidal compounds that activate the receptors for the regulation of gene expression, particularly in plants and mammals, is limited due to their involvement in many other natural biological pathways in such organisms. In order to overcome such difficulties, an alternative system has been developed using insect ecdysone receptors (EcR).

[0010] Growth, molting, and development in insects are regulated by the ecdysone steroid hormone (molting hormone) and the juvenile hormones (Dhadialla, et al., 1998, Annu. Rev. Entomol. 43: 545-569). The molecular target for ecdysone in insects consists of at least ecdysone receptor (EcR) and ultraspiracle protein (USP). EcR is a member of the nuclear steroid receptor super family that is characterized by signature DNA and ligand binding domains, and an activation domain (Koelle et al. 1991, Cell, 67: 59-77). EcR receptors are responsive to a number of steroidal compounds such as ponasterone A and muristerone A. Recently, non-steroidal compounds with ecdysteroid agonist activity have been described, including the commercially available insecticides tebufenozide and methoxyfenozide that are marketed world wide by Rohm and Haas Company (see International Patent Application No. PGT/EP96/00686 and US Patent 5,530,028). Both analogs have exceptional safety profiles to other organisms.

[0011] International Patent Applications No. PCT/US97/05330 (WO 97/38117) and PGT/US99/08381 (WO99/58155) disclose methods for modulating the expression of an exogenous gene in which a DNA construct comprising the exogenous gene and an ecdysone response element is activated by a second DNA construct comprising an ecdysone receptor that, in the presence of a ligand therefor, and optionally in the presence of a receptor capable of acting as a silent partner, binds to the ecdysone response element to induce gene expression. The ecdysone receptor of choice was isolated from *Drosophila melanogaster*. Typically, such systems require the presence of the silent partner, preferably retinoid X receptor (RXR), in order to provide optimum activation. In mammalian cells, insect ecdysone receptor (EcR) heterodimerizes with retinoid X receptor (RXR) and regulates expression of target genes in a ligand dependent manner. International Patent Application No. PCT/US98/14215 (WO 99/02683) discloses that the ecdysone receptor isolated from the silk moth *Bombyx mori* is functional in mammalian systems without the need for an exogenous dimer partner.

[0012] U.S. Patent No. 5,880,333 discloses a *Drosophila melanogaster* EcR and ultraspiracle (USP) heterodimer system used in plants in which the transactivation domain and the DNA binding domain are positioned on two different hybrid proteins. Unfortunately, this system is not effective for inducing reporter gene expression in animal cells (for comparison, see Example 12, below).

[0013] In each of these cases, the transactivation domain and the DNA binding domain (either as native EcR as in International Patent Application No. PGT/US98/14215 or as modified EcR as in International Patent Application No. PCT/US97/05330) were incorporated into a single molecule and the other heterodimeric partners, either USP or RXR, were used in their native state.

[0014] Drawbacks of the above described EcR-based gene regulation systems include a considerable background activity in the absence of ligands and non-applicability of these systems for use in both plants and animals (see U.S. Patent No. 5,880,333). For most applications that rely on modulating gene expression, these EcR-based systems are undesirable. Therefore, a need exists in the art for improved systems to precisely modulate the expression of exogenous genes in both plants and animals. Such improved systems would be useful for applications such as gene therapy, large-scale production of proteins and antibodies, cell-based high throughput screening assays, functional genomics and regulation of traits in transgenic animals. Improved systems that are simple, compact, and dependent on ligands that are relatively inexpensive, readily available, and of low toxicity to the host would prove useful for regulating biological systems.

[0015] Recently, Applicants have shown that an ecdysone receptor-based inducible gene expression system in which the transactivation and DNA binding domains are separated from each other by placing them on two different proteins results in greatly reduced background activity in the absence of a ligand and significantly increased activity over background in the presence of a ligand (pending application PCT/US01/09050. This two-hybrid system is a significantly improved inducible gene expression modulation system compared to the two systems disclosed in applications PCT7US97/05330 and PCT/US98/14215.

[0016] Applicants previously demonstrated that an ecdysone receptor-based gene expression system in partnership with a dipteran (*Drosophila melanogaster*) or a lepidopteran (*Choristoneura fumiferana*) ultraspiracle protein (USP) is constitutively expressed in mammalian cells, while an ecdysone receptor-based gene expression system in partnership with a vertebrate retinoid X receptor (RXR) is inducible in mammalian cells (pending application PCT/US01/09050). Applicants have now made the surprising discovery that a non-dipteran and non-lepidopteran invertebrate RXR homolog can function similar to vertebrate RXR in an ecdysone receptor-based inducible gene expression system. As described herein, Applicants' novel ecdysone receptor/invertebrate retinoid X receptor-based inducible gene expression system provides an improved inducible gene expression system in yeast and mammalian cells that is characterized by increased ligand sensitivity and magnitude of transactivation.

SUMMARY OF THE INVENTION

[0017] The present invention relates to a novel ecdysone receptor/invertebrate retinoid X receptor-based inducible gene expression system, novel receptor polynucleotides and polypeptides for use in the novel inducible gene expression system, and methods of modulating the expression of a gene within a host cell using this inducible gene expression system. In particular, Applicants' invention relates to an improved gene expression modulation system comprising a polynucleotide encoding a ligand binding domain of an invertebrate retinoid X receptor (RXR) polypeptide.

[0018] Specifically, the present invention relates to a gene expression modulation system comprising: a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide that encodes a first hybrid polypeptide comprising: i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) an ecdysone receptor ligand binding domain; and b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide sequence that encodes a second hybrid polypeptide comprising: i) a transactivation domain; and ii) an invertebrate retinoid X receptor ligand binding domain.

[0019] The present invention also relates to a gene expression modulation system comprising: a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide that encodes a first hybrid polypeptide comprising: i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) an invertebrate retinoid X receptor ligand binding domain; and b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide sequence that encodes a second hybrid polypeptide comprising: i) a transactivation domain; and ii) an ecdysone receptor ligand binding domain.

[0020] The present invention also relates to a gene expression modulation system according to the invention further comprising c) a third gene expression cassette comprising: i) a response element to which the DNA-binding domain of the first hybrid polypeptide binds; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated.

[0021] The present invention also relates to a gene expression cassette that is capable of being expressed in a host cell, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising either i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated, or ii) a transactivation domain; and an invertebrate retinoid X receptor ligand binding domain.

[0022] The present invention also relates to an isolated polynucleotide that encodes a hybrid polypeptide comprising either i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated, or ii) a transactivation domain; and an invertebrate retinoid X receptor ligand binding domain.

[0023] The present invention also relates to an isolated polynucleotide encoding a truncated invertebrate RXR polypeptide, wherein the truncation mutation affects ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide.

[0024] The present invention also relates to an isolated polynucleotide encoding a truncated invertebrate RXR polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the truncated invertebrate RXR polypeptide and a dimerization partner. In a specific embodiment, the dimerization partner is an ecdysone receptor polypeptide.

[0025] The present invention also relates to an isolated polypeptide encoded by a polynucleotide according to Applicants' invention.

[0026] The present invention also relates to an isolated hybrid polypeptide comprising either i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated, or ii) a transactivation domain; and an invertebrate retinoid X receptor ligand binding domain.

[0027] The present invention relates to an isolated truncated invertebrate RXR polypeptide comprising a truncation mutation, wherein the invertebrate RXR polypeptide is encoded by a polynucleotide according to the invention.

[0028] Thus, the present invention also relates to an isolated truncated invertebrate RXR polypeptide comprising a truncation mutation that affects ligand binding activity or ligand sensitivity of said invertebrate RXR polypeptide.

[0029] The present invention also relates to an isolated truncated invertebrate RXR polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the truncated invertebrate RXR polypeptide and a dimerization partner. In a specific embodiment, the dimerization partner is an ecdysone receptor polypeptide.

[0030] Applicants' invention also relates to methods of modulating gene expression in a host cell using a gene expression modulation system according to the invention. Specifically, Applicants' invention provides a method of modulating the expression of a gene in a host cell comprising the steps of: a) introducing into the host cell a gene expression modulation system according to the invention; b) introducing into the host cell a gene expression cassette comprising i) a response element comprising a domain to which the DNA binding domain from the first hybrid polypeptide of the gene expression modulation system binds; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide of the gene expression modulation system; and iii) a gene whose expression is to be modulated; and c) introducing into the host cell a ligand; whereby upon introduction of the ligand into the host cell, expression of the gene is modulated.

[0031] Applicants' invention also provides a method of modulating the expression of a gene in a host cell comprising a gene expression cassette comprising a response element comprising a domain to which the DNA binding domain from the first hybrid polypeptide of the gene expression modulation system binds; a promoter that is activated by the transactivation domain of the second hybrid polypeptide of the gene expression modulation system; and a gene whose expression is to be modulated; wherein the method comprises the steps of: a) introducing into the host cell a gene expression modulation system according to the invention; and b) introducing into the host cell a ligand; whereby upon introduction of the ligand into the host, expression of the gene is modulated.

[0032] Applicants' invention also provides an isolated host cell comprising an inducible gene expression system according to the invention. The present invention also relates to an isolated host cell comprising a gene expression cassette, a polynucleotide, or a polypeptide according to the invention. Accordingly, Applicants' invention also relates to a non-human organism comprising a host cell according to the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033]

Figure 1: Transactivation of reporter genes through VP16MmRXRDEF, VP16MmRXREF, VP16LmUSP, and VP16CfUSP constructs transfected into NIH3T3 cells along with GAL4CfEcRCDEF, pFRLuc and pTKRL plasmid DNAs by a non-steroidal ligand.

Figure 2: Transactivation of reporter genes through VP16NUnRXRDEF, VP16MmRXREF, VP16LmUSP, and VP16CfUSP constructs transfected into NIH3T3 cells along with GAL4CfEcRDEF, pFRLuc and pTKRL plasmid DNAs by a non-steroidal ligand.

Figure 3: Amino acid sequence alignments of the EF domains of six vertebrate RXRs (A) and six invertebrate RXRs (B). Helices 1-12 are denoted as H1-H12 and β pleated sheets are denoted as S1 and S2. F denotes the F domain junction.

Figure 4: Expression data of various truncations of CfEcR, GAL4CfEcRA/BCDEF, GAL4CfEcRCDEF, GAL4CfEcR1/2CDEF, OAL4CfEcRDEF, GAL4CfEcREF, GAL4CfEcRDE transfected into NIH3T3 cells along with VP16MmRXRDEF, pFRLUc and pTKRL plasmid DNAs in the presence of non-steroidal ligand or PonA ligand.

Figure 5: Expression data of various truncations of CfEcR, GAL4CfEcRA/BCDEF, GAL4CfEcRCDEF, GAL4CfEcR1/2CDEF, GAL4CfEcRDEF, GAL4CfEcREF, GAL4CfEcRDE transfected into NIH3T3 cells along with VP16MmRXREF, pFRLUc and pTKRL plasmid DNAs in the presence of non-steroidal ligand or PonA ligand.

Figure 6: Expression data of various truncations of CfEcR, GAL4CfEcRA/BCDEF, GAL4CfEcRCDEF, GAL4CfEcR1/2CDEF, GAL4CtEcRDEF, GAL4CfEcREF, GAL4CfEcRDE transfected into NIH3T3 cells along with VP16LmUSPDEF, pFRLUc and pTKRL plasmid DNAs in the presence of non-steroidal ligand or PonA ligand.

Figure 7: Expression data of various, truncations of CfEcR, GAL4CfEcRA/BCDEF, GAL4CfEcRCDEF, GALACfEcR1/2CDEF, GAL4CfEcRDEF, GALACfEcREF, GAL4CfEcRDE transfected into NIH3T3 cells along with VP16LmUSPEF, pFRLUc and pTKRL plasmid DNAs in the presence of non-steroidal ligand or PonA ligand.

Figure 8: Expression data of various truncated MmRXR/LmUSP receptor constructs transfected into NIH3T3 cells along with GALACfEcRDEF, pFRLUc and pTKRL plasmid DNAs in the presence of non-steroidal ligand or PonA ligand.

Figure 9: Expression data of CfLJSP-EF, DmUSP-EF, LmUSP-EF, MmRXRα-EF, AmaRXR1-EF and AmaRXR2-EF ligand binding domains fused to VP16 along with GAL4/CfEcR-DEF and pFRLuc in NIH3T3 cells in the presence of non-steroidal (GSE) ligand or PonA ligand.

Figure 10: Expression data of GAL4:CfEcR-DEF/VP16:LmUSP-EF in stably transfected CHO cells comprising a reporter plasmid pFRLuc in the presence of non-steroidal ligand or PonA ligand.

Figure 11: Expression data of a LexA:CfEcR-CDEF receptor construct transfected into NIH3T3 cells along with 8XLexAopFRLuc and VP16:CfUSP-EF, VP16:LmUSP-EF, VP16:MmRXRα-EF or VP16:DmUSP-EF in the presence of non-steroidal ligand or PonA ligand.

Figure 12: NIH3T3 cells were transfected with different combinations of GAL4:CfEcR-CDEF or LexA:CfEcR-CDEF, 8XLexAopFRLuc and VP16:LmUSP-EF or B42:LmUSP-EF in the presence of non-steroidal ligand.

Figure 13: Effect of 9-cis-retinoic acid on transactivation potential of the GAL4CWcR-DEF/VPI6LmUSP-EF gene switch along with pFRLuc in NIH 3T3 cells in the presence of non-steroid (GSE) and 9-cis-retinoic acid (9Cis) for 48 hours.

DETAILED DESCRIPTION OF THE INVENTION

[0034] Applicants have developed a novel ecdysone receptor-based inducible gene expression system comprising an invertebrate retinoid X receptor polypeptide. Applicants have also shown that truncations of an invertebrate RXR polypeptide are also functional within this gene expression system and that these mutational effects may increase or reduce ligand binding activity or ligand sensitivity and may be steroid or non-steroid specific. Thus, Applicants' invention provides an ecdysone receptor/invertebrate RXR-based inducible gene expression system useful for modulating expression of a gene of interest in a host cell. In a particularly desirable embodiment, Applicants' invention provides an inducible gene expression system that has a reduced level of background gene expression and responds to submicromolar concentrations of non-steroidal ligand. Thus, Applicants' novel inducible gene expression system and its use in methods of modulating gene expression in a host cell overcome the limitations of currently available inducible expression systems and provide the skilled artisan with an effective means to control gene expression.

[0035] The present invention is useful for applications such as gene therapy, large scale production of proteins and antibodies, cell-based high throughput screening assays, functional genomics, proteomics, metabolomics, and regulation of traits in transgenic organisms, where control of gene expression levels is desirable. An advantage of Applicants' invention is that it provides a means to regulate gene expression and to tailor expression levels to suit the user's requirements.

DEFINITIONS

[0036] In this disclosure, a number of terms and abbreviations are used The following definitions are provided and should be helpful in understanding the scope and practice of the present invention.

[0037] In a specific embodiment, the term "about" or "approximately" means within 20%, preferably within 10%, more preferably within 5%, and even more preferably within 1% of a given value or range.

[0038] The term "substantially free" means that a composition comprising "A" (where "A" is a single protein, DNA molecule, vector, recombinant host cell, etc.) is substantially free of "B" (where "B" comprises one or more contaminating proteins, DNA molecules, vectors, etc.) when at least about 75% by weight of the proteins, DNA, vectors (depending on the category of species to which A and B belong) in the composition is "A". Preferably, "A" comprises at least about 90% by weight of the A + B species in the composition, most preferably at least about 99% by weight. It is also preferred that a composition, which is substantially free of contamination, contain only a single molecular weight species having the activity or characteristic of the species of interest.

[0039] The term "isolated" for the purposes of the present invention designates a biological material (nucleic acid or protein) that has been removed from its original environment (the environment in which it is naturally present). For example, a polynucleotide present in the natural state in a plant or an animal is not isolated, however the same polynucleotide separated from the adjacent nucleic acids in which it is naturally present, is considered "isolated". The term "purified" does not require the material to be present in a form exhibiting absolute purity, exclusive of the presence of other compounds. It is rather a relative definition.

[0040] A polynucleotide is in the "purified" state after purification of the starting material or of the natural material by at least one order of magnitude, preferably 2 or 3 and preferably 4 or 5 orders of magnitude.

[0041] A "nucleic acid" is a polymeric compound comprised of covalently linked subunits called nucleotides. Nucleic acid includes polyribonucleic acid (RNA) and polydeoxyribonucleic acid (DNA), both of which may be single-stranded or double-stranded. DNA includes but is not limited to cDNA, genomic DNA, plasmids DNA, synthetic DNA, and semi-synthetic DNA. DNA may be linear, circular, or supercoiled.

[0042] A "nucleic acid molecule" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia,* in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.

[0043] The term "fragment" will be understood to mean a nucleotide sequence of reduced length relative to the reference nucleic acid and comprising, over the common portion, a nucleotide sequence identical to the reference nucleic acid. Such a nucleic acid fragment according to the invention may be, where appropriate, included in a larger polynucleotide of which it is a constituent. Such fragments comprise, or alternatively consist of, oligonucleotides ranging in length from at least 6, 8, 9, 10, 12, 15, 18, 20, 21, 22, 23, 24, 25, 30, 39, 40, 42, 45, 48, 50, 51, 54, 57, 60, 63, 66, 70, 75, 78, 80, 90, 100, 105, 120, 135, 150, 200, 300, 500, 720, 900,1000 or 1500 consecutive nucleotides of a nucleic acid according to the invention.

[0044] As used herein, an "isolated nucleic acid fragment" is a polymer of RNA or DNA that is single-or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

[0045] A "gene" refers to an assembly of nucleotides that encode a polypeptide, and includes cDNA and genomic DNA nucleic acids. "Gene" also refers to a nucleic acid fragment that expresses a specific protein or polypeptide, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers to any gene that is not a native gene, comprising regulatory and/or coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. A chimeric gene may comprise coding sequences derived from different sources and/or regulatory sequences derived from different sources. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene or "heterologous" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

[0046] "Heterologous" DNA refers to DNA not naturally located in the cell, or in a chromosomal site of the cell. Preferably, the heterologous DNA includes a gene foreign to the cell.

[0047] The term "genome" includes chromosomal as well as mitochondrial, chloroplast and viral DNA or RNA.

[0048] A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (see Sambrook *et al.*, 1989 *infra*). Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989), particularly Chapter 11 and Table 11.1 therein The conditions of temperature and ionic strength determine the "stringency" of the hybridization.

[0049] Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T_m of 55°, can be used, *e.g.,* 5x SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5x SSC, 0.5% SDS). Moderate stringency hybridization conditions correspond to a higher T_m, *e.g.*, 40% formamide, with 5x or 6x SCC. High stringency hybridization conditions correspond to the highest T_m, e.g., 50% formamide, 5x or 6x SCC. Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible.

[0050] The term "complementary" is used to describe the relationship between nucleotide bases that are capable of hybridizing to one another. For example, with respect to DNA, adenosine is complementary to thymine and cytosine is complementary to guanine. Accordingly, the instant invention also includes isolated nucleic acid fragments that are complementary to the complete sequences as disclosed or used herein as well as those substantially similar nucleic acid sequences.

[0051] In a specific embodiment, the term "standard hybridization conditions" refers to a T_m of 55°C, and utilizes conditions as set forth above. In a preferred embodiment, the T_m is 60°C; in a more preferred embodiment, the T_m is 65°C.

[0052] Post-hybridization washes also determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 minutes (min), then repeated with 2X SSC, 0.5% SDS at 45°C for 30 minutes, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 minutes. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS at 65°C. Hybridization requires that the two nucleic acids comprise complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible.

[0053] The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA: RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (see Sambrook *et al.*, *supra*, 9.50-0.51). For hybridization with shorter nucleic acids, *i.e.*, oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*, 11.7-11.8).

[0054] In one embodiment the length for a hybridizable nucleic acid is at least about 10 nucleotides. Preferable a minimum length for a hybridizable nucleic acid is at least about 15 nucleotides; more preferably at least about 20 nucleotides; and most preferably the length is at least 30 nucleotides. Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as length of the probe.

[0055] The term "probe" refers to a single-stranded nucleic acid molecule that can base pair with a complementary single stranded target nucleic acid to form a double-stranded molecule. As used herein, the term "oligonucleotide" refers to a nucleic acid, generally of at least 18 nucleotides, that is hybridizable to a genomic DNA molecule, a cDNA molecule, a plasmid DNA or an mRNA molecule. Oligonucleotides can be labeled, *e.g.*, with ^{32}P-nucleotides or nucleotides to which a label, such as biotin, has been covalently conjugated. A labeled oligonucleotide can be used as a probe to detect the presence of a nucleic acid. Oligonucleotides (one or both of which may be labeled) can be used as PCR primers, either for cloning full length or a fragment of a nucleic acid, or to detect the presence of a nucleic acid. An oligonucleotide can also be used to form a triple helix with a DNA molecule. Generally, oligonucleotides are prepared synthetically, preferably on a nucleic acid synthesizer. Accordingly, oligonucleotides can be prepared with non-naturally occurring phosphoester analog bonds, such as thioester bonds, etc.

[0056] A "primer" is an oligonucleotide that hybridizes to a target nucleic acid sequence to create a double stranded nucleic acid region that can serve as an initiation point for DNA synthesis under suitable conditions. Such primers may be used in a polymerase chain reaction.

[0057] "Polymerase chain reaction" is abbreviated PCR and means an *in vitro* method for enzymatically amplifying specific nucleic acid sequences. PCR involves a repetitive series of temperature cycles with each cycle comprising three stages: denaturation of the template nucleic acid to separate the strands of the target molecule, annealing a single stranded PCR oligonucleotide primer to the template nucleic acid, and extension of the annealed primer(s) by DNA polymerase. PCR provides a means to detect the presence of the target molecule and, under quantitative or semi-quantitative conditions, to determine the relative amount of that target molecule within the starting pool of nucleic acids.

[0058] "Reverse transcription-polymerase chain reaction" is abbreviated RT-PCR and means an *in vitro* method for enzymatically producing a target cDNA molecule or molecules from an RNA molecule or molecules, followed by enzymatic amplification of a specific nucleic acid sequence or sequences within the target cDNA molecule or molecules as described above. RT-PCR also provides a means to detect the presence of the target molecule and, under quantitative or semi-quantitative conditions, to determine the relative amount of that target molecule within the starting pool of nucleic acids.

[0059] A DNA "coding sequence" is a double-stranded DNA sequence that is transcribed and translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. "Suitable regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, polyadenylation recognition sequences, RNA processing site, effector binding site and stem-loop structure. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from mRNA, genomic DNA sequences, and even synthetic DNA sequences. If the coding sequence is intended for expression in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[0060] "Open reading frame" is abbreviated ORF and means a length of nucleic acid sequence, either DNA, cDNA or RNA, that comprises a translation start signal or initiation codon, such as an ATG or AUG, and a termination codon and can be potentially translated into a polypeptide sequence.

[0061] The term "head-to-head" is used herein to describe the orientation of two polynucleotide sequences in relation to each other. Two polynucleotides are positioned in a head-to-head orientation when the 5' end of the coding strand of one polynucleotide is adjacent to the 5' end of the coding strand of the other polynucleotide, whereby the direction of transcription of each polynucleotide proceeds away from the 5' end of the other polynucleotide. The term "head-to-head" may be abbreviated (5')-to-(5') and may also be indicated by the symbols (←→) or (3'←5'5'→3').

[0062] The term "tail-to-tail" is used herein to describe the orientation of two polynucleotide sequences in relation to each other. Two polynucleotides are positioned in a tail-to-tail orientation when the 3' end of the coding strand of one polynucleotide is adjacent to the 3' end of the coding strand of the other polynucleotide, whereby the direction of transcription of each polynucleotide proceeds toward the other polynucleotide. The term "tail-to-tail" may be abbreviated (3')-to-(3') and may also be indicated by the symbols (→ ←) or (5'→3'3'←5').

[0063] The term "head-to-tail" is used herein to describe the orientation of two polynucleotide sequences in relation to each other. Two polynucleotides are positioned in a head-to-tail orientation when the 5' end of the coding strand of one polynucleotide is adjacent to the 3' end of the coding strand of the other polynucleotide, whereby the direction of transcription of each polynucleotide proceeds in the same direction as that of the other polynucleotide. The term "head-to-tail" may be abbreviated (5')-to-(3') and may also be indicated by the symbols (→ →) or (5'→3'5'→3').

[0064] The term "downstream" refers to a nucleotide sequence that is located 3' to reference nucleotide sequence. In particular, downstream nucleotide sequences generally relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

[0065] The term "upstream" refers to a nucleotide sequence that is located 5' to reference nucleotide sequence. In particular, upstream nucleotide sequences generally relate to sequences that are located on the 5' side of a coding sequence or starting point of transcription. For example, most promoters are located upstream of the start site of transcription.

[0066] The terms "restriction endonuclease" and "restriction enzyme" refer to an enzyme that binds and cuts within a specific nucleotide sequence within double stranded DNA.

[0067] "Homologous recombination" refers to the insertion of a foreign DNA sequence into another DNA molecule, e.g., insertion of a vector in a chromosome. Preferably, the vector targets a specific chromosomal site for homologous recombination. For specific homologous recombination, the vector will contain sufficiently long regions of homology to sequences of the chromosome to allow complementary binding and incorporation of the vector into the chromosome. Longer regions of homology, and greater degrees of sequence similarity, may increase the efficiency of homologous recombination.

[0068] Several methods known in the art may be used to propagate a polynucleotide according to the invention. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As described herein, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (*e.g.*, lambda), and plasmid and cosmid DNA vectors, to name but a few.

[0069] A "vector" is any means for the cloning of and/or transfer of a nucleic acid into a host cell. A vector may be a replicon to which another DNA segment may be attached so as to bring about the replication of the attached segment. A "replicon" is any genetic element (e.g., plasmid, phage, cosmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*, *i.e.*, capable of replication under its own control. The term "vector" includes both viral and nonviral means for introducing the nucleic acid into a cell *in vitro, ex vivo* or *in vivo*. A large number of vectors known in the art may be used to manipulate nucleic acids, incorporate response elements and promoters into genes, etc. Possible vectors include, for example, plasmids or modified viruses including, for example bacteriophages such as lambda derivatives, or plasmids such as PBR322 or pUC plasmid derivatives, or the Bluescript vector. For example, the insertion of the DNA fragments corresponding to response elements and promoters into a suitable vector can be accomplished by ligating the appropriate DNA fragments into a chosen vector that has complementary cohesive termini. Alternatively, the ends of the DNA molecules may be enzymatically modified or any site may be produced by ligating nucleotide sequences (linkers) into the DNA termini. Such vectors may be engineered to contain selectable marker genes that provide for the selection of cells that have incorporated the marker into the cellular genome. Such markers allow identification and/or selection of host cells that incorporate and express the proteins encoded by the marker.

[0070] Viral vectors, and particularly retroviral vectors, have been used in a wide variety of gene delivery applications in cells, as well as living animal subjects. Viral vectors that can be used include but are not limited to retrovirus, adeno-associated virus, pox, baculovirus, vaccinia, herpes simplex, Epstein-Barr, adenovirus, geminivirus, and caulimovirus vectors. Non-viral vectors include plasmids, liposomes, electrically charged lipids (cytofectins), DNA-protein complexes, and biopolymers. In addition to a nucleic acid, a vector may also comprise one or more regulatory regions, and/or selectable markers useful in selecting, measuring, and monitoring nucleic acid transfer results (transfer to which tissues, duration of expression, etc.).

[0071] The term "plasmid" refers to an extra chromosomal element often carrying a gene that is not part of the central metabolism of the cell, and usually in the form of circular double-stranded DNA molecules. Such elements may be autonomously replicating sequences, genome integrating sequences, phage or nucleotide sequences, linear, circular, or supercoiled, of a single- or double-stranded DNA or RNA, derived from any source, in which a number of nucleotide sequences have been joined or recombined into a unique construction which is capable of introducing a promoter fragment and DNA sequence for a selected gene product along with appropriate 3' untranslated sequence into a cell.

[0072] A "cloning vector" is a "replicon", which is a unit length of a nucleic acid, preferably DNA, that replicates sequentially and which comprises an origin of replication, such as a plasmid, phage or cosmid, to which another nucleic acid segment may be attached so as to bring about the replication of the attached segment. Cloning vectors may be capable of replication in one cell type and expression in another ("shuttle vector").

[0073] Vectors may be introduced into the desired host cells by methods known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion), use of a gene gun, or a DNA vector transporter (see, *e.g.*, Wu et al., 1992, J. Biol. Chem. 267: 963-967; Wu and Wu, 1988, J. Biol. Chem. 263: 14621-14624; and Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990).

[0074] A polynucleotide according to the invention can also be introduced *in vivo* by lipofection. For the past decade, there has been increasing use of liposomes for encapsulation and transfection of nucleic acids *in vitro*. Synthetic cationic lipids designed to limit the difficulties and dangers encountered with liposome-mediated transfection can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., 1987, PNAS 84: 7413; Mackey, et al., 1988, Proc. Natl. Acad. Sci. U.S.A 85: 8027-8031; and Ulmer et al., 1993, Science 259: 1745-1748). The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes (Felgner and Ringold, 1989, Science 337: 387-388). Particularly useful lipid compounds and compositions for transfer of nucleic acids are described in International Patent Publications WO95/18863 and

WO96/17823, and in U.S. Patent No. 5,459,127. The use of lipofection to introduce exogenous genes into the specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. It is clear that directing transfection to particular cell types would be particularly preferred in a tissue with cellular heterogeneity, such as pancreas, liver, kidney, and the brain. Lipids may be chemically coupled to other molecules for the purpose of targeting (Mackey, et al., 1988, *supra*). Targeted peptides, *e.g.*, hormones or neurotransmitters, and proteins such as antibodies, or non-peptide molecules could be coupled to liposomes chemically.

[0075] Other molecules are also useful for facilitating transfection of a nucleic acid *in vivo*, such as a cationic oligopeptide (*e.g.*, WO95/21931), peptides derived from DNA binding proteins (*e.g.*, WO96/25508), or a cationic polymer (*e.g.*, WO95/21931).

[0076] It is also possible to introduce a vector *in vivo* as a naked DNA plasmid (see U.S. Patents 5,693,622, 5,589,466 and 5,580,859). Receptor-mediated DNA delivery approaches can also be used (Curiel et al., 1992, Hum. Gene Ther. 3: 147-154; and Wu and Wu, 1987, J. Biol. Chem 262: 4429-4432).

[0077] The term "transfection" means the uptake of exogenous or heterologous RNA or DNA by a cell. A cell has been "transfected" by exogenous or heterologous RNA or DNA when such RNA or DNA has been introduced inside the cell. A cell has been "transformed" by exogenous or heterologous RNA or DNA when the transfected RNA or DNA effects a phenotypic change. The transforming RNA or DNA can be integrated (covalently linked) into chromosomal DNA making up the genome of the cell.

[0078] "Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" or "recombinant" or "transformed" organisms.

[0079] The term "genetic region" will refer to a region of a nucleic acid molecule or a nucleotide sequence that comprises a gene encoding a polypeptide.

[0080] In addition, the recombinant vector comprising a polynucleotide according to the invention may include one or more origins for replication in the cellular hosts in which their amplification or their expression is sought, markers or selectable markers.

[0081] The term "selectable marker" means an identifying factor, usually an antibiotic or chemical resistance gene, that is able to be selected for based upon the marker gene's effect, *i.e.*, resistance to an antibiotic, resistance to a herbicide, colorimetric markers, enzymes, fluorescent markers, and the like, wherein the effect is used to track the inheritance of a nucleic acid of interest and/or to identify a cell or organism that has inherited the nucleic acid of interest. Examples of selectable marker genes known and used in the art include: genes providing resistance to ampicillin, streptomycin, gentamycin, kanamycin, hygromycin, bialaphos herbicide, sulfonamide, and the like; and genes that are used as phenotypic markers, *i.e.*, anthocyanin regulatory genes, isopentanyl transferase gene, and the like.

[0082] The term "reporter gene" means a nucleic acid encoding an identifying factor that is able to be identified based upon the reporter gene's effect, wherein the effect is used to track the inheritance of a nucleic acid of interest, to identify a cell or organism that has inherited the nucleic acid of interest, and/or to measure gene expression induction or transcription. Examples of reporter genes known and used in the art include: luciferase (Luc), green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), β-galactosidase (LacZ), β-glucuronidase (Gus), and the like. Selectable marker genes may also be considered reporter genes.

[0083] "Promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental or physiological conditions. Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". Promoters that cause a gene to be expressed in a specific cell type are commonly referred to as "cell-specific promoters" or "tissue-specific promoters". Promoters that cause a gene to be expressed at a specific stage of development or cell differentiation are commonly referred to as "developmentally-specific promoters" or "cell differentiation-specific promoters". Promoters that are induced and cause a gene to be expressed following exposure or treatment of the cell with an agent, biological molecule, chemical, ligand, light, or the like that induces the promoter are commonly referred to as "inducible promoters" or "regulatable promoters". It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of different lengths may have identical promoter activity.

[0084] A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

[0085] A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-RNA spliced (if the coding sequence contains introns) and translated into the protein encoded by the coding sequence.

[0086] "Transcriptional and translational control sequences" are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

[0087] The term "response element" means one or more cis-acting DNA elements which confer responsiveness on a promoter mediated through interaction with the DNA-binding domains of the first chimeric gene. This DNA element may be either palindromic (perfect or imperfect) in its sequence or composed of sequence motifs or half sites separated by a variable number of nucleotides. The half sites can be similar or identical and arranged as either direct or inverted repeats or as a single half site or multimers of adjacent half sites in tandem. The response element may comprise a minimal promoter isolated from different organisms depending upon the nature of the cell or organism into which the response element will be incorporated. The DNA binding domain of the first hybrid protein binds, in the presence or absence of a ligand, to the DNA sequence of a response element to initiate or suppress transcription of downstream gene(s) under the regulation of this response element Examples of DNA sequences for response elements of the natural ecdysone receptor include: RRGG/TTCANTGAC/ACYY (see Cherbas L., et. al., (1991), Genes Dev. 5: 120-131); AGOTCAN$_{(n)}$AGGTCA,where N$_{(n)}$ can be one or more spacer nucleotides (see D'Avino PP., et. al., (1995), Mol. Cell. Endocrinol 113: 1-9); and GGGTTGAATGAATTT (see Antoniewski C., et. al., (1994), Mol. Cell Biol. 14:4465-4474).

[0088] The term "operably linked" refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

[0089] The term "expression", as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from a nucleic acid or polynucleotide. Expression may also refer to translation of mRNA into a protein or polypeptide.

[0090] The terms "cassette", "expression cassette" and "gene expression cassette" refer to a segment of DNA that can be inserted into a nucleic acid or polynucleotide at specific restriction sites or by homologous recombination. The segment of DNA comprises a polynucleotide that encodes a polypeptide of interest, and the cassette and restriction sites are designed to ensure insertion of the cassette in the proper reading frame for transcription and translation. "Transformation cassette" refers to a specific vector comprising a polynucleotide that encodes a polypeptide of interest and having elements in addition to the polynucleotide that facilitate transformation of a particular host cell. Cassettes, expression cassettes, gene expression cassettes and transformation cassettes of the invention may also comprise elements that allow for enhanced expression of a polynucleotide encoding a polypeptide of interest in a host cell. These elements may include, but are not limited to: a promoter, a minimal promoter, an enhancer, a response element, a terminator sequence, a polyadenylation sequence, and the like.

[0091] For purposes of this invention, the term "gene switch" refers to the combination of a response element associated with a promoter, and an EcR based system which, in the presence of one or more ligands, modulates the expression of a gene into which the response element and promoter are incorporated.

[0092] The terms "modulate" and "modulates" mean to induce, reduce or inhibit nucleic acid or gene expression, resulting in the respective induction, reduction or inhibition of protein or polypeptide production.

[0093] The plasmids or vectors according to the invention may further comprise at least one promoter suitable for driving expression of a gene in a host cell. The term "expression vector" means a vector, plasmid or vehicle designed to enable the expression of an inserted nucleic acid sequence following transformation into the host. The cloned gene, i.e., the inserted nucleic acid sequence, is usually placed under the control of control elements such as a promoter, a minimal promoter, an enhancer, or the like. Initiation control regions or promoters, which are useful to drive expression of a nucleic acid in the desired host cell are numerous and familiar to those skilled in the art. Virtually any promoter capable of driving these genes is suitable for the present invention including but not limited to: viral promoters, bacterial promoters, animal promoters, mammalian promoters, synthetic promoters, constitutive promoters, tissue specific promoter, developmental specific promoters, inducible promoters, light regulated promoters; *CYC1, HIS3, GAL1, GAL4, GAL10, ADH1, PGK PHO5, GAPDH, ADC1, TRP1, URA3, LEU2, ENO, TPI,* alkaline phosphatase promoters (useful for expression in *Saccharomyces*); *AOX1* promoter (useful for expression in *Pichia*); β-lactamase, *lac, ara, tet, trp,* IP_L, IP_R, *T7, tac, and* trc promoters (useful for expression in *Escherichia coli*); light regulated-promoters; animal and mammalian promoters known in the art include, but are not limited to, the SV40 early (SV40e) promoter region, the promoter contained in the 3' long terminal repeat (LTR) ofRous sarcoma virus (RSV), the promoters of the E1A or major late promoter (MLP) genes of adenoviruses (Ad), the cytomegalovirus (CMV) early promoter, the herpes simplex virus (HSV) thymidine kinase (TK) promoter, an elongation factor 1 alpha (EF1) promoter, a phosphoglycerate kinase (PGK) promoter, a ubiquitin (Ubc) promoter, an albumin promoter, the regulatory sequences of the mouse metallothionein-L promoter and transcriptional control regions, the ubiquitous promoters (HPRT, vimentin, α-actin, tubulin and the like), the promoters of the intermediate filaments (desmin, neurofilaments, keratin, GFAP, and the like), the promoters of therapeutic genes (of the MDR, CFTR or factor VIII type, and the like), pathogenesis or disease related-promoters, and promoters that exhibit tissue specificity and have been utilized in transgenic animals, such as the elastase I gene control region which is active in pancreatic acinar cells; insulin gene control region active in pancreatic beta cells, immunoglobulin gene control region active in lymphoid cells, mouse mammary tumor virus control region active in testicular, breast, lymphoid and mast cells; albumin gene, Apo AI and Apo AII control regions active in liver, alpha-fetoprotein gene control region active in liver, alpha 1-antitrypsin gene control region active in the liver, beta-globin gene control region active in myeloid cells, myelin basic protein gene control region active in oligodendrocyte cells in the brain, myosin light chain-2 gene control region active in skeletal muscle, and gonadotropic releasing hormone gene control region active in the hypothalamus, pyruvate kinase promoter, villin promoter, promoter of the fatty acid binding intestinal protein, promoter of the smooth muscle cell α-actin, and the like. In addition, these expression sequences may be modified by addition of enhancer or regulatory sequences and the like.

[0094] Enhancers that may be used in embodiments of the invention include but are not limited to: an SV40 enhancer, a cytomegalovirus (CMV) enhancer, an elongation factor 1 (EF1) enhancer, yeast enhancers, viral gene enhancers, and the like.

[0095] Termination control regions, *i.e.*, terminator or polyadenylation sequences, may also be derived from various genes native to the preferred hosts. Optionally, a termination site may be unnecessary, however, it is most preferred if included. In a preferred embodiment of the invention, the termination control region may be comprise or be derived from a synthetic sequence, synthetic polyadenylation signal, an SV40 late polyadenylation signal, an SV40 polyadenylation signal, a bovine growth hormone (BGH) polyadenylation signal, viral terminator sequences, or the like.

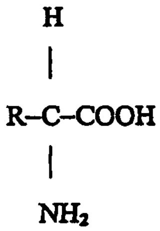
[0096] The terms "3' non-coding sequences" or "3' untranslated region (UTR)" refer to DNA sequences located downstream (3') of a coding sequence and may comprise polyadenylation [poly(A)] recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor.

[0097] "Regulatory region" means a nucleic acid sequence which regulates the expression of a second nucleic acid sequence. A regulatory region may include sequences which are naturally responsible for expressing a particular nucleic acid (a homologous region) or may include sequences of a different origin that are responsible for expressing different proteins or even synthetic proteins (a heterologous region). In particular, the sequences can be sequences of prokaryotic, eukaryotic, or viral genes or derived sequences that stimulate or repress transcription of a gene in a specific or non-specific manner and in an inducible or non-inducible manner. Regulatory regions include origins of replication, RNA splice sites, promoters, enhancers, transcriptional termination sequences, and signal sequences which direct the polypeptide into the secretory pathways of the target cell.

[0098] A regulatory region from a "heterologous source" is a regulatory region that is not naturally associated with the expressed nucleic acid. Included among the heterologous regulatory regions are regulatory regions from a different species, regulatory regions from a different gene, hybrid regulatory sequences, and regulatory sequences which do not occur in nature, but which are designed by one having ordinary skill in the art.

[0099] "RNA transcript" refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from post-transcriptional processing of the primary transcript and is referred to as the mature RNA. "Messenger RNA (mRNA)" refers to the RNA that is without introns and that can be translated into protein by the cell. "cDNA" refers to a double-stranded DNA that is complementary to and derived from mRNA. "Sense" RNA refers to RNA transcript that includes the mRNA and so can be translated into protein by the cell. "Antisense RNA" refers to a RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene. The complementarity of an antisense RNA may be with any part of the specific gene transcript, i.e., at the 5' non-coding sequence, 3' non-coding sequence, or the coding sequence. "Functional RNA" refers to antisense RNA, ribozyme RNA, or other RNA that is not translated yet has an effect on cellular processes.

[0100] A "polypeptide" is a polymeric compound comprised of covalently linked amino acid residues. Amino acids have the following general structure:



$$R-\overset{\displaystyle H}{\underset{\displaystyle NH_2}{C}}-COOH$$

Amino acids are classified into seven groups on the basis of the side chain R: (1) aliphatic side chains, (2) side chains containing a hydroxylic (OH) group, (3) side chains containing sulfur atoms, (4) side chains containing an acidic or amide group, (5) side chains containing a basic group, (6) side chains containing an aromatic ring, and (7) proline, an imino acid in which the side chain is fused to the amino group. A polypeptide of the invention preferably comprises at least about 14 amino acids.

[0101] A "protein" is a polypeptide that performs a structural or functional role in a living cell.

[0102] An "isolated polypeptide" or "isolated protein" is a polypeptide or protein that is substantially free of those compounds that are normally associated therewith in its natural state (e.g., other proteins or polypeptides, nucleic acids, carbohydrates, lipids). "Isolated" is not meant to exclude artificial or synthetic mixtures with other compounds, or the presence of impurities which do not interfere with biological activity, and which may be present, for example, due to incomplete purification, addition of stabilizers, or compounding into a pharmaceutically acceptable preparation.

[0103] "Fragment" of a polypeptide according to the invention will be understood to mean a polypeptide whose amino acid sequence is shorter than that of the reference polypeptide and which comprises, over the entire portion with these reference polypeptides, an identical amino acid sequence. Such fragments may, where appropriate, be included in a larger polypeptide of which they are a part. Such fragments of a polypeptide according to the invention may have a length of at least 2, 3, 4, 5, 6, 8, 10,13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 25, 26, 30, 35, 40, 45, 50, 100, 200, 240, or 300 amino acids.

[0104] A "variant" of a polypeptide or protein is any analogue, fragment, derivative, or mutant which is derived from a polypeptide or protein and which retains at least one biological property of the polypeptide or protein. Different variants of the polypeptide or protein may exist in nature. These variants may be allelic variations characterized by differences in the nucleotide sequences of the structural gene coding for the protein, or may involve differential splicing or post-translational modification. The skilled artisan can produce variants having single or multiple amino acid substitutions, deletions, additions, or replacements. These variants may include, *inter alia*: (a) variants in which one or more amino acid residues are substituted with conservative or non-conservative amino acids, (b) variants in which one or more amino acids are added to the polypeptide or protein, (c) variants in which one or more of the amino acids includes a substituent group, and (d) variants in which the polypeptide or protein is fused with another polypeptide such as serum albumin. The techniques for obtaining these variants, including genetic (suppressions, deletions, mutations, etc.), chemical, and enzymatic techniques, are known to persons having ordinary skill in the art. A variant polypeptide preferably comprises at least about 14 amino acids.

[0105] A "heterologous protein" refers to a protein not naturally produced in the cell.

[0106] A "mature protein" refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. "Precursor" protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

[0107] The term "signal peptide" refers to an amino terminal polypeptide preceding the secreted mature protein. The signal peptide is cleaved from and is therefore not present in the mature protein. Signal peptides have the function of directing and translocating secreted proteins across cell membranes. Signal peptide is also referred to as signal protein.

[0108] A "signal sequence" is included at the beginning of the coding sequence of a protein to be expressed on the surface of a cell. This sequence encodes a signal peptide, N-terminal to the mature polypeptide, that directs the host cell to translocate the polypeptide. The term "translocation signal sequence" is used herein to refer to this sort of signal sequence. Translocation signal sequences can be found associated with a variety of proteins native to eukaryotes and prokaryotes, and are often functional in both types of organisms.

[0109] The term "homology" refers to the percent of identity between two polynucleotide or two polypeptide moieties. The correspondence between the sequence from one moiety to another can be determined by techniques known to the art. For example, homology can be determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions that form stable duplexes between homologous regions, followed by digestion with single-stranded-specific nuclease(s) and size determination of the digested fragments.

[0110] As used herein, the term "homologous" in all its grammatical forms and spelling variations refers to the relationship between proteins that possess a "common evolutionary origin," including proteins from superfamilies (*e.g.*, the inununoglobulin superfamily) and homologous proteins from different species (*e.g.*, myosin light chain, etc.) (Reeck et al., 1987, Cell 50: 667.). Such proteins (and their encoding genes) have sequence homology, as reflected by their high degree of sequence similarity. However, in common usage and in the instant application, the term "homologous," when modified with an adverb such as "highly," may refer to sequence similarity and not a common evolutionary origin.

[0111] Accordingly, the term "sequence similarity" in all its grammatical forms refers to the degree of identity or correspondence between nucleic acid or amino acid sequences of proteins that may or may not share a common evolutionary origin (see Reeck et al., 1987, Cell 50:667).

[0112] In a specific embodiment, two DNA sequences are "substantially homologous" or "substantially similar" when at least about 50% (preferably at least about 75%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Sambrook *et al*., 1989, *supra*.

[0113] As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the protein encoded by the DNA sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by antisense or co-suppression technology. "Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotide bases that do not substantially affect the functional properties of the resulting transcript. It is therefore understood that the invention encompasses more than the specific exemplary sequences. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products.

[0114] Moreover, the skilled artisan recognizes that substantially similar sequences encompassed by this invention are also defined by their ability to hybridize, under stringent conditions (0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS), with the sequences exemplified herein. Substantially similar nucleic acid fragments of the instant invention are those nucleic acid fragments whose DNA sequences are at least 70% identical to the DNA sequence of the nucleic acid fragments reported herein. Preferred substantially nucleic acid fragments of the instant invention are those nucleic acid fragments whose DNA sequences are at least 80% identical to the DNA sequence of the nucleic acid fragments reported herein. More preferred nucleic acid fragments are at least 90% identical to the DNA sequence of the nucleic acid fragments reported herein. Even more preferred are nucleic acid fragments that are at least 95% identical to the DNA sequence of the nucleic acid fragments reported herein.

[0115] Two amino acid sequences are "substantially homologous" or "substantially similar" when greater than about 40% of the amino acids are identical, or greater than 60% are similar (functionally identical). Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, *Version 7,* Madison, Wisconsin) pileup program.

[0116] The term "corresponding to" is used herein to refer to similar or homologous sequences, whether the exact position is identical or different from the molecule to which the similarity or homology is measured. A nucleic acid or amino acid sequence alignment may include spaces. Thus, the term "corresponding to" refers to the sequence similarity, and not the numbering of the amino acid residues or nucleotide bases.

[0117] A "substantial portion" of an amino acid or nucleotide sequence comprises enough of the amino acid sequence of a polypeptide or the nucleotide sequence of a gene to putatively identify that polypeptide or gene, either by manual evaluation of the sequence by one skilled in the art, or by computer-automated sequence comparison and identification using algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) J. Mol. Biol. 215: 403-410; see also www.ncbi.nlm.nlh.gov/BLAST/). In general, a sequence of ten or more contiguous amino acids or thirty or more nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene specific oligonucleotide probes comprising 20-30 contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12-15 bases may be used as amplification primers in PCR in order to obtain a particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises enough of the sequence to specifically identify and/or isolate a nucleic acid fragment comprising the sequence.

[0118] The term "percent identity", as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford

University Press, New York (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, New York (1993); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, New Jersey (1994); Sequence Analysis in Molecular Biology (von Heinje, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribskov, M. and Devereux, J., eds.) Stockton Press, New York (1991). Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences may be performed using the Clustal method of alignment (Higgins and Sharp (1989) CABIOS. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method may be selected: KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

[0119] The term "sequence analysis software" refers to any computer algorithm or software program that is useful for the analysis of nucleotide or amino acid sequences. "Sequence analysis software" may be commercially available or independently developed. Typical sequence analysis software will include but is not limited to the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WI), BLASTP, BLASTN, BLASTX (Altschul et al., J. Mol. Biol. 215:403-410 (1990), and DNASTAR (DNASTAR, Inc. 1228 S. Park St. Madison, WI 53715 USA). Within the context of this application it will be understood that where sequence analysis software is used for analysis, that the results of the analysis will be based on the "default values" of the program referenced, unless otherwise specified. As used herein "default values" will mean any set of values or parameters which originally load with the software when first initialized.

[0120] "Synthetic genes" can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form gene segments that are then enzymatically assembled to construct the entire gene. "Chemically synthesized", as related to a sequence of DNA, means that the component nucleotides were assembled *in vitro.* Manual chemical synthesis of DNA may be accomplished using well-established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the genes can be tailored for optimal gene expression based on optimization of nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

GENE EXPRESSION MODULATION SYSTEM OF THE INVENTION

[0121] Applicants have previously shown that separating the transactivation and DNA binding domains by placing them on two different proteins results in greatly reduced background activity in the absence of a ligand and significantly increased activity over background in the presence of a ligand (pending application PCT7US01/09050). This two-hybrid system is a significantly improved inducible gene expression modulation system compared to the two systems disclosed in International Patent Applications PGT/US97/05330 and PCT/US98/14215. The two-hybrid system exploits the ability of a pair of interacting proteins to bring the transcription activation domain into a more favorable position relative to the DNA binding domain such that when the DNA binding domain binds to the DNA binding site on the gene, the transactivation domain more effectively activates the promoter (see, for example, U.S. Patent No. 5,283,173). Briefly, the two-hybrid gene expression system comprises two gene expression cassettes; the first encoding a DNA binding domain fused to a nuclear receptor polypeptide, and the second encoding a transactivation domain fused to a different nuclear receptor polypeptide. In the presence of ligand, the interaction of the first polypeptide with the second polypeptide effectively tethers the DNA binding domain to the transactivation domain. Since the DNA binding and transactivation domains reside on two different molecules, the background activity in the absence of ligand is greatly reduced.

[0122] The two-hybrid ecdysone receptor-based gene expression modulation system may be either heterodimeric and homodimeric. A functional EcR complex generally refers to a heterodimeric protein complex consisting of two members of the steroid receptor family, an ecdysone receptor protein obtained from various insects, and an ultraspiracle (USP) protein or the vertebrate homolog of USP, retinoid X receptor protein (see Yao, et al. (1993) Nature 366, 476-479; Yao, et al., (1992) Cell 71, 63-72). However, the complex may also be a homodimer as detailed below. The functional ecdysteroid receptor complex may also include additional protein(s) such as immunophilins. Additional members of the steroid receptor family of proteins, known as transcriptional factors (such as DHR38 or *betaFTZ-1*), may also be ligand dependent or independent partners for EcR, USP, and/or RXR. Additionally, other cofactors may be required such as proteins generally known as coactivators (also termed adapters or mediators). These proteins do not bind sequence-specifically to DNA and are not involved in basal transcription. They may exert their effect on transcription activation through various mechanisms, including stimulation of DNA-binding of activators, by affecting chromatin structure, or by mediating activator-initiation complex interactions. Examples of such coactivators include RIP140, TIF1. RAP46/Bag-

1, ARA70, SRG1/NCoA-1, TIF2/GRIP/NCoA-2, ACTR/AIB1/R.AC3/pCIP as well as the promiscuous coactivator C response element B binding protein, CBP/p300 (for review see Glass et al., Curr. Opin. Cell Biol. 9: 222-232, 1997). Also, protein cofactors generally known as corepressors (also known as repressors, silencers, or silencing mediators) may be required to effectively inhibit transcriptional activation in the absence of ligand. These corepressors may interact with the unliganded ecdysone receptor to silence the activity at the response element. Current evidence suggests that the binding of ligand changes the conformation of the receptor, which results in release of the corepressor and recruitment of the above described coactivators, thereby abolishing their silencing activity. Examples of corepressors include N-CoR and SMRT (for review, see Horwitz et al. Mol Endocrinol. 10: 1167-1177, 1996). These cofactors may either be endogenous within the cell or organism, or may be added exogenously as transgenes to be expressed in either a regulated or unregulated fashion. Homodimer complexes of the ecdysone receptor protein, USP, or RXR may also be functional under some circumstances.

[0123] The ecdysone receptor complex typically includes proteins that are members of the nuclear receptor superfamily wherein all members are generally characterized by the presence of an amino-terminal transactivation domain, a DNA binding domain ("DBD"), and a ligand binding domain ("LBD") separated from the DBD by a hinge region. As used herein, the term "DNA binding domain" comprises a minimal polypeptide sequence of a DNA binding protein, up to the entire length of a DNA binding protein, so long as the DNA binding domain functions to associate with a particular response element. Members of the nuclear receptor superfamily are also characterized by the presence of four or five domains: A/B, C, D, E, and in some members F (see US patent 4,981,784 and Evans, Science 240: 889-895 (1988)). The "AB" domain corresponds to the transactivation domain, "C" corresponds to the DNA binding domain, "D" corresponds to the hinge region, and "E" corresponds to the ligand binding domain. Some members of the family may also have another transactivation domain on the carboxy-terminal side of the LBD corresponding to "F".

[0124] The DBD is characterized by the presence of two cysteine zinc fingers between which are two amino acid motifs, the P-box and the D-box, which confer specificity for ecdysone response elements. These domains may be either native, modified, or chimeras of different domains of heterologous receptor proteins. This EcR receptor, like a subset of the steroid receptor family, also possesses less well-defined regions responsible for heterodimerization properties. Because the domains of EcR, USP, and RXR are modular in nature, the LBD, DBD, and transactivation domains may be interchanged.

[0125] Gene switch systems are known that incorporate components from the ecdysone receptor complex. However, in these known systems, whenever EcR is used it is associated with native or modified DNA binding domains and transactivation domains on the same molecule. USP or RXR are typically used as silent partners. Applicants have previously shown that when DNA binding domains and transactivation domains are on the same molecule the background activity in the absence of ligand is high and that such activity is dramatically reduced when DNA binding domains and transactivation domains are on different molecules, that is, on each of two partners of a heterodimeric or homodimeric complex (see PCT/US01/09050). This two-hybrid system also provides improved sensitivity to non-steroidal ligands for example, diacylhydrazines, when compared to steroidal ligands for example, ponasterone A ("PonA") or muristerone A ("MurA"). That is, when compared to steroids, the non-steroidal ligands provide higher activity at a lower concentration. In addition, since transactivation based on EcR gene switches is often cell-line dependent, it is easier to tailor switching systems to obtain maximum transactivation capability for each application. Furthermore, the two-hybrid system avoids some side effects due to overexpression of RXR that often occur when unmodified RXR is used as a switching partner. In a specific embodiment of the two-hybrid system, native DNA binding and transactivation domains of EcR or RXR are eliminated and as a result, these hybrid molecules have less chance of interacting with other steroid hormone receptors present in the cell resulting in reduced side effects.

[0126] Applicants have previously shown that an ecdysone receptor in partnership with a dipteran (fruit fly *Drosophila melanogaster*) or a lepidopteran (spruce bud worm *Choristonieura fumiferana*) ultraspiracle protein (USP) is constitutively expressed in mammalian cells, while an ecdysone receptor in partnership with a vertebrate retinoid X receptor (RXR) is inducible in mammalian cells (pending application PCT/US01/09050). Applicants have now made the surprising discovery that the ultraspiracle protein *of Locusta migratoria* ("LmUSP") and the RXR homolog 1 and RXR homolog 2 of the ixodid tick *Amblyomma americanum* ("AmaRXR1" and "AmaRXR2", respectively) can function similar to vertebrate retinoid X receptor (RXR) in an inducible ecdysone receptor-based inducible gene expression system. Thus, Applicants' findings that LmUSP, AmaRXR1, AmaRXR2, and their non-Dipteran, non-Lepidopteran homologs including, but not limited to: fiddler crab *Celuca pugilator* RXR homolog ("CpRXR"), beetle *Tenebrio molitor* RXR homolog ("TmRXR"), honeybee *Apis mellifera* RXR homolog ("AmRXR"), and aphid *Myzus persicae* RXR homolog ("MpRXR"), all of which are referred to herein collectively as invertebrate RXRs, can be substituted for vertebrate RXR in ecdysone receptor-based inducible gene expression systems can only be regarded as unexpected and surprising. As described herein, Applicants' novel ecdysone receptor/invertebrate RXR -based inducible gene expression system provides an improved inducible gene expression system in yeast and mammalian cells that is characterized by increased ligand sensitivity and magnitude of transactivation.

[0127] In particular, Applicants describe herein a novel two-hybrid system that comprises an invertebrate RXR ligand binding domain. This novel gene expression system demonstrates for the first time that an invertebrate ultraspiracle protein/RXR homolog can function as a component of an inducible EcR-based inducible gene expression system in yeast and mammalian cells. As discussed herein, this finding is both unexpected and surprising.

[0128] Specifically, Applicants' invention relates to a gene expression modulation system comprising: a) a first gene expression cassette that is capable of being expressed in a host cell, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) an ecdysone receptor ligand binding domain; and b) a second gene expression cassette that is capable of being expressed in the host cell, wherein the second gene expression cassette comprises a polynucleotide sequence that encodes a second hybrid polypeptide comprising i) a transactivation domain; and ii) an invertebrate retinoid X receptor ligand binding domain.

[0129] The present invention also relates to a gene expression modulation system comprising. a) a first gene expression cassette that is capable of being expressed in a host cell, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and ii) an invertebrate retinoid X receptor ligand binding domain; and b) a second gene expression cassette that is capable of being expressed in the host cell, wherein the second gene expression cassette comprises a polynucleotide sequence that encodes a second hybrid polypeptide comprising i) a transactivation domain; and ii) an ecdysone receptor ligand binding domain.

[0130] The present invention also relates to a gene expression modulation system according to the present invention further comprising c) a third gene expression cassette comprising. i) a response element to which the DNA-binding domain of the first hybrid polypeptide binds; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated.

[0131] In a specific embodiment, the gene whose expression is to be modulated is a homologous gene with respect to the host cell. In another specific embodiment, the gene whose expression is to be modulated is a heterologous gene with respect to the host cell.

[0132] The ligands for use in the present invention as described below, when combined with the ligand binding domains of an EcR and an invertebrate RXR, which in turn are bound to the response element linked to a gene, provide the means for external temporal regulation of expression of the gene. The binding mechanism or the order in which the various components of this invention bind to each other, that is, for example, ligand to receptor, first hybrid polypeptide to response element, second hybrid polypeptide to promoter, etc., is not critical. Binding of the ligand to the ligand binding domains of an EcR and invertebrate RXR enables expression or suppression of the gene. This mechanism does not exclude the potential for ligand binding to EcR or invertebrate RXR, and the resulting formation of active homodimer complexes (e.g. EcR+ EcR or invertebrate RXR+ invertebrate RXR). Preferably, one or more of the receptor domains is varied producing a chimeric or hybrid gene switch. Typically, one or more of the three domains, DBD, LBD, and transactivation domain, may be chosen from a source different than the source of the other domains so that the hybrid genes and the resulting hybrid proteins are optimized in the chosen host cell or organism for transactivating activity, complementary binding of the ligand, and recognition of a specific response element. In addition, the response element itself can be modified or substituted with response elements for other DNA binding protein domains such as the GAL-4 protein from yeast (see Sadowski, et al., (1988) Nature 335: 563-564) or LexA protein from *Escherichia coli* (see Brent and Ptashne, (1985), Cell 43: 729-736), or synthetic response elements specific for targeted interactions with proteins designed, modified, and selected for such specific interactions (see, for example, Kim, et al. (1997), Proc. Natl. Acad Sci., USA 94: 3616-3620) to accommodate hybrid receptors. Another advantage of two-hybrid systems is that they allow choice of a promoter used to drive the gene expression according to a desired end result. Such double control can be particularly important in areas of gene therapy, especially when cytotoxic proteins are produced, because both the timing of expression as well as the cells wherein expression occurs can be controlled. When genes, operably linked to a suitable promoter, are introduced into the cells of the subject, expression of the exogenous genes is controlled by the presence of the system of this invention. Promoters may be constitutively or inducibly regulated or may be tissue-specific (that is, expressed only in a particular type of cells) or specific to certain developmental stages of the organism.

GENE EXPRESSION CASSETTES OF THE INVENTION

[0133] The novel EcR/invertebrate RXR-based inducible gene expression system of the invention comprises gene expression cassettes that are capable of being expressed in a host cell, wherein the gene expression cassettes each comprise a polynucleotide encoding a hybrid polypeptide. Thus, Applicants' invention also provides novel gene expression cassettes for use in the gene expression system of the invention.

[0134] Specifically, the present invention provides a gene expression cassette comprising a polynucleotide encoding a hybrid polypeptide. In particular, the present invention provides a gene expression cassette that is capable of being expressed in a host cell, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising either i) a DNA-binding domain that recognizes a response element, or ii) a transactivation domain; and an ecdysone receptor ligand binding domain or an invertebrate retinoid X receptor ligand binding domain.

[0135] In a specific embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain that recognizes a response element and an EcR ligand binding domain.

[0136] In another specific embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain that recognizes a response element and an invertebrate RXR ligand binding domain.

[0137] In another specific embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain and an EcR ligand binding domain.

[0138] In another specific embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain and an invertebrate RXR ligand binding domain.

[0139] In a preferred embodiment, the ligand binding domain (LBD) is an EcR LBD, an invertebrate RXR LBD, or a related steroid/thyroid hormone nuclear receptor family member LBD, or analogs, combinations, or modifications thereof. In a specific embodiment, the LBD is from an EcR or an invertebrate RXR In another specific embodiment, the LBD is from a truncated EcR LBD or a truncated invertebrate RXR LBD. A truncation mutation may be made by any method used in the art, including but not limited to restriction endonuclease digestion/deletion, PCR-mediated/oligonucleotide-directed deletion, chemical mutagenesis, DNA strand breakage, and the like.

[0140] The EcR may be an invertebrate EcR, preferably selected from the class Arthropod. Preferably the EcR is selected from the group consisting of a Lepidopteran EcR, a Dipteran EcR, an Orthopteran EcR, a Homopteran EcR and a Hemipteran EcR More preferably, the EcR for use is a spruce budworm *Chorisioneura fuiniferana* EcR ("CfEcR"), a beetle *Tenebrio molitor* EcR ("TmEcR"), a *Manduca sexta* EcR ("MsEcR"), a *Heliothies virescens* EcR ("HvEcR"), a midge *Chironomus tentans* EcR ("CtEcR"), a silk moth *Bombyx mori* EcR ("BmEcR"), a fruit fly *Drosophila melanogaster* EcR ("DmEcR"), a mosquito *Aedes aegypti* EcR ("AaEcR"), a blowfly *Lucilia capitata* ("LcEcR"), a blowfly *Lucilia cuprina* EcR ("LucEcR"), a Mediterranean fruit fly *Ceratitis capitata* EcR ("CcEcR"), a locust *Locusta migratoria* EcR ("LmEcR"), an aphid *Myzus persicae* EcR ("MpEcR"), a fiddler crab *Celuca pugilator* EcR ("CpEcR"), an ixodid tick *Amblyomma americanum* EcR ("AmaEcR"), a whitefly *Bainecia argentifoli* EcR ("BaBeR", SEQ ID NO: 57), or a leafhopper *Nephotetix cincticeps* EcR ("NcEcR", SEQ ID NO: 58). Most preferably, the LBD is from spruce budworm (*Choristoneura fumiferana*) EcR ("CfEcR"), fruit fly *Drosophila melanogaster* EcR ("DmEcR"), whitefly *Bamecia argentifoli* EcR ("BaEcR"), leafhopper *Nephotetix citicticeps* EcR ("NcEcR"), beetle *Tenebrio molitor* EcR ("TmEcR"), or ixodid tick *Amblyomma americanum* EcR ("AmaEcR").

[0141] In a specific embodiment, the LBD is from a truncated EcR polypeptide. The EcR polypeptide truncation results in a deletion of at least 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190,195, 200, 205, 210, 215, 220, 225, 230, 235, 240, 245, 250, 255, 260, or 265 amino acids. Preferably, the EcR polypeptide truncation results in a deletion of at least a partial polypeptide domain. More preferably, the EcR polypeptide truncation results in a deletion of at least an entire polypeptide domain. In a specific embodiment, the EcR polypeptide truncation results in a deletion of at least an A/B-domain, a C-domain, a D-domain, an F-domain, an A/B/C-domains, an A/B/1/2-C-domains, an AB/C/D-domains, an A/B/C/D/F-domains, an A/B/F-domains, an A/B/C/F-domains, a partial E domain, or a partial F domain. A combination of several complete and/or partial domain deletions may also be performed.

[0142] In one embodiment, the ecdysone receptor ligand binding domain is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 (DmEcR-EF), SEQ ID NO: 3 (CfEcR-DE), and SEQ ID NO: 4 (DmEcR-DE). In a preferred embodiment, the ecdysone receptor ligand binding domain is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 (CfEcR-EF), SEQ ID NO: 53 (CfEcR-DEF), and SEQ ID NO: 45 (CfEcR-CDEF).

[0143] In one embodiment, the ecdysone receptor ligand binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6 (DmEcR-EF), SEQ ID NO: 7 (CfEcR-DE), and SEQ ID NO: 8 (DmEcR-DE). In a preferred embodiment, the ecdysone receptor ligand binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5 (CfEcR EF), SEQ ID NO: 43 (CFEcR-DEF), and SEQ ID NO: 59 (CfEcR-CDEF).

[0144] Preferably, the invertebrate RXR polypeptide is a locust *Locusta migratoria* ultraspiracle polypeptide ("LmUSP"), an ixodid tick *Amblyomma americanum* RXR homolog I ("AmaRXR1"), a ixodid tick *Amblyomma americanum* RXR homolog 2 ("AmaRXR2"), a fiddler crab *Celuca pugilator* RXR homolog ("CpRXR"), a beetle *Tenebrio molitor* RXR homolog ("TmRXR"), a honeybee *Apis mellifera* RXR homolog ("AmRXR"), an aphid *Myzus persicae* RXR homolog ("MpRXR"), or a non-Dipteran/non-Lepidopteran RXR homolog.

[0145] In a specific embodiment, the LBD is from a truncated invertebrate RXR The invertebrate RXR polypeptide truncation results in a deletion of at least 1, 2, 3, 4, 5, 6, 8, 10,13, 14,15, 16,17, 18,19, 20, 21, 22, 25, 26, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100,105, 110,115, 120,125, 130, 135, 140, 145,150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, 220, 225, 230, 235, or 240 amino acids. Preferably, the invertebrate RXR polypeptide truncation results in a deletion of at least a partial polypeptide domain. More preferably, the invertebrate RXR polypeptide truncation results in a deletion of at least an entire polypeptide domain. In a specific embodiment, the invertebrate RXR polypeptide truncation results in a deletion of at least a partial E-domain, a complete E-domain, a partial F-domain, a complete F-domain, an EF-domain helix 1, an EF-domain helix 2, an EF-domain helix 3, an EF-domain helix 4, an EF-domain helix 5, an EF-domain helix 6, an EF-domain helix 7, an EF-domain helix 8, and EF-domain helix 9, an EF-domain helix 10, an EF-domain helix 11, an EF-domain helix 12, an EF-domain β-pleated sheet, an AB-domain, a C-domain, a D-domain, A/B/C-domains, AB/1/2-C-domains, A/B/C/D-domains, A/B/C/D/F-domains, A/B/P-domains, or A/B/C/F-domains. A combination of several complete and/or partial domain deletions may also be performed.

[0146] In a preferred embodiment, the invertebrate RXR ligand binding domain is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9 (LmUSP-EF), SEQ ID NO: 10 (AmaRXR1-EF), SEQ ID NO: 11 (AmaRXR2-EF), SEQ ID NO: 12 (CpRXR-EF), SEQ ID NO: 13 (TmRXR-EF), SEQ ID NO: 14 (AmRXR-EF), SEQ ID NO: 15 (LmUSP-EF, BamHI-deleted), SEQ ID NO: 16 (AmaRXR1-EF, BamHI-deleted), SEQ ID NO: 17 (AmaRXR2-EF, BamHI-deleted), SEQ ID NO: 18 (CpRXR-EF, BamHI-deleted), SEQ ID NO: 19 (TmRXR EF, BamHI-deleted), and SEQ ID NO: 20 (AmRXR-EF, BamHI-deleted).

[0147] In another preferred embodiment, the invertebrate RXR ligand binding domain comprises a polypeptide sequence selected from the group consisting of SEQ ID NO: 21 (LmUSP-EF), SEQ ID NO: 22 (AmaRXR1-EF), SEQ ID NO: 23 (AmaRXR2-EF), SEQ ID NO: 24 (CpRXR-EF), SEQ ID NO: 25 (TmRXR-EF), SEQ ID NO: 26 (AmRXR-EF), SEQ ID NO: 27 (LmUSP-EF, BamHI-deleted), SEQ ID NO: 28 (AmaRXR1-EF, BamHI-deleted), SEQ ID NO: 29 (AmaRXR2-EF, BamHI-deleted), SEQ ID NO: 30 (CpRXR-EF, BamHI-deleted), SEQ ID NO: 31 (TmRXR-EF, BamHI-deleted), and SEQ ID NO: 32 (AmRXR-EF, BamHI-deleted).

[0148] For purposes of this invention, EcR and invertebrate RXR also include synthetic and chimeric EcR and invertebrate RXR and their homologs.

[0149] The DNA binding domain can be any DNA binding domain with a known response element, including synthetic and chimeric DNA binding domains, or analogs, combinations, or modifications thereof Preferably, the DBD is a GAL4 DBD, a LexA DBD, a transcription factor DBD, a steroid/thyroid hormone nuclear receptor superfamily member DBD, a bacterial LacZ DBD, or a yeast put DBD. More preferably, the DBD is a GAL4 DBD [SEQ ID NO: 33 (polynucleotide) or SEQ ID NO: 34 (polypeptide)] or a LexA DBD [(SEQ ID NO: 35 (polynucleotide) or SEQ ID NO: 36 (polypeptide)].

[0150] The transactivation domain (abbreviated "AD" or "TA") may be any steroid/thyroid hormone nuclear receptor AD, synthetic or chimeric AD, polyglutamine AD, basic or acidic amino acid AD, a VP16 AD, a GAL4 AD, an NF-κB AD, a BP64 AD, a B42 acidic activation domain (B42AD), or an analog, combination, or modification thereof. In a specific embodiment, the AD is a synthetic or chimeric AD, or is obtained from a VP16, GAL4, NF-kB, or B42 acidic activation domain AD. Preferably, the AD is a VP16 AD [SEQ ID NO: 37 (polynucleotide) or SEQ ID NO: 38 (polypeptide)] or a B42 AD [SEQ ID NO: 39 (polynucleotide) or SEQ ID NO: 40 (polypeptide)].

[0151] In a preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ m NO: 33) and a LexA DBD (SEQ m NO: 35), and an EcR ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53, and SEQ ID NO: 45.

[0152] In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 34) and a LexA DBD (SEQ ID NO: 36), and an EcR ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43, and SEQ ID NO: 59.

[0153] In another preferred embodiment; the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 33) or a LexA DBD (SEQ ID NO: 35) and an invertebrate RXR ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

[0154] In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 34) and a LexA DBD (SEQ ID NO: 36), and an invertebrate RXR ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

[0155] In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 37 or SEQ ID NO: 39, and an EcR ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53, and SEQ ID NO: 45.

[0156] In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence of SEQ ID NO: 38 or SEQ ID NO: 40, and an EcR ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43, and SEQ ID NO: 59.

[0157] In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence of SEQ ID NO: 37 or SEQ ID NO: 39, and an invertebrate RXR ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

[0158] In another preferred embodiment, the gene expression cassette encodes a hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence of SEQ ID NO: 38 or SEQ ID NO: 40 and an invertebrate RXR ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

[0159] The response element ("RE") may be any response element with a known DNA binding domain, or an analog, combination, or modification thereof. A single RE may be employed or multiple REs, either multiple copies of the same RE or two or more different REs, may be used in the present invention. In a specific embodiment, the RE is an RE from GAL4 ("GAL4RE"), LexA, a steroid/thyroid hormone nuclear receptor RE, or a synthetic RE that recognizes a synthetic DNA binding domain. Preferably, the RE is a GAL4RE comprising a polynucleotide sequence of SEQ ID NO: 41 or a LexA RE (operon, "op") comprising a polynucleotide sequence of SEQ ID NO: 42 ("2XLexAopRB"). Preferably, the first hybrid protein is substantially free of a transactivation domain and the second hybrid protein is substantially free of a DNA binding domain. For purposes of this invention, "substantially free" means that the protein in question does not contain a sufficient sequence of the domain in question to provide activation or binding activity.

[0160] Thus, the present invention also relates to a gene expression cassette comprising: i) a response element comprising a domain to which a polypeptide comprising a DNA binding domain binds; ii) a promoter that is activated by a polypeptide comprising a transactivation domain; and iii) a gene whose expression is to be modulated.

[0161] Genes of interest for use in Applicants' gene expression cassettes may be endogenous genes or heterologous genes. Nucleic acid or amino acid sequence information for a desired gene or protein can be located in one of many public access databases, for example, GENBANK, EMBL, Swiss-Prot, and PIR, or in many biology related journal publications. Thus, those skilled in the art have access to nucleic acid sequence information for virtually all known genes. Such information can then be used to construct the desired constructs for the insertion of the gene of interest within the gene expression cassettes used in Applicants' methods described herein.

[0162] Examples of genes of interest for use in Applicants' gene expression cassettes include, but are not limited to: genes encoding therapeutically desirable polypeptides or products that may be used to treat a condition, a disease, a disorder, a dysfunction, a genetic defect, such as monoclonal antibodies, enzymes, proteases, cytokines, interferon, insulin, erthropoietin, clotting factors, other blood factors or components, viral vectors for gene therapy, virus for vaccines, targets for drug discovery, functional genomics, and proteomics analyses and applications, and the like.

POLYNUCLEOTIDES OF THE INVENTION

[0163] The novel ecdysone receptor/invertebrate retinoid X receptor-based inducible gene expression system of the invention comprises a gene expression cassette comprising a polynucleotide that encodes a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain, and b) an EcR ligand binding domain or an invertebrate RXR ligand binding domain. These gene expression cassettes, the polynucleotides they comprise, and the hybrid polypeptides they encode are useful as components of an EcR-based gene expression system to modulate the expression of a gene within a host cell.

[0164] Thus, the present invention provides an isolated polynucleotide that encodes a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain according to the invention, and b) an EcR ligand binding domain or an invertebrate RXR ligand binding domain according to the invention.

[0165] The present invention also relates to an isolated polynucleotide that encodes a truncated EcR or a truncated invertebrate RXR polypeptide comprising a truncation mutation according to the invention. Specifically, the present invention relates to an isolated polynucleotide encoding an EcR or an invertebrate RXR polypeptide comprising a truncation mutation that affects ligand binding activity or ligand sensitivity that is useful in modulating gene expression in a host cell.

[0166] In a specific embodiment, the isolated truncated EcR polynucleotide comprises a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53 and SEQ ID NO: 45.

[0167] In another specific embodiment, the isolated truncated EcR polynucleotide encodes a truncated ecdysone receptor polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43 and SEQ ID NO: 59.

[0168] In another specific embodiment, the isolated truncated invertebrate RXR polynucleotide according to the invention comprises a polynucleotide sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

[0169] In another specific embodiment, the isolated truncated invertebrate RXR polynucleotide according to the invention encodes a truncated invertebrate RXR polypeptide comprising an amino acid sequence consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

[0170] In particular, the present invention relates to an isolated polynucleotide encoding an invertebrate RXR polypeptide comprising a truncation mutation, wherein the mutation reduces ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide. In a specific embodiment, the present invention relates to an isolated polynucleotide encoding an invertebrate RXR polypeptide comprising a truncation mutation that reduces steroid binding activity or steroid sensitivity of the invertebrate RXR polypeptide.

[0171] In another specific embodiment, the present invention relates to an isolated polynucleotide encoding an invertebrate RXR polypeptide comprising a truncation mutation that reduces non-steroid binding activity or non-steroid sensitivity of the invertebrate RXR polypeptide.

[0172] The present invention also relates to an isolated polynucleotide encoding an invertebrate RXR polypeptide comprising a truncation mutation, wherein the mutation enhances ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide. In a specific embodiment, the present invention relates to an isolated polynucleotide encoding an invertebrate RXR polypeptides comprising a truncation mutation that enhances steroid binding activity or steroid sensitivity of the invertebrate RXR polypeptide.

[0173] In another specific embodiment, the present invention relates to an isolated polynucleotide encoding an invertebrate RXR polypeptide comprising a truncation mutation that enhances non-steroid binding activity or non-steroid sensitivity of the invertebrate RXR polypeptide.

[0174] The present invention also relates to an isolated polynucleotide encoding an invertebrate retinoid X receptor polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the mutated invertebrate retinoid X receptor polypeptide and a dimerization partner. Preferably, the isolated polynucleotide encoding an invertebrate retinoid X receptor polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprises a polynucleotide sequence selected from the group consisting of SEQ ID NO: 9 (LmUSP-EF), SEQ ID NO: 10 (AmaRXR1-EF), SEQ ID NO: 11 (AmaRXR2-EF), SEQ ID NO: 12 (CpRXR-EF), SEQ ID NO: 13 (TmRXR-EF), and SEQ ID NO: 14 (AmRXR-EF). In a specific embodiment, the dimerization partner is an ecdysone receptor polypeptide. Preferably, the dimerization partner is a truncated EcR polypeptide. More preferably, the dimerization partner is an EcR polypeptide in which domain A/B has been deleted. Even more preferably, the dimerization partner is an EcR polypeptide comprising an amino acid sequence of SEQ ID NO: 5 (CfEcR-EF), SEQ ID NO: 43 (CfEcR-DEF) or SEQ ID NO: 59 (CfEcR-CDEF).

POLYPEPTIDES OF THE INVENTION

[0175] The novel ecdysone receptor/invertebrate retinoid X receptor-based inducible gene expression system of the invention comprises a gene expression cassette comprising a polynucleotide that encodes a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain, and b) an EcR ligand binding domain or an invertebrate RXR ligand binding domain. These gene expression cassettes, the polynucleotides they comprise, and the hybrid polypeptides they encode are useful as components of an EcR-based gene expression system to modulate the expression of a gene within a host cell.

[0176] Thus, the present invention also relates to a hybrid polypeptide comprising a) a DNA binding domain or a transactivation domain according to the invention, and b) an EcR ligand binding domain or an invertebrate RXR ligand binding domain according to the invention.

[0177] The present invention also relates to an isolated truncated EcR or an isolated truncated invertebrate RXR polypeptide comprising a truncation mutation according to the invention. Specifically, the present invention relates to an isolated truncated EcR or an isolated truncated invertebrate RXR polypeptide comprising a truncation mutation that affects ligand binding activity or ligand sensitivity.

[0178] In a specific embodiment, the isolated truncated EcR polypeptide is encoded by a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53 and SEQ ID NO: 45.

[0179] In another specific embodiment, the isolated truncated EcR polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43 and SEQ ID NO: 59.

[0180] In another specific embodiment, the isolated truncated invertebrate RXR polypeptide is encoded by a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

[0181] In another specific embodiment, the isolated truncated invertebrate RXR polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

[0182] The present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that reduces ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide, wherein the polypeptide is encoded by a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

[0183] Thus, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that reduces ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

[0184] In a specific embodiment, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that reduces steroid binding activity or steroid sensitivity of the invertebrate RXR polypeptide.

[0185] In another specific embodiment, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that reduces non-steroid binding activity or non-steroid sensitivity of the invertebrate RXR polypeptide.

[0186] In addition, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that enhances ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide.

[0187] The present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that enhances ligand binding activity or ligand sensitivity of the invertebrate RXR polypeptide. In a specific embodiment, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that enhances steroid binding activity or steroid sensitivity of the invertebrate RXR polypeptide.

[0188] In another specific embodiment, the present invention relates to an isolated invertebrate RXR polypeptide comprising a truncation mutation that enhances non-steroid binding activity or non-steroid sensitivity of the invertebrate RXR polypeptide.

[0189] The present invention also relates to an isolated invertebrate retinoid X receptor polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprising the mutated invertebrate retinoid X receptor polypeptide and a dimerization partner. Preferably, the isolated invertebrate retinoid X receptor polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9 (LmUSP-EF), SEQ ID NO: 10 (AmaRXR1-EF), SEQ ID NO: 11 (AmaRXR2-EF), SEQ ID NO: 12 (CpRXR-EF), SEQ ID NO: 13 (TmRXR-EF), and SEQ ID NO: 14 (AmRXR-EF). More preferably, the isolated polynucleotide encoding an invertebrate retinoid X receptor polypeptide comprising a truncation mutation that increases ligand sensitivity of a heterodimer comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 21 (LmUSP-EF), SEQ ID NO: 22 (AmaRXR1-EF), SEQ ID NO: 23 (AmaRXR2-EF), SEQ ID NO: 24 (CpRXR-EF), SEQ ID NO: 25 (TmRXR-EF), and SEQ ID NO: 26 (AmRXR-EF).

[0190] In a specific embodiment, the dimerization partner is an ecdysone receptor polypeptide. Preferably, the dimerization partner is a truncated EcR polypeptide. More preferably, the dimerization partner is an EcR polypeptide in which domain A/B has been deleted. Even more preferably, the dimerization partner is an EcR polypeptide comprising an amino acid sequence of SEQ ID NO: 5 (CfEcR-EF), SEQ ID NO: 43 (CfEcR DEF) or SEQ ID NO: 59 (CtEcR-CDEF).

METHOD OF MODULATING GENE EXPRESSION OF THE INVENTION

[0191] Applicants' invention also relates to methods of modulating gene expression in a host cell using a gene expression modulation system according to the invention. Specifically, Applicants' invention provides a method of modulating the expression of a gene in a host cell comprising the steps of: a) introducing into the host cell a gene expression modulation system according to the invention; and b) introducing into the host cell a ligand; wherein the gene to be modulated is a component of a gene expression cassette comprising: i) a response element comprising a domain recognized by the DNA binding domain of the first hybrid polypeptide; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated, whereby upon introduction of the ligand into the host cell, expression of the gene is modulated.

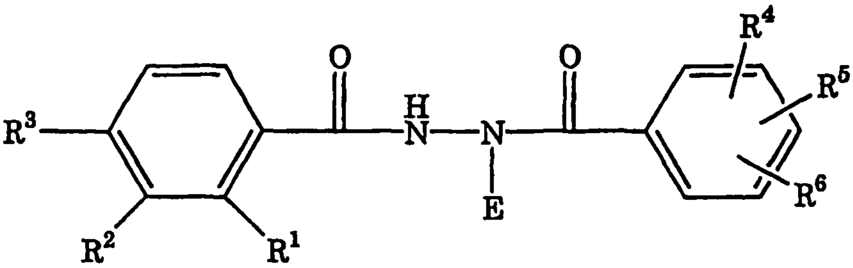
[0192] The invention also provides a method of modulating the expression of a gene in a host cell comprising the steps of: a) introducing into the host cell a gene expression modulation system according to the invention; b) introducing into the host cell a gene expression cassette comprising i) a response element comprising a domain recognized by the DNA binding domain from the first hybrid polypeptide; ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and iii) a gene whose expression is to be modulated; and c) introducing into the host cell a ligand; whereby upon introduction of the ligand into the host cell, expression of the gene is modulated.

[0193] Genes of interest for expression in a host cell using Applicants' methods may be endogenous genes or heterologous genes. Nucleic acid or amino acid sequence information for a desired gene or protein can be located in one of many public access databases, for example, GENBANK, EMBL, Swiss-Prot, and PIR, or in many biology related journal publications. Thus, those skilled in the art have access to nucleic acid sequence information for virtually all known genes. Such information can then be used to construct the desired constructs for the insertion of the gene of interest within the gene expression cassettes used in Applicants' methods described herein.

[0194] Examples of genes of interest for expression in a host cell using Applicants' methods include, but are not limited to: genes encoding therapeutically desirable polypeptides or products that may be used to treat a condition, a disease, a disorder, a dysfunction, a genetic defect, such as monoclonal antibodies, enzymes, proteases, cytokines, interferons, insulin, erthropoietin, clotting factors, other blood factors or components, viral vectors for gene therapy, virus for vaccines, targets for drug discovery, functional genomics, and proteomics analyses and applications, and the like.

[0195] Acceptable ligands are any that modulate expression of the gene when binding of the DNA binding domain of the two-hybrid system to the response element in the presence of the ligand results in activation or suppression of expression of the genes. Preferred ligands include ponasterone, muristerone A, 9-cis-retinoic acid, synthetic analogs of retinoic acid, N,N'-diacylhydrazines such as those disclosed in U. S. Patents No. 6,013,836; 5,117,057; 5,530,028; and 5,378,726; dibenzoylalkyl cyanohydrazines such as those disclosed in European Application No. 461,809; N-alkyl-N, N'-diaroylhydrazines such as those disclosed in U. S. Patent No. 5,225,443; N-acyl-N-alkylcarbonylhydrazines such as those disclosed in European Application No. 234,994; N-aroyl-N-alkyl-N'-aroylhydrazines such as those described in U. S. Patent No. 4,985,461; and other similar materials including 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, and the like.

[0196] In a preferred embodiment, the ligand for use in Applicants' method of modulating expression of gene is a compound of the formula:



wherein:

E	is a $(C_4\text{-}C_6)$alkyl containing a tertiary carbon or a cyano$(C_3\text{-}C_5)$alkyl containing a tertiary carbon;
R^1	is H, Me, Et, i-Pr, F, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF_2CF_3, CH=CHCN, allyl, azido, SCN, or SCHF;
R^2	is H, Me, Et, n-Pr, i-Pr, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe_2, NEt_2, SMe, SEt, $SOCF_3$, OCF_2CF_2H, COEt, cyclopropyl, CF_2CF_3, CH=CHCN, allyl, azido, OCF_3, $OCBF_2$, O-i-Pr, SCN, $SCHF_2$, SOMe, NH-CN, or joined with R^3 and the phenyl carbons to which R^2 and R^3 are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;
R^3	is H, Et, or joined with R^2 and the phenyl carbons to which R^2 and R^3 are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;
R^4, R^5, and R^6	are independently H, Me, Et, F, Cl, Br, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

[0197] In another preferred embodiment, a second ligand may be used in addition to the first ligand discussed above in Applicants' method of modulating expression of a gene, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of retinoic acid

[0198] Applicants' invention provides for modulation of gene expression in prokaryotic and eukaryotic host cells. Thus, the present invention also relates to a method for modulating gene expression in a host cell selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell. Preferably, the host cell is a yeast cell, a hamster cell, a mouse cell, a monkey cell, or a human cell.

[0199] Expression in transgenic host cells may be useful for the expression of various polypeptides of interest including but not limited to therapeutic polypeptides, pathway intermediates; for the modulation of pathways already existing in the host for the synthesis of new products heretofore not possible using the host; cell based assays; functional genomics assays, biotherapeutic protein production, proteomics assays, and the like. Additionally the gene products may be useful for conferring higher growth yields of the host or for enabling an alternative growth mode to be utilized.

HOST CELLS AND NON-HUMAN ORGANISMS OF THE INVENTION

[0200] As described above, the gene expression modulation system of the present invention may be used to modulate gene expression in a host cell. Expression in transgenic host cells may be useful for the expression of various genes of interest. Thus, Applicants' invention provides an isolated host cell comprising a gene expression system according to the invention. The present invention also provides an isolated host cell comprising a gene expression cassette according to the invention. Applicants' invention also provides an isolated host cell comprising a polynucleotide or a polypeptide according to the invention. The isolated host cell may be either a prokaryotic or a eukaryotic host cell.

[0201] Preferably, the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell. Examples of preferred host cells include, but are not limited to, fungal or yeast species such as *Aspergillus, Trichoderma, Saccharomyces, Pichia, Candida, Hansenula,* or bacterial species such as those in the genera *Synechocystis, Synechococcus, Salmonella, Bacillus, Acinetobacter, Rhodococcus, Streptomyces, Escherichia, Pseudomonas, Methylomonas, Methylobacter, Alcaligenes, Synechocystis, Anabaena, Thiobacillus, Methanobacterium* and *Klebsiella,* animal, and mammalian host cells.

[0202] In a specific embodiment, the host cell is a yeast cell selected from the group consisting of a *Saccharomyces, a Pichia,* and a *Candida* host cell.

[0203] In another specific embodiment, the host cell is a hamster cell.

[0204] In another specific embodiment, the host cell is a murine cell.

[0205] In another specific embodiment, the host cell is a monkey cell.

[0206] In another specific embodiment, the host cell is a human cell.

[0207] Host cell transformation is well known in the art and may be achieved by a variety of methods including but not limited to electroporation, viral infection, plasmid/vector transfection, non-viral vector mediated transfection, particle bombardment, and the like. Expression of desired gene products involves culturing the transformed host cells under suitable conditions and inducing expression of the transformed gene. Culture conditions and gene expression protocols in prokaryotic and eukaryotic cells are well known in the art (see General Methods section of Examples). Cells may be harvested and the gene products isolated according to protocols specific for the gene product.

[0208] In addition, a host cell may be chosen which modulates the expression of the inserted polynucleotide, or modifies and processes the polypeptide product in the specific fashion desired. Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification [*e.g.*, glycosylation, cleavage (*e.g.*, of signal sequence)] of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce a non-glycosylated core protein product. However, a polypeptide expressed in bacteria may not be properly folded. Expression in yeast can produce a glycosylated product. Expression in eukaryotic cells can increase the likelihood of "native" glycosylation and folding of a heterologous protein. Moreover, expression in mammalian cells can provide a tool for reconstituting, or constituting, the polypeptide's activity. Furthermore, different vector/host expression systems may affect processing reactions, such as proteolytic cleavages, to a different extent

[0209] Applicants' invention also relates to a non-human organism comprising an isolated host cell according to the invention. Preferably, the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, an animal, and a mammal. More preferably, the non-human organism is a yeast, a mouse, a rat, a rabbit, a cat, a dog, a bovine, a goat, a pig, a horse, a sheep, a monkey, or a chimpanzee.

[0210] In a specific embodiment, the non-human organism is a yeast selected from the group consisting of *Saccharomyces, Pichia,* and *Candida.*

[0211] In another specific embodiment, the non-human organism is a *Mus musculus* mouse.

MEASURING GENE EXPRESSION/TRANSCRIPTION

[0212] One useful measurement of Applicants' methods of the invention is that of the transcriptional state of the cell including the identities and abundances of RNA, preferably mRNA species. Such measurements are conveniently conducted by measuring cDNA abundances by any of several existing gene expression technologies.

[0213] Nucleic acid array technology is a useful technique for determining differential mRNA expression. Such technology includes, for example, oligonucleotide chips and DNA microarrays. These techniques rely on DNA fragments or oligonucleotides which correspond to different genes or cDNAs which are immobilized on a solid support and hybridized to probes prepared from total mRNA pools extracted from cells, tissues, or whole organisms and converted to cDNA. Oligonucleotide chips are arrays of oligonucleotides synthesized on a substrate using photolithographic techniques. Chips have been produced which can analyze for up to 1700 genes. DNA microarrays are arrays of DNA samples, typically PCR products, that are robotically printed onto a microscope slide. Each gene is analyzed by a full-or partial-length target DNA sequence. Microarrays with up to 10,000 genes are now routinely prepared commercially. The primary difference between these two techniques is that oligonucleotide chips typically utilize 25-mer oligonucleotides which allow fractionation of short DNA molecules whereas the larger DNA targets of microarrays, approximately 1000 base pairs, may provide more sensitivity in fractionating complex DNA mixtures.

[0214] Another useful measurement of Applicants' methods of the invention is that of determining the translation state of the cell by measuring the abundances of the constituent protein species present in the cell using processes well known in the art.

[0215] Where identification of genes associated with various physiological functions is desired, an assay may be employed in which changes in such functions as cell growth, apoptosis, senescence, differentiation, adhesion, binding to a specific molecules, binding to another cell, cellular organization, organogenesis, intracellular transport, transport facilitation, energy conversion, metabolism, myogenesis, neurogenesis, and/or hematopoiesis is measured.

[0216] In addition, selectable marker or reporter gene expression may be used to measure gene expression modulation using Applicants' invention.

[0217] Other methods to detect the products of gene expression are well known in the art and include Southern blots (DNA detection), dot or slot blots (DNA, RNA), northern blots (RNA), RT-PCR (RNA), western blots (polypeptide detection), and ELISA (polypeptide) analyses. Although less preferred, labeled proteins can be used to detect a particular nucleic acid sequence to which it hybridizes.

[0218] In some cases it is necessary to amplify the amount of a nucleic acid sequence. This may be carried out using one or more of a number of suitable methods including, for example, polymerase chain reaction ("PCR"), ligase chain reaction ("LCR"), strand displacement amplification ("SDA"), transcription-based amplification, and the like. PCR is carried out in accordance with known techniques in which, for example, a nucleic acid sample is treated in the presence of a heat stable DNA polymerase, under hybridizing conditions, with one pair of oligonucleotide primers, with one primer hybridizing to one strand (template) of the specific sequence to be detected. The primers are sufficiently complementary to each template strand of the specific sequence to hybridize therewith. An extension product of each primer is synthesized and is complementary to the nucleic acid template strand to which it hybridized. The extension product synthesized from each primer can also serve as a template for further synthesis of extension products using the same primers. Following a sufficient number of rounds of synthesis of extension products, the sample may be analyzed as described above to assess whether the sequence or sequences to be detected are present.

[0219] The present invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention.

EXAMPLES

GENERAL METHODS

[0220] Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described by Sambrook, J., Fritsch, E. F. and Maniatis, T. Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, N.Y. (1989) (Maniatis) and by T. J. Silhavy, M. L. Bennan, and L. W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1984) and by Ausubel, F. M. et al., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley-Interscience (1987).

[0221] Materials and methods suitable for the maintenance and growth of bacterial cultures are well known in the art. Techniques suitable for use in the following examples may be found as set out in Manual of Methods for General Bacteriology (Phillipp Gerhardt, R. G. E. Murray, Ralph N. Costilow, Eugene W. Nester, Willis A. Wood, Noel R. Krieg and G. Briggs Phillips, eds), American Society for Microbiology, Washington, DC. (1994)) or by Thomas D. Brock in Biotechnology: A Textbook of Industrial Microbiology. Second Edition, Sinauer Associates, Inc., Sunderland, MA (1989). All reagents, restriction enzymes and materials used for the growth and maintenance of host cells were obtained from Aldrich Chemicals (Milwaukee, WI), DIFCO Laboratories (Detroit, MI), GIBCO/BRL (Gaithersburg, MD), or Sigma Chemical Company (St. Louis, MO) unless otherwise specified.

[0222] Manipulations of genetic sequences may be accomplished using the suite of programs available from the Genetics Computer Group Inc. (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WI). Where the GCG program "Pileup" is used the gap creation default value of 12, and the gap extension default value of 4 may be used. Where the CGC "Gap" or "Bestfit" program is used the default gap creation penalty of 50 and the default gap extension penalty of 3 may be used. In any case where GCG program parameters are not prompted for, in these or any other GCG program, default values may be used.

[0223] The meaning of abbreviations is as follows: "h" means hour(s), "min" means minute(s), "sec" means second (s), "d" means day(s), "μl" means microliter(s), "ml" means milliliter(s), "L" means liter(s), "μM" means micromolar, "mM" means millimolar, "μg" means microgram(s), "mg" means milligram(s), "A" means adenine or adenosine, "T" means thymine or thymidine, "G" means guanine or guanosine, "C" means cytidine or cytosine, "x g" means times gravity, "nt" means nucleotide(s), "aa" means amino acid(s), "bp" means base pair(s), "kb" means kilobase(s), "k" means kilo, "μ" means micro, and "°C" means degrees Celsius.

EXAMPLE 1

[0224] Applicants' EcR/invertebrate RXR-based inducible gene modulation system is useful in various applications including gene therapy, expression of proteins of interest in host cells, production of transgenic organisms, and cell-based assays. In various cellular backgrounds, including mammalian cells, invertebrate EcR heterodimerizes with vertebrate RXR and, upon binding of ligand, transactivates genes under the control of ecdysone response elements. Applicants have made the surprising discovery that invertebrate RXR can substitute for vertebrate RXR and provide a novel inducible gene expression system for yeast and animal cell applications. This Example describes the construction of several gene expression cassettes for use in the EcR-based inducible gene expression system of the invention

[0225] Applicants constructed several EcR-based gene expression cassettes based on the spruce budworm *Choristoneura fumiferana* EcR ("CfEcR"), *C.fumiferana* ultraspiracle ("CfUSP"), *Drosophila melanogaster* USP ("DmUSP"), mouse *Mus musculus* retinoid X receptor α ("MmRXRα"), locust *Locusta migratoria* USP ("LmUSP"), an invertebrate homolog of vertebrate RXR, *Amblyomma americanum* RXR homolog 1 ("AmaRXR1"), an invertebrate homolog of vertebrate RXR, and *Amblyomma americanum* RXR homolog 2 ("AmaRXR2"), an invertebrate homolog of vertebrate RXR. The prepared receptor constructs comprise a ligand binding domain of either an EcR, a vertebrate RXR, an invertebrate USP, or an invertebrate RXR; and a GAL4 or LexA DNA binding domain (DBD) or a VP16 or B42 acidic activator transactivation domain (AD). The reporter constructs include a reporter gene, luciferase or LacZ, operably linked to a synthetic promoter construct that comprises either a GALA response element or a LexA response element to which the Gal4 DBD or LexA DBD binds, respectively. Various combinations of these receptor and reporter constructs were cotransfected into mammalian cells as described in Examples 2-9 *infra.*

Gene Expression Cassettes: Ecdysone receptor-based gene expression cassettes (switches) were constructed as followed, using standard cloning methods available in the art. The following is brief description of preparation and composition of each switch used in the Examples described herein.

1.1 - GALACfEcR-CDEF/VP16MmRXRα-DEF: The C, D, E, and F domains from spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-CDEF"; SEQ ID NO: 45) were fused to a GAL4 DNA binding domain ("Gal4DNABD" or "Gal4DBD"; SEQ ID NO: 33) and placed under the control of an SV40e promoter (SEQ ID NO: 46). The DEF domains from mouse *(Mus musculus)* RXRa ("MmRXRα-DEF"; SEQ ID NO: 47) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 37) and placed under the control of an SV40e promoter (SEQ ID NO: 46). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 41) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 48) and placed upstream of the luciferase gene (SEQ ID NO: 49).

1.2 - GAL4CfEcR-CDEF/VP16MmRXCRα-EF: This construct was prepared in the same way as in switch 1.1 above except MmRXRα-DEF was replaced with MmRXRα-EF (SEQ ID NO: 50).

1.3- GAL4CfEcR-CDEF/VP16CfUSP-DEF: This construct was prepared in the same way as in switch 1.1 above except MmRXRα-DEF was replaced with the D, E and F domains from spruce budworm USP ("CfUSP-DEF"; SEQ ID NO: 51). The constructs used in this example are similar to those disclosed in U. S. Patent No. 5,880,333 except that *Choristoneura funiferana* USP rather than *Drosophila melanogaster* USP was utilized.

1.4 - GAL4CfEcR-CDEF/VP16LmUSP-DEF: This construct was prepared in the same way as in switch 1.1 above except MmRXRα-DEF was replaced with the D, E and F domains *of Locusta migratoria* ultraspiracle ("LmUSP-DEF"; SEQ ID NO: 52).

1.5 - GALACfEcR-DEF/VP16MmRXRα :This construct was prepared in the same way as switch 1.1 except CfEcR-CDEF was replaced with CfEcR-DEF (SEQ ID NO: 53).

1.6 - GAL4CfEcR-DEF/VP16MmRXα-EF: This construct was prepared in the same way as switch 1.5 except MmRXRα-DEF was replaced with MmRXRα-EF (SEQ ID NO: 50).

1.7 - GAL4CfEcR-DEF/VP16CfUSP-DEF: This construct was prepared in the same way as in switch 1.5 above except MmRXRα-DEF was replaced with the D, E and F domains from spruce budworm *C fumiferana* USP ("CfUSP-DEF"; SEQ ID NO: 51).

1.8-GAL4CfEcR-DEF/VP16LmUSP-DEF: This construct was prepared in the same way as in switch 1.5 above except MmRXRα-DEF was replaced with the D, E, and F domains *of Locusta migratoria* ultraspiracle ("LmUSP-DEF"; SEQ ID NO: 52).

1.9-Gal4CfEcR-A/BCDEF/VP16LmUSP-DEF: The full-length spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-A/BCDEF"; SEQ ID NO: 54) was fused to a GAL4 DNA binding domain ("Gal4DNABD" or "Gal4DBD"; SEQ ID NO: 33) and placed under the control of an SV40e promoter (SEQ ID NO: 46). The DEF domains from *Locusta migratoria* ultraspiracle ("LmUSP-DEF"; SEQ ID NO: 52) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 37) and placed under the control of an SV40e promoter (SEQ ID NO: 46). Five consensus GALA response element binding sites ("5XGAL4RE"; comprising 5 copies of a GALARE comprising SEQ ID NO: 41) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 48) and placed upstream of the luciferase gene

(SEQ ID NO: 49).

1.10-Gal4CfEcR-1/2CDEF/VP16LmUSP-DEF: This construct, was prepared in the same way as switch 1.9 except CfEcR-A/BCDEF was replaced with CfEcR-1/2CDEF (SEQ ID NO: 55).

1.11- GAL4CfEcR-CDEF/VP16LmUSP-DEF: This construct was prepared in the same way as switch 1.9 except CfEcR-A/BCDEF was replaced with CfEcR-CDEF (SEQ ID NO: 45).

1.12 - Gal4CfEcR-DEF/VP16LmUSP-DEF: This construct was prepared in the same way as switch 1.9 except CfEcR-A/BCDEF was replaced with CfEcR-DEF (SEQ ID NO: 53).

1.13 - Gal4CfEcR-EF/VP16LmUSP-DEF: This construct was prepared in the same way as switch 1.9 except CfEcR-A/BCDEF was replaced with CfEcR-EF (SEQ ID NO: 1).

1.14 - Gal4CfEcR-DE/VP16LmUSP-DEF: This construct was prepared in the same way as switch 1.9 except CfEcR-CDEF was replaced with CfEcR-DE (SEQ ID NO: 3).

1,15 - Gal4CfEcR-A/BCDEF/VP16LmUSP-EF: The full-length spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-A/BCDEF"; SEQ ID NO: 54) was fused to a GAL4 DNA binding domain ("Gal4DNABD" or "Gal4DBD"; SEQ ID NO: 33) and placed under the control of an SV40e promoter (SEQ ID NO: 46). The EF domains *from Locusta migratoria* ultraspiracle ("LmUSP-EF"; SEQ ID NO: 9) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 37) and placed under the control of an SV40e promoter (SEQ ID NO: 46). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 41) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 48) and placed upstream of the luciferase gene (SEQ ID NO: 49).

1.16 - Gal4CfEcR-1/2CDEF/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.15 except CfEcR-A/BCDEF was replaced with CfEcR-1/2CDEF (SEQ ID NO: 55).

1.17 - Gal4CfEcR-CDEF/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.15 except CfEcR-A/BCDEF was replaced with CfEcR-CDEF (SEQ ID NO: 45).

1.18 - Gal4CfEcR-DEF/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.15 except CfEcR-A/BCDEF was replaced with CfEcR-DEF (SEQ ID NO: 53).

1.19 - Gal4CfEcR-EF/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.15 except CfEcR-A/BCDEF was replaced with CfEcR-EF (SEQ ID NO: 1).

1.20 - Gal4CfEcR-DE/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.15 except CfEcR-CDEF was replaced with CfEcR-DE (SEQ ID NO: 3).

1.21- Gal4CfEcR-DEF/VP16AmaRXR1-EF: This construct was prepared in the same way as switch 1.18 except LmUSP-EF was replaced with the E and F domains of ixodid tick *Amblyomma americanum* RXR homolog I ("AmaRXR1-EF"; SEQ ID NO: 10).

1.22 - Gal4CfEcR-DEF/VP16AmaRXR2-EF: This construct was prepared in the same way as switch 1.21 except AmaRXR1-EF was replaced with the E and F domains of ixodid tick *Amblyomma americanum* RXR homolog 2 ("AmaRXR2-EF"; SEQ ID NO: 11).

1.23 - LexACfEcR-CDEF/VP16CfUSP-EF: The C, D, E, and F domains from spruce budworm *Choristoneura fumiferana* EcR ("CfEcR-CDEF"; SEQ ID NO: 45) were fused to a LexA DNA binding domain ("LexADNABD" or "LexADBD"; SEQ ID NO: 35) and placed under the control of an SV40e promoter (SEQ ID NO: 46). The E and F domains from spruce budworm *C. fumiferana* USP ("CfUSP-EF"; SEQ ID NO: 56) were fused to the transactivation domain from VP16 ("VP16AD"; SEQ ID NO: 37) and placed under the control of an SV40e promoter (SEQ ID NO: 46). Eight consensus LexA response element binding sites ("8XLexAop"; comprising 4 copies of a LexA response element binding site comprising SEQ ID NO: 42) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 48) and placed upstream of the luciferase gene (SEQ ID NO: 49).

1.24-LexACfEcR-CDEF/VP16LmUSP-EF: This construct was prepared in the same way as switch 1.23 except CfUSP-EF was replaced with LmUSP-EF (SEQ ID NO: 9).

1.25 - LexACfEcR-CDEF/VP16MmRXRα-EF: This construct was prepared in the same way as switch 1.23 except CfUSP-EF was replaced with MmRXRα-EF (SEQ ID NO: 50).

1.26 - LexACfEcR-CDEF/VP16DmUSP-EF: This construct was prepared in the same way as switch 1.23 except CfUSP-EF was replaced with the corresponding EF domains of DmUSP-EF (SEQ ID NO: 60).

1.27 - Gal4CfEcR-CDEF/BA2LmUSP-EF: The C, D, E, and F domains from spruce budworm *Choristoneura fumiferana* EcR ("CfEcR- CDEF"; SEQ ID NO: 45) were fused to a GAL4 DNA binding domain ("GAL4DNABD" or "GAL4DBD"; SEQ ID NO: 33) and placed under the control of an SV40e promoter (SEQ ID NO: 46). The E and F domains from locust *Locusta migratoria* USP ("LmUSP-EF"; SEQ ID NO: 9) were fused to the transactivation domain from B42 ("B42AD"; SEQ ID NO: 39) and placed under the control of an SV40e promoter (SEQ ID NO: 46). Five consensus GAL4 response element binding sites ("5XGAL4RE"; comprising 5 copies of a GAL4RE comprising SEQ ID NO: 41) were fused to a synthetic E1b minimal promoter (SEQ ID NO: 48) and placed upstream of the luciferase gene (SEQ ID NO: 49).

1.28 - LexACfEcR-CDEF/B42LmUSP-EF: This construct was prepared in the same way as switch 1.27 except the

GAL4 DNA binding domain was replaced with a LexA DNA binding domain (SEQ ID NO: 35).

1.29 - GAL4CfEcR DEF/VP16DmUSP-EF: This construct was prepared in the same way as switch 1.7 except CfUSP-DEF was replaced with the corresponding EF domains of DmUSP-EF (SEQ ID NO: 60).

1.30 - GAL4CfEcR-DEF/VP16CfUSP-EF: This construct was prepared in the same way as switch 1.7 except CfUSP-DEF was replaced with CfUSP-EF (SEQ ID NO: 56).

EXAMPLE 2

[0226] In a two-hybrid switch format, CfUSP and DmUSP in partnership with CfEcR are constitutively active in both yeast and mammalian cells. On the other hand, vertebrate RXR in partnership with CfEcR is a ligand dependent transactivator in mammalian cells. Applicants tested an invertebrate RXR, LmUSP in a two-hybrid format in mouse NIH3T3 cells to determine if it would function as a USP (constitutively) or as a vertebrate RXR (inducibly) in mammalian cells. Gal4:CfEcR-CDEF (Figure 1) or Gal4:CfEcR-DEF (Figure 2) were paired with VP16:MmRXR-DEF; YP16:MmRXR-EF; VP16:LmUSP-DEF; or VP16:CfUSP-EF and analyzed in mammalian cells. Briefly, gene induction potential (magnitude of induction) and ligand specificity and sensitivity were examined using two different ligands: a steroidal ligand (Ponasterone A, "PonA") and a non-steroidal ligand [N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N-tert-butylhydrazine] in a dose-dependent induction of reporter gene expression in the transfected NIH3T3 cells. Reporter gene expression activities were assayed at 48 hours after ligand addition. Standard methods for culture and maintenance of the cells were followed. Transfections: DNAs corresponding to the various switch constructs outlined in Example 1, specifically switches 1.1 through 1.8, were transfected into mouse NIH3T3 cells (ATCC) as follows. Cells were harvested when they reached 50% confluency and plated in 6-, 12- or 24- well plates at 125,000, 50,000, or 25,000 cells, respectively, in 2.5,1.0, or 0.5 ml of growth medium containing 10% fetal bovine serum (FBS), respectively. The next day, the cells were rinsed with growth medium and transfected for four hours. Superfect™ (Qiagen Inc.) was found to be the best transfection reagent for 3T3 cells. For 12- well plates, 4 μl of Superfec™ was mixed with 100 μl of growth medium. 1.0 μg of reporter construct and 0.25 μg of each receptor construct of the receptor pair to be analyzed were added to the transfection mix. A second reporter construct was added [pTKRL (Promega), 0.1 μg/transfection mix] that comprises a *Renilla* luciferase gene operably linked and placed under the control of a thymidine kinase (TK) constitutive promoter and was used for normalization. The contents of the transfection mix were mixed in a vortex mixer and let stand at room temperature for 30 min. At the end of incubation, the transfection mix was added to the cells maintained in 400 μl growth medium. The cells were maintained at 37°C and 5% CO_2 for four hours. At the end of incubation, 500 μl of growth medium containing 20% FBS and either dimethylsulfoxide (DMSO; control) or a DMSO solution of 0.1, 1, 5,10, and 50 μM PonA steroidal ligand or N-(2-ethyl-3-methoxybenzoyl)N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine non-steroidal ligand was added and the cells were maintained at 37°C and 5% CO_2 for 48 hours. The cells were harvested and reporter activity was assayed. The same procedure was followed for 6 and 24 well plates as well except all the reagents were doubled for 6 well plates and reduced to half for 24-well plates.

Ligands: The steroidal ligand Ponasterone A (PonA) was purchased from Sigma Chemical Company. The non-steroidal ligand N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-t-butylhydrazine (GS™-E non-steroidal ligand) is a synthetic stable ecdysteroid ligand synthesized at Rohm and Haas Company. All ligands were dissolved in DMSO and the final concentration of DMSO was maintained at 0.1 % in both controls and treatments.

Reporter Assays: Cells were harvested 48 hours after adding ligands. 125, 250, or 500 μl of passive lysis buffer (part of Dual-luciferase™ reporter assay system from Promega Corporation) were added to each well of 24- or 12- or 6-well plates respectively. The plates were placed on a rotary shaker for 15 minutes. Twenty μl of lysate were assayed. Luciferase activity was measured using Dual-luciferase™ reporter assay system from Promega Corporation following the manufacturer's instructions. β-Galactosidase was measured using Galacto-Star™ assay kit from TROPIX following the manufacturer's instructions. All luciferase and β-galactosidase activities were normalized using *Renilla* luciferase as a standard. Fold activities were calculated by dividing normalized relative light units ("RLU") in ligand treated cells with normalized RLU in DMSO treated cells (untreated control). Results: As shown in Figures 1 and 2, LmUSP in partnership with CfEcR functions as a ligand-inducible gene expression system in mammalian cells. This result is surprising since Applicants' previous experiments with CfUSP and DmUSP in partnership with CfEcR demonstrated constitutive expression activity (see PCT/US01/09050 application and Figures 1 and 2 for CfUSP results; DmUSP results are not shown). In addition, LmUSP worked better than vertebrate RXR as a CfEcR partner. In particular, both the sensitivity, i.e. the concentration of ligand required for transactivation, and the magnitude of transactivation were increased with LmUSP compared to vertebrate RXR. Thus, Applicants have demonstrated for the first time that invertebrate RXRs can function effectively in partnership with an ecdysone receptor in an inducible gene expression system in mammalian cells. This EcR/invertebrate RXR inducible gene expression system is an improvement over the EcR/vertebrate RXR gene expression system since less ligand is required for transactivation and increased levels of transactivation can be achieved.

[0227] Based upon Applicant's discovery described herein, one of ordinary skill in the art is able to predict that other invertebrate RXRs and their homologs, with the exception of Dipteran RXR homologs (example DmUSP) and Lepidopteran RXR homologs (example CfUSP), will also function in Applicants' EcR/invertebrate RXR-based inducible gene expression system. In addition, one of ordinary skill in the art is also able to predict that Applicants' novel inducible gene expression system will also work to modulate gene expression in yeast cells. Since the Dipteran RXR homolog/ and Lepidopteran RXR homolog/EcR gene expression systems function constitutively in yeast cells (data not shown), similar to how they function in mammalian cells, and Applicants have shown herein that non-Dipteran and non-Lepidopteran invertebrate RXRs function inducibly in partnership with an EcR in mammalian cells, the EcR/invertebrate RXR-based gene expression system is also predicted to function inducibly in yeast cells. Thus, the EcR/invertebrate RXR inducible gene expression system of the present invention is useful in applications where modulation of gene expression levels is desired in both yeast and mammalian cells. Further, there is no reason not to expect that the present invention would also work in other cells.

EXAMPLE 3

[0228] This Example describes the comparison of vertebrate RXR and invertebrate RXR-based two-hybrid gene expression systems comprising full length or truncated EcR, vertebrate RXR, and invertebrate RXR polypeptides. An amino acid sequence alignment, comparing the EF domains of twelve different vertebrate and invertebrate RXRs is shown in Figure 3A and B. As described below, Applicants compared different GAL4/CfEcR-based switches comprising MmRXRα-EF (a vertebrate RXR), LmUSP-EF (an invertebrate RXR), AmaRXR1-EF (an invertebrate RXR), and AmaRXR2-EF (an invertebrate RXR) fused to a VP16 activation domain to identify the receptors that give a switch with a) maximum induction in the presence of ligand; b) minimum background in the absence of ligand; c) highly sensitive to ligand concentration; and/or d) minimum cross-talk among ligands and receptors in mammalian cells.

[0229] Briefly, full-length EcR and truncated EcRs, created by a truncation mutation at the junctions of A/B, C, D, E and F domains and fused to a GAL4 DNA binding domain encoding polynucleotide (SEQ ID NO: 33) as described in Example 1 above. A VP16 activation domain encoding polynucleotide (SEQ ID NO: 37) was fused to the E and F domains of MmRXRα, LmUSP, AmaRXR1, and AmaRXR2 as described in Example 1. The resulting hybrid EcR/vertebrate or invertebrate RXR-encoding gene expression cassettes were assayed in NIH3T3 cells in pairwise comparisons. Plasmid pFRLUC (Stratagene) encoding a luciferase polypeptide was used as a reporter gene construct and pTKRL (Promega) encoding a *Renilla* luciferase polypeptide under the control of the constitutive TK promoter was used to normalize the transfections as described above. The transfected cells were grown in the presence 0, 1, 5 or 25 μM of the non-steroid N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine or the steroid PonA for 48 hours. The cells were harvested, lysed and luciferase reporter activity was measured in the cell lysates. Total fly luciferase relative light units are presented. The number on the top of each bar is the maximum fold induction for that treatment. The analysis was performed in triplicate and mean luciferase counts [total relative light units (RLU)] were determined as described above.

[0230] As shown in the Figures 4-7, CfEcR-CDEF performs better than any other CfEcR truncation. In particular, Gal4CfEcR-CDEF showed better induction than Gal4CfEcR-DEF using VP16LmUSP-EF. The EF domain of CfEcR in combination with LmUSP-DEF showed fairly good induced levels with very low uninduced levels. Most of EcR-EF domains described in patents and publications include D, E, and F domains (about 300 amino acids). This particular truncation includes only 230 amino acids and may rely on the D domain of LmUSP for heterodimerization.

[0231] Of all the truncations of LmUSP tested, Applicants' results show that the VP16LmUSP-EF hybrid receptor polypeptide was the best partner for Gal4CfEcR-based hybrid polypeptides, with GAL4CfEcRCDEF/VP16LmUSP-EF (switch 1.17) performing better than any other receptor combination and more sensitive to non-steroids than steroids (Figures 6 and 7). In general, the CfEcR/LmUSP-based switch was more sensitive to the non-steroid N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine than to the steroid PonA. Thus, the EF domain of LmUSP is sufficient and performs better than DEF domains of this receptor in partnership with CfEcR constructs.

[0232] Applicants' results show that the magnitude and fold induction of MmRXRα and LmUSP are similar but LmUSP improves sensitivity to ligand by at least 10 fold. Thus, the EcR/invertebrate system is an improvement over the EcR/vertebrate system.

EXAMPLE 4

[0233] This Example describes Applicants' further analysis of gene expression cassettes encoding truncated EcR or RXR receptor polypeptides that affect either ligand binding activity or ligand sensitivity, or both. Briefly, eleven different combinations of two-hybrid receptor pairs, constructed as described in Example 1, were further analyzed in a single experiment in NIH3T3 cells. These eleven receptor pair combinations and their corresponding sample numbers are depicted in Table 1.

Table 1

CfEcR+MmRXRα/LmUSP Truncation Receptor Combinations in NIH3T3 Cells		
Figure 8 X-Axis Sample No.	**CfEcR Polypeptide Construct**	**MmRXRa or LmUSP Polypeptide Construct**
Samples 1 and 2	GAL4CfEcR-CDEF	VP16MmRXRα-A/BCDEF
Samples 3 and 4	GAL4CfEcR-CDEF	VP16MmRXRα-DEF
Samples 5 and 6	GAL4CfEcR-CDEF	VP16MmRXRα-EF
Samples 7 and 8	GAL4CfEcR-DEF	VP16MmRXRα-A/BCDEF
Samples 9 and 10	GAL4CfEcR-DEF	VP16MmRXRα-DEF
Samples 11 and 12	GAL4CfEcR-DEF	VP16MmRXRα-EF
Samples 13 and 14	GAL4:CfEcR-CDEF	VP16:LmUSP-DEF
Samples 15 and 16	GAL4:CfEcR-CDEF	VP16:LmUSP-EF
Samples 17 and 18	GAL4:CfEcR-DEF	VP16:LmUSP-DEF
Samples 19 and 20	GAL4:CfEcR-DEF	VP16:LmUSP-EF
Samples 21 and 22	GAL4:CfEcR-EF	VP16:LmUSP-DEF

[0234] The above receptor construct pairs, along with the reporter plasmid pFRLuc were constructed as described above and transfected into NIH3T3 cells as described above. The eleven CfEcR truncation receptor combinations were duplicated into two groups and treated with either steroid (odd numbers on x-axis of Figure 8) or non-steroid (even numbers on x-axis of Figure 8). In particular, the cells were cultured in media containing 0, 1, 5 or 25 uM PonA (steroid) or N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine (non-steroid) ligand. The reporter gene activity was measured and total RLU are shown. The number on top of each bar is the maximum fold induction for that treatment and is the mean of three replicates.

[0235] As shown in Figure 8, the CfEcR-CDEF/LmUSP-EF receptor combination (columns 15 and 16) was the best format both in terms of total RLU and fold induction. This result is consistent with Applicants' results presented above in Example 3. These eleven receptor pair combinations were also assayed in a human lung carcinoma cell line A549 (ATCC) and similar results were observed (data not shown).

EXAMPLE 5

[0236] This Example describes Applicants' analysis of additional invertebrate retinoid X receptor homologs for use within the EcR/invertebrate RXR-based inducible gene expression system of the present invention. Briefly, two-hybrid receptor gene switches were constructed as described in Example 1 comprising a GAL4/CfEcR-DEF gene expression cassette and VP16AmaRXR1-EF or a VP16AmaRXR2-EF. These AmaRXR1- and AmaRXR2-based gene switches (switches 1.21 and 1.22 of Example 1) were compared to GAL4/CfEcR-DEF gene switches comprising VP16MmRXRα-EF (switch 1.6), VP16LmUSP-EF (switch 1.18), VP16DmUSP-EF (switch 1.29), and VP16CfUSP-EF (switch 1.30) along with pFRLuc in NIH3T3 cells.

[0237] The above receptor construct pairs, along with the reporter plasmid pFRLuc were constructed as described above and transfected into NIH3T3 cells as described above. The transactivation potential of these six CfEcR-DEF receptor-based gene switches were determined in the transfected cells in the presence of 0, 0.2, 1, or 10 μM PonA (steroid) or 0, 0.4, 0.2, 1, or 10 μM non-steroid ligand N-(2-ethyl-3-methoxybenzoyl)-N'-(3,5-dimethylbenzoyl)-N'-tert-butylhydrazine. The reporter gene activity was measured and total RLU are shown. The number on top of each bar is the maximum fold induction for that treatment and is the mean of three replicates.

[0238] As shown in Figure 9, both AmaRXR1-EF and AmaRXR2-EF based switches performed better than the vertebrate MmRXRα-EF based switch, demonstrating that these non-dipteran, non-lepidopteran invertebrate RXR homologs can also function in the EcR/invertebrate RXR-based inducible gene expression system of the present invention. Thus, based upon Applicants' surprising discovery that an invertebrate RXR (LmUSP) can substitute for a vertebrate RXR and the findings borne out in this Example regarding additional invertebrate species RXRs, one of ordinary skill in the art is able to predict that other invertebrate species, non-dipteran and non-lepidopteran RXR homologs will work in Applicant's gene expression system.

EXAMPLE 6

[0239] This Example describes the construction of host cells comprising the EcR/invertebrate RXR based gene expression modulation system according to the invention. To make stable cells expressing GAL4:CfEcR-DEF/VP16: LmUSP-EF (switch 1.18, prepared as described in Example 1), Applicants transfected the gene expression cassettes encoding the hybrid GAL4:CfEcR-DEF and VP16:LmUSP-EF polypeptides into Chinese hamster ovary CHO cells comprising a stably transfected reporter plasmid pFRLuc. Briefly, CHO cells were harvested when they reach 60-80% confluency and plated in 6- or 12-or 24- well plates at 250,000,100,000, or 50,000 cells in 2.5,1.0, or 0.5 ml of growth medium containing 10% Fetal bovine serum respectively. The next day, the cells were rinsed with growth medium and transfected for four hours. LipofectAMINE™ 2000 (Life Technologies Inc,) was found to be the best transfection reagent for these cells. For 12- well plates, 4 μl of LipofectAMINE™ 2000 was mixed with 100 μl of growth medium. 1.0 μg of reporter construct and 0.25 μg of each receptor construct GAL4:CfEcR-DEF and VP16:LmUSP-EF were added to the transfection mix. A second reporter construct was added (0.1 μg/transfection mix) and comprised a *Renilla* luciferase gene operably linked and placed under the control of a thymidine kinase (TK) constitutive promoter and was used for normalization. The contents of the transfection mix were mixed in a vortex mixer and let stand at room temperature for 30 min. At the end of incubation, the transfection mix was added to the cells maintained in 400 μl growth medium. The cells were maintained at 37°C and 5% CO_2 for four hours. At the end of incubation, 500 μl of growth medium containing 20% FBS and either DMSO (control) or a DMSO solution of appropriate ligands were added and the cells were maintained at 37 °C and 5% CO_2 for 24-48 hr. The cells were harvested and reporter activity was assayed. The same procedure was followed for 6 and 24 well plates as well except all the reagents were doubled for 6 well plates and reduced to half for 24-well plates.

[0240] The transfected CHO cells were grown in the presence of 0,1, 5, or 25 μM PonA steroid ligand or GS™- E non-steroid ligand for 48 hours. The cells were harvested, lysed and the reporter activity was measured. Total fly luciferase relative light units, (RLU) are presented. The numbers on the top of the bars correspond to the maximum fold induction for each treatment Bulk populations of cells were selected for resistance to the antibiotic neomycin (vp16:LmUSP/VP16: RXR constructs have the neomycin resistance gene incorporated). Several clones from each population were isolated by end point dilution. Three clones of stably transfected GAL4:CfEcR-DEF/VP16:LmUSP-EF cells were analyzed (see Figure 10, clone 1A2; the data related to the two other clones are not shown).

[0241] Of the three clones analyzed, the GAL4:CfEcR-DEF/VP16:LmUSP-EF stable clone 1A2 exhibited the highest fold induction, 162 fold, in the presence of non-steroidal ligand and 42 fold induction in the presence of steroid PonA (see Figure 10).

EXAMPLE 7

[0242] This Example describes the development of another embodiment of the EcR/invertebrate RXR gene expression modulation system of the invention. Specifically, Applicants have constructed LexA DNA binding domain (DBD) based-EcR/invertebrate RXR gene switches for use in the gene expression modulation system of the invention This embodiment can be useful as an alternate switch for a GAL4DBD-based switch and can also be used in multiple switch formats. While the LexA DBD has been used in yeast and plant expression systems, Applicants are not aware of its use in mammalian applications.

[0243] Briefly, a gene expression cassette comprising the LexA DNA binding domain (SEQ ID NO: 35) fused to CfEcR-CDEF domains (SEQ ID NO: 45) was prepared as described in Example 1. The LexA:CfEcR-CDEF gene expression cassette, along with a VP16:MmRXRα-EF, a VP16:CfUSP-EF, a VP16:DmUSP-EF, or a VP16:LmUSP-EF gene expression cassette, and a reporter construct (8opFRLuc) comprising an 8XLexA operator (4 copies of LexA response element; SEQ ID NO: 42), a minimal promoter (synthetic E1b minimal promoter SEQ ID NO: 48), and a luciferase gene (SEQ ID NO: 49) were transfected into mouse NIH3T3 cells. The transfected cells were cultured in the presence of 0, 0.1, 1,5,10, and 50 μM GS™-E non-steroidal ligand or PonA steroid ligand for 48 hours as described above. The cells were harvested, lysed and reporter activity was measured and total relative light units (RLU) are presented in Figure 11. The number on the top of each bar corresponds to the maximum fold induction of each treatment.

[0244] The LexA:CfEcR-DEF construct functioned well in these mammalian cells with all partners examined (see Figure 11). The fold induction is comparable to what was observed with Applicants' GAL4 system (see Figure 4). The 8opFRLuc reporter (control) showed very little activity in these cells. The results presented in Figure 11 show that the LexA DNA binding domain functions well in Applicants' two-hybrid system, demonstrating that the DNA binding domain is portable in these gene expression cassettes.

EXAMPLE 8

[0245] This Example describes the development of another embodiment of the EcR/invertebrate RXR gene expression modulation system of the invention. Specifically, Applicants have constructed gene expression cassettes for use in the gene modulation system of the invention comprising a B42 acidic activator domain as a transactivation domain. The B42 acidic activator domain ("B42AD"; see Gyuris et al., (1993) Cell 75: 791-803) works well as a transactivator in yeast and as Applicants have now shown, works well in mammalian cells. The B42 acidic activator domain may be used in the gene expression cassettes of the present invention as an alternative to VP16 transactivation domain.

[0246] Briefly, Applicants have constructed a gene expression cassette comprising a polynucleotide encoding a B42AD (SEQ ID NO: 39) fused to a polynucleotide encoding LmUSP-EF domains (SEQ ID NO: 9) as described in Example 1. This B42AD:LmUSP-EF gene expression cassette was evaluated in mouse NIH3T3 cells in partnership with either a GAL4:CfEcR-CDEF or a LexA:CfEcR-CDEF gene expression cassette and compared to a VP16:LmUSP-EF-based switch. All gene expression cassettes were prepared as described in Example 1. The appropriate reporter constructs were transfected into NIH3T3 cells. The transfected cells were cultured in the presence of 0, 0.1, 1, 5, 10, and 50 μM GS™-E non-steroidal ligand for 48 hours as described above. Reporter activity is plotted as total RLU (see Figure 12). The numbers on the top of the bars correspond to the maximum fold induction observed for that combination.

[0247] The results show that the B42 acidic activation domain works as well as the VP16 transactivation domain in Applicants' two-hybrid system, demonstrating that the transactivation domain is also portable in these gene expression cassettes.

EXAMPLE 9

[0248] This Example demonstrates the effect of introduction of a second ligand into the host cell comprising an EcR/invertebrate RXR-based inducible gene expression modulation system of the invention. In particular, Applicants have determined the effect of 9-cis-retinoic acid on the transactivation potential of the GAL4CfEcR-DEF/VP16LmUSP-EF (switch 1.18) gene switch along with pFRLuc in NIH3T3 cells in the presence of non-steroid (GSE) for 48 hours.

[0249] Briefly, GAL4CfEcR-DEF, pFRLuc and VP16MUSP-EF were transfected into NIH3T3 cells and the transfected cells were treated with 0, 0.04, 0.2,1, 5 and 25 μM non-steroidal ligand (GSE) and 0, 1, 5 and 25 μM 9-Cis-retinoic acid (Sigma Chemical Company). The reporter activity was measured at 48 hours after adding ligands.

[0250] As shown in Figure 13, the presence ofretinoic acid increased the sensitivity of CfEcR-DEF to non-steroidal ligand. At a non-steroid ligand concentration of 1 μM or less, there is very little induction in the absence of 9-Cis-retinoic acid, but when 1 μM 9-Cis-retinoic acid is added in addition to non-steroid, induction is greatly increased.

SEQUENCE LISTING

[0251]

<110> Rohm and Haas Company
Palli, Subba R.
Kapitskaya, Marianna Z.

<120> Novel ecdysone receptor/invertebrate retinoid X receptor-based inducible gene expression system

<130> A01237

<150> US 60/294,814
<151> 2001-05-31

<160> 60

<170> PatentIn version 3.1

<210> 1
<211> 735
<212> DNA
<213> Choristoneura fumiferana

<400> 1

taccaggacg ggtacgagca gccttctgat gaagatttga agaggattac gcagacgtgg 60

cagcaagcgg acgatgaaaa cgaagagtct gacactccct tccgccagat cacagagatg 120

actatcctca cggtccaact tatcgtggag ttcgcgaagg gattgccagg gttcgccaag 180

atctcgcagc ctgatcaaat tacgctgctt aaggcttgct caagtgaggt aatgatgctc 240

cgagtcgcgc gacgatacga tgcggcctca gacagtgttc tgttcgcgaa caaccaagcg 300

tacactcgcg acaactaccg caaggctggc atggcctacg tcatcgagga tctactgcac 360

ttctgccggt gcatgtactc tatggcgttg gacaacatcc attacgcgct gctcacggct 420

gtcgtcatct tttctgaccg gccagggttg gagcagccgc aactggtgga agaaatccag 480

cggtactacc tgaatacgct ccgcatctat atcctgaacc agctgagcgg gtcggcgcgt 540

tcgtccgtca tatacggcaa gatcctctca atcctctctg agctacgcac gctcggcatg 600

caaaactcca acatgtgcat ctccctcaag ctcaagaaca gaaagctgcc gcctttcctc 660

gaggagatct gggatgtggc ggacatgtcg cacacccaac cgccgcctat cctcgagtcc 720

cccacgaatc tctag 735

<210> 2
<211> 1338
<212> DNA
<213> Drosophila melanogaster

<400> 2

tatgagcagc catctgaaga ggatctcagg cgtataatga gtcaacccga tgagaacgag 60

agccaaacgg acgtcagctt tcggcatata accgagataa ccatactcac ggtccagttg 120

attgttgagt ttgctaaagg tctaccagcg tttacaaaga taccccagga ggaccagatc 180
acgttactaa aggcctgctc gtcggaggtg atgatgctgc gtatggcacg acgctatgac 240
cacagctcgg actcaatatt cttcgcgaat aatagatcat atacgcggga ttcttacaaa 300
atggccggaa tggctgataa cattgaagac ctgctgcatt tctgccgcca aatgttctcg 360
atgaaggtgg acaacgtcga atacgcgctt ctcactgcca ttgtgatctt ctcggaccgg 420
ccgggcctgg agaaggccca actagtcgaa gcgatccaga gctactacat cgacacgcta 480
cgcatttata tactcaaccg ccactgcggc gactcaatga gcctcgtctt ctacgcaaag 540
ctgctctcga tcctcaccga gctgcgtacg ctgggcaacc agaacgccga gatgtgtttc 600
tcactaaagc tcaaaaaccg caaactgccc aagttcctcg aggagatctg ggacgttcat 660
gccatcccgc catcggtcca gtcgcacctt cagattaccc aggaggagaa cgagcgtctc 720
gagcgggctg agcgtatgcg ggcatcggtt gggggcgcca ttaccgccgg cattgattgc 780
gactctgcct ccacttcggc ggcggcagcc gcggcccagc atcagcctca gcctcagccc 840
cagccccaac cctcctccct gacccagaac gattcccagc accagacaca gccgcagcta 900
caacctcagc taccacctca gctgcaaggt caactgcaac cccagctcca accacagctt 960
cagacgcaac tccagccaca gattcaacca cagccacagc tccttcccgt ctccgctccc 1020
gtgcccgcct ccgtaaccgc acctggttcc ttgtccgcgg tcagtacgag cagcgaatac 1080
atgggcggaa gtgcggccat aggacccatc acgccggcaa ccaccagcag tatcacggct 1140
gccgttaccg ctagctccac cacatcagcg gtaccgatgg gcaacggagt tggagtcggt 1200
gttggggtgg gcggcaacgt cagcatgtat gcgaacgccc agacggcgat ggccttgatg 1260
ggtgtagccc tgcattcgca ccaagagcag cttatcgggg gagtggcggt taagtcggag 1320
cactcgacga ctgcatag 1338

<210> 3
<211> 960
<212> DNA
<213> Choristoneura fumiferana

<400> 3

cctgagtgcg tagtacccga gactcagtgc gccatgaagc ggaaagagaa gaaagcacag 60

aaggagaagg acaaactgcc tgtcagcacg acgacggtgg acgaccacat gccgcccatt 120

atgcagtgtg aacctccacc tcctgaagca gcaaggattc acgaagtggt cccaaggttt 180

ctctccgaca agctgttgga gacaaaccgg cagaaaaaca tcccccagtt gacagccaac 240

cagcagttcc ttatcgccag gctcatctgg taccaggacg ggtacgagca gccttctgat 300

gaagatttga agaggattac gcagacgtgg cagcaagcgg acgatgaaaa cgaagagtct 360

gacactccct tccgccagat cacagagatg actatcctca cggtccaact tatcgtggag 420

ttcgcgaagg gattgccagg gttcgccaag atctcgcagc ctgatcaaat tacgctgctt 480

aaggcttgct caagtgaggt aatgatgctc cgagtcgcgc gacgatacga tgcggcctca 540

gacagtgttc tgttcgcgaa caaccaagcg tacactcgcg acaactaccg caaggctggc 600

atggcctacg tcatcgagga tctactgcac ttctgccggt gcatgtactc tatggcgttg 660

gacaacatcc attacgcgct gctcacggct gtcgtcatct tttctgaccg gccagggttg 720

gagcagccgc aactggtgga agaaatccag cggtactacc tgaatacgct ccgcatctat 780

atcctgaacc agctgagcgg gtcggcgcgt tcgtccgtca tatacggcaa gatcctctca 840

atcctctctg agctacgcac gctcggcatg caaaactcca acatgtgcat ctccctcaag 900

ctcaagaaca gaaagctgcc gcctttcctc gaggagatct gggatgtggc ggacatgtcg 960

<210> 4
<211> 969
<212> DNA
<213> Drosophila melanogaster

<400> 4

cggccggaat gcgtcgtccc ggagaaccaa tgtgcgatga agcggcgcga aaagaaggcc 60

cagaaggaga aggacaaaat gaccacttcg ccgagctctc agcatggcgg caatggcagc 120

ttggcctctg gtggcggcca agactttgtt aagaaggaga ttcttgacct tatgacatgc 180

gagccgcccc agcatgccac tattccgcta ctacctgatg aaatattggc caagtgtcaa 240

gcgcgcaata taccttcctt aacgtacaat cagttggccg ttatatacaa gttaatttgg 300

taccaggatg gctatgagca gccatctgaa gaggatctca ggcgtataat gagtcaaccc 360

gatgagaacg agagccaaac ggacgtcagc tttcggcata taaccgagat aaccatactc 420

acggtccagt tgattgttga gtttgctaaa ggtctaccag cgtttacaaa gataccccag 480

gaggaccaga tcacgttact aaaggcctgc tcgtcggagg tgatgatgct gcgtatggca 540

cgacgctatg accacagctc ggactcaata ttcttcgcga ataatagatc atatacgcgg 600

gattcttaca aaatggccgg aatggctgat aacattgaag acctgctgca tttctgccgc 660

caaatgttct cgatgaaggt ggacaacgtc gaatacgcgc ttctcactgc cattgtgatc 720

ttctcggacc ggccgggcct ggagaaggcc caactagtcg aagcgatcca gagctactac 780

atcgacacgc tacgcattta tatactcaac cgccactgcg gcgactcaat gagcctcgtc 840

ttctacgcaa agctgctctc gatcctcacc gagctgcgta cgctgggcaa ccagaacgcc 900

gagatgtgtt tctcactaaa gctcaaaaac cgcaaactgc ccaagttcct cgaggagatc 960

tgggacgtt 969

<210> 5
<211> 244
<212> PRT
<213> Choristoneura fumiferana

<400> 5

```
Tyr Gln Asp Gly Tyr Glu Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile
1               5                   10                  15

Thr Gln Thr Trp Gln Gln Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr
            20                  25                  30

Pro Phe Arg Gln Ile Thr Glu Met Thr Ile Leu Thr Val Gln Leu Ile
        35                  40                  45

Val Glu Phe Ala Lys Gly Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro
    50                  55                  60

Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met Leu
65                  70                  75                  80

Arg Val Ala Arg Arg Tyr Asp Ala Ala Ser Asp Ser Val Leu Phe Ala
                85                  90                  95

Asn Asn Gln Ala Tyr Thr Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala
            100                 105                 110

Tyr Val Ile Glu Asp Leu Leu His Phe Cys Arg Cys Met Tyr Ser Met
        115                 120                 125

Ala Leu Asp Asn Ile His Tyr Ala Leu Leu Thr Ala Val Val Ile Phe
    130                 135                 140

Ser Asp Arg Pro Gly Leu Glu Gln Pro Gln Leu Val Glu Glu Ile Gln
145                 150                 155                 160

Arg Tyr Tyr Leu Asn Thr Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser
                165                 170                 175

Gly Ser Ala Arg Ser Ser Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu
            180                 185                 190

Ser Glu Leu Arg Thr Leu Gly Met Gln Asn Ser Asn Met Cys Ile Ser
        195                 200                 205

Leu Lys Leu Lys Asn Arg Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp
```

```
    210                 215                 220

Asp Val Ala Asp Met Ser His Thr Gln Pro Pro Pro Ile Leu Glu Ser
225                 230                 235                 240

Pro Thr Asn Leu
```

<210> 6
<211> 445
<212> PRT
<213> Drosophila melanogaster

<400> 6

```
Tyr Glu Gln Pro Ser Glu Glu Asp Leu Arg Arg Ile Met Ser Gln Pro
1               5                   10                  15

Asp Glu Asn Glu Ser Gln Thr Asp Val Ser Phe Arg His Ile Thr Glu
            20                  25                  30

Ile Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Gly Leu
        35                  40                  45

Pro Ala Phe Thr Lys Ile Pro Gln Glu Asp Gln Ile Thr Leu Leu Lys
    50                  55                  60

Ala Cys Ser Ser Glu Val Met Met Leu Arg Met Ala Arg Arg Tyr Asp
65                  70                  75                  80

His Ser Ser Asp Ser Ile Phe Phe Ala Asn Asn Arg Ser Tyr Thr Arg
                85                  90                  95

Asp Ser Tyr Lys Met Ala Gly Met Ala Asp Asn Ile Glu Asp Leu Leu
            100                 105                 110

His Phe Cys Arg Gln Met Phe Ser Met Lys Val Asp Asn Val Glu Tyr
        115                 120                 125

Ala Leu Leu Thr Ala Ile Val Ile Phe Ser Asp Arg Pro Gly Leu Glu
    130                 135                 140

Lys Ala Gln Leu Val Glu Ala Ile Gln Ser Tyr Tyr Ile Asp Thr Leu
145                 150                 155                 160

Arg Ile Tyr Ile Leu Asn Arg His Cys Gly Asp Ser Met Ser Leu Val
                165                 170                 175
```

```
Phe Tyr Ala Lys Leu Leu Ser Ile Leu Thr Glu Leu Arg Thr Leu Gly
            180                 185                 190

Asn Gln Asn Ala Glu Met Cys Phe Ser Leu Lys Leu Lys Asn Arg Lys
        195                 200                 205

Leu Pro Lys Phe Leu Glu Glu Ile Trp Asp Val His Ala Ile Pro Pro
    210                 215                 220

Ser Val Gln Ser His Leu Gln Ile Thr Gln Glu Glu Asn Glu Arg Leu
225                 230                 235                 240

Glu Arg Ala Glu Arg Met Arg Ala Ser Val Gly Gly Ala Ile Thr Ala
                245                 250                 255

Gly Ile Asp Cys Asp Ser Ala Ser Thr Ser Ala Ala Ala Ala Ala Ala
            260                 265                 270

Gln His Gln Pro Gln Pro Gln Pro Gln Pro Gln Pro Ser Ser Leu Thr
        275                 280                 285

Gln Asn Asp Ser Gln His Gln Thr Gln Pro Gln Leu Gln Pro Gln Leu
    290                 295                 300

Pro Pro Gln Leu Gln Gly Gln Leu Gln Pro Gln Leu Gln Pro Gln Leu
305                 310                 315                 320

Gln Thr Gln Leu Gln Pro Gln Ile Gln Pro Gln Pro Gln Leu Leu Pro
                325                 330                 335

Val Ser Ala Pro Val Pro Ala Ser Val Thr Ala Pro Gly Ser Leu Ser
            340                 345                 350

Ala Val Ser Thr Ser Ser Glu Tyr Met Gly Gly Ser Ala Ala Ile Gly
        355                 360                 365

Pro Ile Thr Pro Ala Thr Thr Ser Ser Ile Thr Ala Ala Val Thr Ala
    370                 375                 380

Ser Ser Thr Thr Ser Ala Val Pro Met Gly Asn Gly Val Gly Val Gly
385                 390                 395                 400

Val Gly Val Gly Gly Asn Val Ser Met Tyr Ala Asn Ala Gln Thr Ala
                405                 410                 415
```

```
Met Ala Leu Met Gly Val Ala Leu His Ser His Gln Glu Gln Leu Ile
            420                 425                 430

Gly Gly Val Ala Val Lys Ser Glu His Ser Thr Thr Ala
        435                 440                 445
```

<210> 7
<211> 320
<212> PRT
<213> Choristoneura fumiferana

<400> 7

```
Pro Glu Cys Val Val Pro Glu Thr Gln Cys Ala Met Lys Arg Lys Glu
1               5                   10                  15

Lys Lys Ala Gln Lys Glu Lys Asp Lys Leu Pro Val Ser Thr Thr Thr
            20                  25                  30

Val Asp Asp His Met Pro Pro Ile Met Gln Cys Glu Pro Pro Pro Pro
        35                  40                  45

Glu Ala Ala Arg Ile His Glu Val Val Pro Arg Phe Leu Ser Asp Lys
    50                  55                  60

Leu Leu Glu Thr Asn Arg Gln Lys Asn Ile Pro Gln Leu Thr Ala Asn
65                  70                  75                  80

Gln Gln Phe Leu Ile Ala Arg Leu Ile Trp Tyr Gln Asp Gly Tyr Glu
                85                  90                  95

Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile Thr Gln Thr Trp Gln Gln
            100                 105                 110

Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr Pro Phe Arg Gln Ile Thr
        115                 120                 125

Glu Met Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Gly
    130                 135                 140

Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro Asp Gln Ile Thr Leu Leu
145                 150                 155                 160

Lys Ala Cys Ser Ser Glu Val Met Met Leu Arg Val Ala Arg Arg Tyr
                165                 170                 175

Asp Ala Ala Ser Asp Ser Val Leu Phe Ala Asn Asn Gln Ala Tyr Thr
            180                 185                 190
```

```
Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala Tyr Val Ile Glu Asp Leu
        195                 200                 205

Leu His Phe Cys Arg Cys Met Tyr Ser Met Ala Leu Asp Asn Ile His
    210                 215                 220

Tyr Ala Leu Leu Thr Ala Val Val Ile Phe Ser Asp Arg Pro Gly Leu
225                 230                 235                 240

Glu Gln Pro Gln Leu Val Glu Glu Ile Gln Arg Tyr Tyr Leu Asn Thr
                245                 250                 255

Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser Gly Ser Ala Arg Ser Ser
            260                 265                 270

Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu Ser Glu Leu Arg Thr Leu
        275                 280                 285

Gly Met Gln Asn Ser Asn Met Cys Ile Ser Leu Lys Leu Lys Asn Arg
    290                 295                 300

Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp Asp Val Ala Asp Met Ser
305                 310                 315                 320
```

<210> 8
<211> 323
<212> PRT
<213> Drosophila melanogaster

<400> 8

```
Arg Pro Glu Cys Val Val Pro Glu Asn Gln Cys Ala Met Lys Arg Arg
1               5                   10                  15

Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Met Thr Thr Ser Pro Ser
                20                  25                  30

Ser Gln His Gly Gly Asn Gly Ser Leu Ala Ser Gly Gly Gly Gln Asp
        35                  40                  45

Phe Val Lys Lys Glu Ile Leu Asp Leu Met Thr Cys Glu Pro Pro Gln
    50                  55                  60

His Ala Thr Ile Pro Leu Leu Pro Asp Glu Ile Leu Ala Lys Cys Gln
65                  70                  75                  80
```

```
Ala Arg Asn Ile Pro Ser Leu Thr Tyr Asn Gln Leu Ala Val Ile Tyr
                85                  90                  95

Lys Leu Ile Trp Tyr Gln Asp Gly Tyr Glu Gln Pro Ser Glu Glu Asp
            100                 105                 110

Leu Arg Arg Ile Met Ser Gln Pro Asp Glu Asn Glu Ser Gln Thr Asp
        115                 120                 125

Val Ser Phe Arg His Ile Thr Glu Ile Thr Ile Leu Thr Val Gln Leu
    130                 135                 140

Ile Val Glu Phe Ala Lys Gly Leu Pro Ala Phe Thr Lys Ile Pro Gln
145                 150                 155                 160

Glu Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met
                165                 170                 175

Leu Arg Met Ala Arg Arg Tyr Asp His Ser Ser Asp Ser Ile Phe Phe
            180                 185                 190

Ala Asn Asn Arg Ser Tyr Thr Arg Asp Ser Tyr Lys Met Ala Gly Met
        195                 200                 205

Ala Asp Asn Ile Glu Asp Leu Leu His Phe Cys Arg Gln Met Phe Ser
    210                 215                 220

Met Lys Val Asp Asn Val Glu Tyr Ala Leu Leu Thr Ala Ile Val Ile
225                 230                 235                 240

Phe Ser Asp Arg Pro Gly Leu Glu Lys Ala Gln Leu Val Glu Ala Ile
                245                 250                 255

Gln Ser Tyr Tyr Ile Asp Thr Leu Arg Ile Tyr Ile Leu Asn Arg His
            260                 265                 270

Cys Gly Asp Ser Met Ser Leu Val Phe Tyr Ala Lys Leu Leu Ser Ile
        275                 280                 285

Leu Thr Glu Leu Arg Thr Leu Gly Asn Gln Asn Ala Glu Met Cys Phe
    290                 295                 300

Ser Leu Lys Leu Lys Asn Arg Lys Leu Pro Lys Phe Leu Glu Glu Ile
305                 310                 315                 320

Trp Asp Val
```

<210> 9
<211> 635
<212> DNA
<213> Locusta migratoria

<400> 9

```
tgcatacaga catgcctgtt gaacgcatac ttgaagctga aaaacgagtg gagtgcaaag      60
cagaaaacca agtggaatat gagctggtgg agtgggctaa acacatcccg cacttcacat     120
ccctacctct ggaggaccag gttctcctcc tcagagcagg ttggaatgaa ctgctaattg     180
cagcattttc acatcgatct gtagatgtta aagatggcat agtacttgcc actggtctca     240
cagtgcatcg aaattctgcc catcaagctg gagtcggcac aatatttgac agagttttga     300
cagaactggt agcaaagatg agagaaatga aaatggataa aactgaactt ggctgcttgc     360
gatctgttat tcttttcaat ccagaggtga ggggtttgaa atccgcccag gaagttgaac     420
ttctacgtga aaaagtatat gccgctttgg aagaatatac tagaacaaca catcccgatg     480
aaccaggaag atttgcaaaa cttttgcttc gtctgccttc tttacgttcc ataggcctta     540
agtgtttgga gcatttgttt ttctttcgcc ttattggaga tgttccaatt gatacgttcc     600
tgatggagat gcttgaatca ccttctgatt cataa                                635
```

<210> 10
<211> 687
<212> DNA
<213> Amblyomma americanum

<400> 10

cctcctgaga tgcctctgga gcgcatactg gaggcagagc tgcgggttga gtcacagacg 60

gggaccctct cggaaagcgc acagcagcag gatccagtga gcagcatctg ccaagctgca 120

gaccgacagc tgcaccagct agttcaatgg gccaagcaca ttccacattt tgaagagctt 180

ccccttgagg accgcatggt gttgctcaag gctggctgga acgagctgct cattgctgct 240

ttctcccacc gttctgttga cgtgcgtgat ggcattgtgc tcgctacagg tcttgtggtg 300

cagcggcata gtgctcatgg ggctggcgtt ggggccatat ttgatagggt tctcactgaa 360

ctggtagcaa agatgcgtga gatgaagatg gaccgcactg agcttggatg cctgcttgct 420

gtggtacttt ttaatcctga ggccaagggg ctgcggacct gcccaagtgg aggccctgag 480

ggagaaagtg tatctgcctt ggaagagcac tgccggcagc agtacccaga ccagcctggg 540

cgctttgcca agctgctgct gcggttgcca gctctgcgca gtattggcct caagtgcctc 600

gaacatctct ttttcttcaa gctcatcggg gacacgccca tcgacaactt tcttctttcc 660

atgctggagg ccccctctga cccctaa 687

<210> 11
<211> 693
<212> DNA
<213> Amblyomma americanum

<400> 11

tctccggaca tgccactcga acgcattctc gaagccgaga tgcgcgtcga gcagccggca 60

ccgtccgttt tggcgcagac ggccgcatcg ggccgcgacc ccgtcaacag catgtgccag 120

gctgccccgc cacttcacga gctcgtacag tgggcccggc gaattccgca cttcgaagag 180

cttcccatcg aggatcgcac cgcgctgctc aaagccggct ggaacgaact gcttattgcc 240

gccttttcgc accgttctgt ggcggtgcgc gacggcatcg ttctggccac cgggctggtg 300

gtgcagcggc acagcgcaca cggcgcaggc gttggcgaca tcttcgaccg cgtactagcc 360

gagctggtgg ccaagatgcg cgacatgaag atggacaaaa cggagctcgg ctgcctgcgc 420

gccgtggtgc tcttcaatcc agacgccaag ggtctccgaa acgccaccag agtagaggcg 480

ctccgcgaga aggtgtatgc ggcgctggag gagcactgcc gtcggcacca cccggaccaa 540

ccgggtcgct tcggcaagct gctgctgcgg ctgcctgcct tgcgcagcat cgggctcaaa 600

tgcctcgagc atctgttctt cttcaagctc atcggagaca ctcccataga cagcttcctg 660

ctcaacatgc tggaggcacc ggcagacccc tag 693

<210> 12
<211> 801
<212> DNA

<213> Celuca pugilator

<400> 12

```
tcagacatgc caattgccag catacgggag gcagagctca gcgtggatcc catagatgag   60
cagccgctgg accaaggggt gaggcttcag gttccactcg cacctcctga tagtgaaaag  120
tgtagcttta ctttaccttt tcatcccgtc agtgaagtat cctgtgctaa ccctctgcag  180
gatgtggtga gcaacatatg ccaggcagct gacagacatc tggtgcagct ggtggagtgg  240
gccaagcaca tcccacactt cacagacctt cccatagagg accaagtggt attactcaaa  300
gccgggtgga acgagttgct tattgcctca ttctcacacc gtagcatggg cgtggaggat  360
ggcatcgtgc tggccacagg gctcgtgatc cacagaagta gtgctcacca ggctggagtg  420
ggtgccatat ttgatcgtgt cctctctgag ctggtggcca agatgaagga gatgaagatt  480
gacaagacag agctgggctg ccttcgctcc atcgtcctgt tcaacccaga tgccaaagga  540
ctaaactgcg tcaatgatgt ggagatcttg cgtgagaagg tgtatgctgc cctggaggag  600
tacacacgaa ccacttaccc tgatgaacct ggacgctttg ccaagttgct tctgcgactt  660

cctgcactca ggtctatagg cctgaagtgt cttgagtacc tcttcctgtt taagctgatt  720
ggagacactc ccctggacag ctacttgatg aagatgctcg tagacaaccc aaatacaagc  780
gtcactcccc ccaccagcta g                                            801
```

<210> 13
<211> 690
<212> DNA
<213> Tenebrio molitor

<400> 13

```
gccgagatgc ccctcgacag gataatcgag gcggagaaac ggatagaatg cacacccgct     60
ggtggctctg gtggtgtcgg agagcaacac gacggggtga acaacatctg tcaagccact    120
aacaagcagc tgttccaact ggtgcaatgg gctaagctca tacctcactt tacctcgttg    180
ccgatgtcgg accaggtgct tttattgagg gcaggatgga atgaattgct catcgccgca    240
ttctcgcaca gatctataca ggcgcaggat gccatcgttc tagccacggg gttgacagtt    300
aacaaaacgt cggcgcacgc cgtgggcgtg ggcaacatct acgaccgcgt cctctccgag    360
ctggtgaaca agatgaaaga gatgaagatg gacaagacgg agctgggctg cttgagagcc    420
atcatcctct acaaccccac gtgtcgcggc atcaagtccg tgcaggaagt ggagatgctg    480
cgtgagaaaa tttacggcgt gctggaagag tacaccagga ccacccaccc gaacgagccc    540
ggcaggttcg ccaaactgct tctgcgcctc ccggccctca ggtccatcgg gttgaaatgt    600
tccgaacacc tctttttctt caagctgatc ggtgatgttc caatagacac gttcctgatg    660
gagatgctgg agtctccggc ggacgcttag                                     690
```

<210> 14
<211> 681
<212> DNA
<213> Apis mellifera

<400> 14

```
cattcggaca tgccgatcga gcgtatcctg gaggccgaga agagagtcga atgtaagatg     60
gagcaacagg gaaattacga gaatgcagtg tcgcacattt gcaacgccac gaacaaacag    120
ctgttccagc tggtagcatg ggcgaaacac atcccgcatt ttacctcgtt gccactggag    180
gatcaggtac ttctgctcag ggccggttgg aacgagttgc tgatagcctc cttttcccac    240
cgttccatcg acgtgaagga cggtatcgtg ctggcgacgg ggatcaccgt gcatcggaac    300
tcggcgcagc aggccggcgt gggcacgata ttcgaccgtg tcctctcgga gcttgtctcg    360
aaaatgcgtg aaatgaagat ggacaggaca gagcttggct gtctcagatc tataatactc    420
ttcaatcccg aggttcgagg actgaaatcc atccaggaag tgaccctgct ccgtgagaag    480
atctacggcg ccctggaggg ttattgccgc gtagcttggc ccgacgacgc tggaagattc    540

gcgaaattac ttctacgcct gcccgccatc cgctcgatcg gattaaagtg cctcgagtac    600
ctgttcttct tcaaaatgat cggtgacgta ccgatcgacg atttctcgt ggagatgtta      660
gaatcgcgat cagatcctta g                                               681
```

<210> 15
<211> 516
<212> DNA

<213> Locusta migratoria

<400> 15

atccctacct ctggaggacc aggttctcct cctcagagca ggttggaatg aactgctaat 60

tgcagcattt tcacatcgat ctgtagatgt taaagatggc atagtacttg ccactggtct 120

cacagtgcat cgaaattctg cccatcaagc tggagtcggc acaatatttg acagagtttt 180

gacagaactg gtagcaaaga tgagagaaat gaaaatggat aaaactgaac ttggctgctt 240

gcgatctgtt attcttttca atccagaggt gaggggtttg aaatccgccc aggaagttga 300

acttctacgt gaaaaagtat atgccgcttt ggaagaatat actagaacaa cacatcccga 360

tgaaccagga agatttgcaa aacttttgct tcgtctgcct tctttacgtt ccataggcct 420

taagtgtttg gagcatttgt tttctttcgc cttattggag atgttccaat tgatacgttc 480

ctgatggaga tgcttgaatc accttctgat tcataa 516

<210> 16
<211> 528
<212> DNA
<213> Amblyomma americanum

<400> 16

attccacatt ttgaagagct tccccttgag gaccgcatgg tgttgctcaa ggctggctgg 60

aacgagctgc tcattgctgc tttctcccac cgttctgttg acgtgcgtga tggcattgtg 120

ctcgctacag gtcttgtggt gcagcggcat agtgctcatg gggctggcgt tggggccata 180

tttgataggg ttctcactga actggtagca aagatgcgtg agatgaagat ggaccgcact 240

gagcttggat gcctgcttgc tgtggtactt tttaatcctg aggccaaggg gctgcggacc 300

tgcccaagtg gaggccctga gggagaaagt gtatctgcct tggaagagca ctgccggcag 360

cagtacccag accagcctgg gcgctttgcc aagctgctgc tgcggttgcc agctctgcgc 420

agtattggcc tcaagtgcct cgaacatctc tttttcttca agctcatcgg ggacacgccc 480

atcgacaact ttcttctttc catgctggag gccccctctg accсctaa 528

<210> 17
<211> 531
<212> DNA
<213> Amblyomma americanum

<400> 17

```
attccgcact tcgaagagct tcccatcgag gatcgcaccg cgctgctcaa agccggctgg      60
aacgaactgc ttattgccgc cttttcgcac cgttctgtgg cggtgcgcga cggcatcgtt     120
ctggccaccg ggctggtggt gcagcggcac agcgcacacg gcgcaggcgt tggcgacatc     180
ttcgaccgcg tactagccga gctggtggcc aagatgcgcg acatgaagat ggacaaaacg     240
gagctcggct gcctgcgcgc cgtggtgctc ttcaatccag acgccaaggg tctccgaaac     300
gccaccagag tagaggcgct ccgcgagaag gtgtatgcgg cgctggagga gcactgccgt     360
cggcaccacc cggaccaacc gggtcgcttc ggcaagctgc tgctgcggct gcctgccttg     420
cgcagcatcg ggctcaaatg cctcgagcat ctgttcttct tcaagctcat cggagacact     480
cccatagaca gcttcctgct caacatgctg gaggcaccgg cagacccctaa g              531
```

<210> 18
<211> 552
<212> DNA
<213> Celuca pugilator

<400> 18

```
atcccacact tcacagacct tcccatagag gaccaagtgg tattactcaa agccgggtgg      60
aacgagttgc ttattgcctc attctcacac cgtagcatgg gcgtggagga tggcatcgtg     120
ctggccacag ggctcgtgat ccacagaagt agtgctcacc aggctggagt gggtgccata     180
tttgatcgtg tcctctctga gctggtggcc aagatgaagg agatgaagat tgacaagaca     240
gagctgggct gccttcgctc catcgtcctg ttcaacccag atgccaaagg actaaactgc     300
gtcaatgatg tggagatctt gcgtgagaag gtgtatgctg ccctggagga gtacacacga     360
accacttacc ctgatgaacc tggacgcttt gccaagttgc ttctgcgact tcctgcactc     420
aggtctatag gcctgaagtg tcttgagtac ctcttcctgt ttaagctgat tggagacact     480
cccctggaca gctacttgat gaagatgctc gtagacaacc caaatacaag cgtcactccc     540
cccaccagct ag                                                         552
```

<210> 19
<211> 531
<212> DNA
<213> Tenebrio molitor

<400> 19

```
atacctcact ttacctcgtt gccgatgtcg gaccaggtgc ttttattgag ggcaggatgg     60
aatgaattgc tcatcgccgc attctcgcac agatctatac aggcgcagga tgccatcgtt    120
ctagccacgg ggttgacagt taacaaaacg tcggcgcacg ccgtgggcgt gggcaacatc    180

tacgaccgcg tcctctccga gctggtgaac aagatgaaag agatgaagat ggacaagacg    240
gagctgggct gcttgagagc catcatcctc tacaacccca cgtgtcgcgg catcaagtcc    300
gtgcaggaag tggagatgct gcgtgagaaa atttacggcg tgctggaaga gtacaccagg    360
accacccacc cgaacgagcc cggcaggttc gccaaactgc ttctgcgcct cccggccctc    420
aggtccatcg ggttgaaatg ttccgaacac ctctttttct tcaagctgat cggtgatgtt    480
ccaatagaca cgttcctgat ggagatgctg gagtctccgg cggacgctta g             531
```

<210> 20
<211> 531
<212> DNA
<213> Apis mellifera

<400> 20

```
atcccgcatt ttacctcgtt gccactggag gatcaggtac ttctgctcag ggccggttgg     60
aacgagttgc tgatagcctc cttttcccac cgttccatcg acgtgaagga cggtatcgtg    120
ctggcgacgg ggatcaccgt gcatcggaac tcggcgcagc aggccggcgt gggcacgata    180
ttcgaccgtg tcctctcgga gcttgtctcg aaaatgcgtg aaatgaagat ggacaggaca    240
gagcttggct gtctcagatc tataatactc ttcaatcccg aggttcgagg actgaaatcc    300
atccaggaag tgaccctgct ccgtgagaag atctacggcg ccctggaggg ttattgccgc    360
gtagcttggc ccgacgacgc tggaagattc gcgaaattac ttctacgcct gcccgccatc    420
cgctcgatcg gattaaagtg cctcgagtac ctgttcttct tcaaaatgat cggtgacgta    480
ccgatcgacg attttctcgt ggagatgtta gaatcgcgat cagatcctta g             531
```

<210> 21
<211> 210
<212> PRT
<213> Locusta migratoria

<400> 21

```
His Thr Asp Met Pro Val Glu Arg Ile Leu Glu Ala Glu Lys Arg Val
1               5                       10              15

Glu Cys Lys Ala Glu Asn Gln Val Glu Tyr Glu Leu Val Glu Trp Ala
            20                  25                  30

Lys His Ile Pro His Phe Thr Ser Leu Pro Leu Glu Asp Gln Val Leu
        35                  40                  45

Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His
    50                  55                  60

Arg Ser Val Asp Val Lys Asp Gly Ile Val Leu Ala Thr Gly Leu Thr
65                  70                  75                  80

Val His Arg Asn Ser Ala His Gln Ala Gly Val Gly Thr Ile Phe Asp
                85                  90                  95

Arg Val Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met Lys Met Asp
            100                 105                 110

Lys Thr Glu Leu Gly Cys Leu Arg Ser Val Ile Leu Phe Asn Pro Glu
        115                 120                 125

Val Arg Gly Leu Lys Ser Ala Gln Glu Val Glu Leu Leu Arg Glu Lys
    130                 135                 140

Val Tyr Ala Ala Leu Glu Glu Tyr Thr Arg Thr Thr His Pro Asp Glu
145                 150                 155                 160

Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ser Leu Arg Ser
                165                 170                 175

Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe Arg Leu Ile Gly
            180                 185                 190

Asp Val Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ser Pro Ser
        195                 200                 205

Asp Ser
    210
```

<210> 22
<211> 228
<212> PRT
<213> Amblyomma americanum

<400> 22

```
Pro Pro Glu Met Pro Leu Glu Arg Ile Leu Glu Ala Glu Leu Arg Val
1               5                   10                  15

Glu Ser Gln Thr Gly Thr Leu Ser Glu Ser Ala Gln Gln Gln Asp Pro
            20                  25                  30

Val Ser Ser Ile Cys Gln Ala Ala Asp Arg Gln Leu His Gln Leu Val
        35                  40                  45

Gln Trp Ala Lys His Ile Pro His Phe Glu Glu Leu Pro Leu Glu Asp
    50                  55                  60

Arg Met Val Leu Leu Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala
65                  70                  75                  80

Phe Ser His Arg Ser Val Asp Val Arg Asp Gly Ile Val Leu Ala Thr
                85                  90                  95

Gly Leu Val Val Gln Arg His Ser Ala His Gly Ala Gly Val Gly Ala
            100                 105                 110

Ile Phe Asp Arg Val Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met
        115                 120                 125

Lys Met Asp Arg Thr Glu Leu Gly Cys Leu Leu Ala Val Val Leu Phe
    130                 135                 140

Asn Pro Glu Ala Lys Gly Leu Arg Thr Cys Pro Ser Gly Gly Pro Glu
145                 150                 155                 160

Gly Glu Ser Val Ser Ala Leu Glu Glu His Cys Arg Gln Gln Tyr Pro
                165                 170                 175

Asp Gln Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu
            180                 185                 190

Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu
        195                 200                 205

Ile Gly Asp Thr Pro Ile Asp Asn Phe Leu Leu Ser Met Leu Glu Ala
    210                 215                 220

Pro Ser Asp Pro
225
```

<210> 23
<211> 230
<212> PRT
<213> Amblyomma americanum

<400> 23

```
Ser Pro Asp Met Pro Leu Glu Arg Ile Leu Glu Ala Glu Met Arg Val
1               5                   10                  15

Glu Gln Pro Ala Pro Ser Val Leu Ala Gln Thr Ala Ala Ser Gly Arg
            20                  25                  30
```

```
Asp Pro Val Asn Ser Met Cys Gln Ala Ala Pro Pro Leu His Glu Leu
        35                  40                  45

Val Gln Trp Ala Arg Arg Ile Pro His Phe Glu Glu Leu Pro Ile Glu
    50                  55                  60

Asp Arg Thr Ala Leu Leu Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala
65                  70                  75                  80

Ala Phe Ser His Arg Ser Val Ala Val Arg Asp Gly Ile Val Leu Ala
                85                  90                  95

Thr Gly Leu Val Val Gln Arg His Ser Ala His Gly Ala Gly Val Gly
            100                 105                 110

Asp Ile Phe Asp Arg Val Leu Ala Glu Leu Val Ala Lys Met Arg Asp
        115                 120                 125

Met Lys Met Asp Lys Thr Glu Leu Gly Cys Leu Arg Ala Val Val Leu
    130                 135                 140

Phe Asn Pro Asp Ala Lys Gly Leu Arg Asn Ala Thr Arg Val Glu Ala
145                 150                 155                 160

Leu Arg Glu Lys Val Tyr Ala Ala Leu Glu Glu His Cys Arg Arg His
                165                 170                 175

His Pro Asp Gln Pro Gly Arg Phe Gly Lys Leu Leu Leu Arg Leu Pro
            180                 185                 190

Ala Leu Arg Ser Ile Gly Leu Lys Cys Leu Glu His Leu Phe Phe Phe
        195                 200                 205

Lys Leu Ile Gly Asp Thr Pro Ile Asp Ser Phe Leu Leu Asn Met Leu
    210                 215                 220

Glu Ala Pro Ala Asp Pro
225                 230
```

<210> 24
<211> 266
<212> PRT
<213> Celuca pugilator

<400> 24

```
Ser Asp Met Pro Ile Ala Ser Ile Arg Glu Ala Glu Leu Ser Val Asp
1               5                   10                  15
```

```
Pro Ile Asp Glu Gln Pro Leu Asp Gln Gly Val Arg Leu Gln Val Pro
            20                  25                  30

Leu Ala Pro Pro Asp Ser Glu Lys Cys Ser Phe Thr Leu Pro Phe His
        35                  40                  45

Pro Val Ser Glu Val Ser Cys Ala Asn Pro Leu Gln Asp Val Val Ser
    50                  55                  60

Asn Ile Cys Gln Ala Ala Asp Arg His Leu Val Gln Leu Val Glu Trp
65                  70                  75                  80

Ala Lys His Ile Pro His Phe Thr Asp Leu Pro Ile Glu Asp Gln Val
                85                  90                  95

Val Leu Leu Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser
            100                 105                 110

His Arg Ser Met Gly Val Glu Asp Gly Ile Val Leu Ala Thr Gly Leu
        115                 120                 125

Val Ile His Arg Ser Ser Ala His Gln Ala Gly Val Gly Ala Ile Phe
    130                 135                 140

Asp Arg Val Leu Ser Glu Leu Val Ala Lys Met Lys Glu Met Lys Ile
145                 150                 155                 160

Asp Lys Thr Glu Leu Gly Cys Leu Arg Ser Ile Val Leu Phe Asn Pro
                165                 170                 175

Asp Ala Lys Gly Leu Asn Cys Val Asn Asp Val Glu Ile Leu Arg Glu
            180                 185                 190

Lys Val Tyr Ala Ala Leu Glu Glu Tyr Thr Arg Thr Thr Tyr Pro Asp
        195                 200                 205

Glu Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg
    210                 215                 220

Ser Ile Gly Leu Lys Cys Leu Glu Tyr Leu Phe Leu Phe Lys Leu Ile
225                 230                 235                 240

Gly Asp Thr Pro Leu Asp Ser Tyr Leu Met Lys Met Leu Val Asp Asn
                245                 250                 255
```

```
            Pro Asn Thr Ser Val Thr Pro Pro Thr Ser
                        260                 265
```

<210> 25
<211> 229
<212> PRT
<213> Tenebrio molitor

<400> 25

```
Ala Glu Met Pro Leu Asp Arg Ile Ile Glu Ala Glu Lys Arg Ile Glu
1               5                   10                  15

Cys Thr Pro Ala Gly Gly Ser Gly Gly Val Gly Glu Gln His Asp Gly
            20                  25                  30

Val Asn Asn Ile Cys Gln Ala Thr Asn Lys Gln Leu Phe Gln Leu Val
        35                  40                  45

Gln Trp Ala Lys Leu Ile Pro His Phe Thr Ser Leu Pro Met Ser Asp
    50                  55                  60

Gln Val Leu Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala
65                  70                  75                  80

Phe Ser His Arg Ser Ile Gln Ala Gln Asp Ala Ile Val Leu Ala Thr
                85                  90                  95

Gly Leu Thr Val Asn Lys Thr Ser Ala His Ala Val Gly Val Gly Asn
            100                 105                 110

Ile Tyr Asp Arg Val Leu Ser Glu Leu Val Asn Lys Met Lys Glu Met
        115                 120                 125

Lys Met Asp Lys Thr Glu Leu Gly Cys Leu Arg Ala Ile Ile Leu Tyr
    130                 135                 140

Asn Pro Thr Cys Arg Gly Ile Lys Ser Val Gln Glu Val Glu Met Leu
145                 150                 155                 160

Arg Glu Lys Ile Tyr Gly Val Leu Glu Glu Tyr Thr Arg Thr Thr His
                165                 170                 175

Pro Asn Glu Pro Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala
            180                 185                 190

Leu Arg Ser Ile Gly Leu Lys Cys Ser Glu His Leu Phe Phe Phe Lys
```

195 200 205

Leu Ile Gly Asp Val Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu
210 215 220

Ser Pro Ala Asp Ala
225

<210> 26
<211> 226
<212> PRT
<213> Apis mellifera

<400> 26

```
His Ser Asp Met Pro Ile Glu Arg Ile Leu Glu Ala Glu Lys Arg Val
1               5                   10                  15

Glu Cys Lys Met Glu Gln Gln Gly Asn Tyr Glu Asn Ala Val Ser His
            20                  25                  30

Ile Cys Asn Ala Thr Asn Lys Gln Leu Phe Gln Leu Val Ala Trp Ala
        35                  40                  45

Lys His Ile Pro His Phe Thr Ser Leu Pro Leu Glu Asp Gln Val Leu
    50                  55                  60

Leu Leu Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His
65                  70                  75                  80

Arg Ser Ile Asp Val Lys Asp Gly Ile Val Leu Ala Thr Gly Ile Thr
                85                  90                  95

Val His Arg Asn Ser Ala Gln Gln Ala Gly Val Gly Thr Ile Phe Asp
            100                 105                 110

Arg Val Leu Ser Glu Leu Val Ser Lys Met Arg Glu Met Lys Met Asp
        115                 120                 125

Arg Thr Glu Leu Gly Cys Leu Arg Ser Ile Ile Leu Phe Asn Pro Glu
    130                 135                 140

Val Arg Gly Leu Lys Ser Ile Gln Glu Val Thr Leu Leu Arg Glu Lys
145                 150                 155                 160

Ile Tyr Gly Ala Leu Glu Gly Tyr Cys Arg Val Ala Trp Pro Asp Asp
                165                 170                 175

Ala Gly Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Ile Arg Ser
            180                 185                 190

Ile Gly Leu Lys Cys Leu Glu Tyr Leu Phe Phe Phe Lys Met Ile Gly
        195                 200                 205

Asp Val Pro Ile Asp Asp Phe Leu Val Glu Met Leu Glu Ser Arg Ser
    210                 215                 220

Asp Pro
225
```

<210> 27
<211> 176
<212> PRT
<213> Locusta migratoria

<400> 27

```
Ile Pro His Phe Thr Ser Leu Pro Leu Glu Asp Gln Val Leu Leu Leu
1               5                   10                  15

Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His Arg Ser
            20                  25                  30

Val Asp Val Lys Asp Gly Ile Val Leu Ala Thr Gly Leu Thr Val His
        35                  40                  45

Arg Asn Ser Ala His Gln Ala Gly Val Gly Thr Ile Phe Asp Arg Val
    50                  55                  60

Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met Lys Met Asp Lys Thr
65                  70                  75                  80

Glu Leu Gly Cys Leu Arg Ser Val Ile Leu Phe Asn Pro Glu Val Arg
                85                  90                  95

Gly Leu Lys Ser Ala Gln Glu Val Glu Leu Leu Arg Glu Lys Val Tyr
            100                 105                 110

Ala Ala Leu Glu Glu Tyr Thr Arg Thr Thr His Pro Asp Glu Pro Gly
        115                 120                 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ser Leu Arg Ser Ile Gly
    130                 135                 140

Leu Lys Cys Leu Glu His Leu Phe Phe Phe Arg Leu Ile Gly Asp Val


145                 150                 155                 160

Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ser Pro Ser Asp Ser
                165                 170                 175
```

<210> 28
<211> 175
<212> PRT
<213> Amblyomma americanum

<400> 28

```
Ile Pro His Phe Glu Glu Leu Pro Leu Glu Asp Arg Met Val Leu Leu
1               5                   10                  15

Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His Arg Ser
            20                  25                  30

Val Asp Val Arg Asp Gly Ile Val Leu Ala Thr Gly Leu Val Val Gln
        35                  40                  45

Arg His Ser Ala His Gly Ala Gly Val Gly Ala Ile Phe Asp Arg Val
    50                  55                  60

Leu Thr Glu Leu Val Ala Lys Met Arg Glu Met Lys Met Asp Arg Thr
65                  70                  75                  80

Glu Leu Gly Cys Leu Leu Ala Val Val Leu Phe Asn Pro Glu Ala Lys
                85                  90                  95

Gly Leu Arg Thr Cys Pro Ser Gly Gly Pro Glu Gly Glu Ser Val Ser
            100                 105                 110

Ala Leu Glu Glu His Cys Arg Gln Gln Tyr Pro Asp Gln Pro Gly Arg
        115                 120                 125

Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly Leu
    130                 135                 140

Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr Pro
145                 150                 155                 160

Ile Asp Asn Phe Leu Leu Ser Met Leu Glu Ala Pro Ser Asp Pro
                165                 170                 175
```

<210> 29
<211> 176
<212> PRT
<213> Amblyomma americanum

<400> 29

```
Ile Pro His Phe Glu Glu Leu Pro Ile Glu Asp Arg Thr Ala Leu Leu
1               5                   10                  15

Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His Arg Ser
            20                  25                  30

Val Ala Val Arg Asp Gly Ile Val Leu Ala Thr Gly Leu Val Val Gln
        35                  40                  45

Arg His Ser Ala His Gly Ala Gly Val Gly Asp Ile Phe Asp Arg Val
    50                  55                  60

Leu Ala Glu Leu Val Ala Lys Met Arg Asp Met Lys Met Asp Lys Thr
65                  70                  75                  80

Glu Leu Gly Cys Leu Arg Ala Val Val Leu Phe Asn Pro Asp Ala Lys
                85                  90                  95

Gly Leu Arg Asn Ala Thr Arg Val Glu Ala Leu Arg Glu Lys Val Tyr
            100                 105                 110

Ala Ala Leu Glu Glu His Cys Arg Arg His His Pro Asp Gln Pro Gly
        115                 120                 125

Arg Phe Gly Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly
    130                 135                 140

Leu Lys Cys Leu Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Thr
145                 150                 155                 160

Pro Ile Asp Ser Phe Leu Leu Asn Met Leu Glu Ala Pro Ala Asp Pro
                165                 170                 175
```

<210> 30
<211> 183
<212> PRT
<213> Celuca pugilator

<400> 30

```
Ile Pro His Phe Thr Asp Leu Pro Ile Glu Asp Gln Val Val Leu Leu
1               5                   10                  15

Lys Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser
            20                  25                  30
```

Met Gly Val Glu Asp Gly Ile Val Leu Ala Thr Gly Leu Val Ile His
35 40 45

Arg Ser Ser Ala His Gln Ala Gly Val Gly Ala Ile Phe Asp Arg Val
50 55 60

Leu Ser Glu Leu Val Ala Lys Met Lys Glu Met Lys Ile Asp Lys Thr
65 70 75 80

Glu Leu Gly Cys Leu Arg Ser Ile Val Leu Phe Asn Pro Asp Ala Lys
85 90 95

Gly Leu Asn Cys Val Asn Asp Val Glu Ile Leu Arg Glu Lys Val Tyr
100 105 110

Ala Ala Leu Glu Glu Tyr Thr Arg Thr Thr Tyr Pro Asp Glu Pro Gly
115 120 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly
130 135 140

Leu Lys Cys Leu Glu Tyr Leu Phe Leu Phe Lys Leu Ile Gly Asp Thr
145 150 155 160

Pro Leu Asp Ser Tyr Leu Met Lys Met Leu Val Asp Asn Pro Asn Thr
165 170 175

Ser Val Thr Pro Pro Thr Ser
180

<210> 31
<211> 176
<212> PRT
<213> Tenebrio molitor

<400> 31

Ile Pro His Phe Thr Ser Leu Pro Met Ser Asp Gln Val Leu Leu Leu
1 5 10 15

Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ala Phe Ser His Arg Ser
20 25 30

Ile Gln Ala Gln Asp Ala Ile Val Leu Ala Thr Gly Leu Thr Val Asn
35 40 45

```
Lys Thr Ser Ala His Ala Val Gly Val Gly Asn Ile Tyr Asp Arg Val
    50                  55                  60

Leu Ser Glu Leu Val Asn Lys Met Lys Glu Met Lys Met Asp Lys Thr
65                  70                  75                  80

Glu Leu Gly Cys Leu Arg Ala Ile Ile Leu Tyr Asn Pro Thr Cys Arg
                85                  90                  95

Gly Ile Lys Ser Val Gln Glu Val Glu Met Leu Arg Glu Lys Ile Tyr
            100                 105                 110

Gly Val Leu Glu Glu Tyr Thr Arg Thr Thr His Pro Asn Glu Pro Gly
        115                 120                 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Leu Arg Ser Ile Gly
    130                 135                 140

Leu Lys Cys Ser Glu His Leu Phe Phe Phe Lys Leu Ile Gly Asp Val
145                 150                 155                 160

Pro Ile Asp Thr Phe Leu Met Glu Met Leu Glu Ser Pro Ala Asp Ala
                165                 170                 175
```

<210> 32
<211> 176
<212> PRT
<213> Apis mellifera

<400> 32

```
Ile Pro His Phe Thr Ser Leu Pro Leu Glu Asp Gln Val Leu Leu Leu
1               5                   10                  15

Arg Ala Gly Trp Asn Glu Leu Leu Ile Ala Ser Phe Ser His Arg Ser
            20                  25                  30

Ile Asp Val Lys Asp Gly Ile Val Leu Ala Thr Gly Ile Thr Val His
        35                  40                  45

Arg Asn Ser Ala Gln Gln Ala Gly Val Gly Thr Ile Phe Asp Arg Val
    50                  55                  60

Leu Ser Glu Leu Val Ser Lys Met Arg Glu Met Lys Met Asp Arg Thr
65                  70                  75                  80

Glu Leu Gly Cys Leu Arg Ser Ile Ile Leu Phe Asn Pro Glu Val Arg
                85                  90                  95

Gly Leu Lys Ser Ile Gln Glu Val Thr Leu Leu Arg Glu Lys Ile Tyr
            100                 105                 110

Gly Ala Leu Glu Gly Tyr Cys Arg Val Ala Trp Pro Asp Asp Ala Gly
        115                 120                 125

Arg Phe Ala Lys Leu Leu Leu Arg Leu Pro Ala Ile Arg Ser Ile Gly
    130                 135                 140

Leu Lys Cys Leu Glu Tyr Leu Phe Phe Phe Lys Met Ile Gly Asp Val
145                 150                 155                 160

Pro Ile Asp Asp Phe Leu Val Glu Met Leu Glu Ser Arg Ser Asp Pro
                165                 170                 175
```

<210> 33
<211> 441
<212> DNA
<213> Saccharomyces cerevisiae

<400> 33

```
atgaagctac tgtcttctat cgaacaagca tgcgatattt gccgacttaa aaagctcaag      60
tgctccaaag aaaaaccgaa gtgcgccaag tgtctgaaga acaactggga gtgtcgctac     120
tctcccaaaa ccaaaaggtc tccgctgact agggcacatc tgacagaagt ggaatcaagg     180
ctagaaagac tggaacagct atttctactg atttttcctc gagaagacct tgacatgatt     240
ttgaaaatgg attctttaca ggatataaaa gcattgttaa caggattatt tgtacaagat     300
aatgtgaata aagatgccgt cacagataga ttggcttcag tggagactga tatgcctcta     360
acattgagac agcatagaat aagtgcgaca tcatcatcgg aagagagtag taacaaaggt     420
caaagacagt tgactgtatc g                                                441
```

<210> 34
<211> 147
<212> PRT
<213> Saccharomyces cerevisiae

<400> 34

```
Met Lys Leu Leu Ser Ser Ile Glu Gln Ala Cys Asp Ile Cys Arg Leu
1               5                   10                  15

Lys Lys Leu Lys Cys Ser Lys Glu Lys Pro Lys Cys Ala Lys Cys Leu
            20                  25                  30

Lys Asn Asn Trp Glu Cys Arg Tyr Ser Pro Lys Thr Lys Arg Ser Pro
```

35 40 45

Leu Thr Arg Ala His Leu Thr Glu Val Glu Ser Arg Leu Glu Arg Leu
50 55 60

Glu Gln Leu Phe Leu Leu Ile Phe Pro Arg Glu Asp Leu Asp Met Ile
65 70 75 80

Leu Lys Met Asp Ser Leu Gln Asp Ile Lys Ala Leu Leu Thr Gly Leu
85 90 95

Phe Val Gln Asp Asn Val Asn Lys Asp Ala Val Thr Asp Arg Leu Ala
100 105 110

Ser Val Glu Thr Asp Met Pro Leu Thr Leu Arg Gln His Arg Ile Ser
115 120 125

Ala Thr Ser Ser Ser Glu Glu Ser Ser Asn Lys Gly Gln Arg Gln Leu
130 135 140

Thr Val Ser
145

<210> 35
<211> 606
<212> DNA
<213> Bscherichia coli

<400> 35

atgaaagcgt taacggccag gcaacaagag gtgtttgatc tcatccgtga tcacatcagc 60
cagacaggta tgccgccgac gcgtgcggaa atcgcgcagc gtttggggtt ccgttcccca 120
aacgcggctg aagaacatct gaaggcgctg gcacgcaaag gcgttattga aattgtttcc 180
ggcgcatcac gcgggattcg tctgttgcag gaagaggaag aagggttgcc gctggtaggt 240
cgtgtggctg ccggtgaacc acttctggcg caacagcata ttgaaggtca ttatcaggtc 300
gatccttcct tattcaagcc gaatgctgat ttcctgctgc gcgtcagcgg gatgtcgatg 360
aaagatatcg gcattatgga tggtgacttg ctggcagtgc ataaaactca ggatgtacgt 420
aacggtcagg tcgttgtcgc acgtattgat gacgaagtta ccgttaagcg cctgaaaaaa 480
cagggcaata aagtcgaact gttgccagaa aatagcgagt ttaaaccaat tgtcgtagat 540
cttcgtcagc agagcttcac cattgaaggg ctggcggttg gggttattcg caacggcgac 600
tggctg 606

<210> 36
<211> 202
<212> PRT
<213> Escherichia coli

<400> 36

```
Met Lys Ala Leu Thr Ala Arg Gln Gln Glu Val Phe Asp Leu Ile Arg
1               5                   10                  15

Asp His Ile Ser Gln Thr Gly Met Pro Pro Thr Arg Ala Glu Ile Ala
            20                  25                  30

Gln Arg Leu Gly Phe Arg Ser Pro Asn Ala Ala Glu Glu His Leu Lys
        35                  40                  45

Ala Leu Ala Arg Lys Gly Val Ile Glu Ile Val Ser Gly Ala Ser Arg
    50                  55                  60

Gly Ile Arg Leu Leu Gln Glu Glu Glu Glu Gly Leu Pro Leu Val Gly
65                  70                  75                  80

Arg Val Ala Ala Gly Glu Pro Leu Leu Ala Gln Gln His Ile Glu Gly
                85                  90                  95

His Tyr Gln Val Asp Pro Ser Leu Phe Lys Pro Asn Ala Asp Phe Leu
            100                 105                 110

Leu Arg Val Ser Gly Met Ser Met Lys Asp Ile Gly Ile Met Asp Gly
        115                 120                 125

Asp Leu Leu Ala Val His Lys Thr Gln Asp Val Arg Asn Gly Gln Val
    130                 135                 140

Val Val Ala Arg Ile Asp Asp Glu Val Thr Val Lys Arg Leu Lys Lys
145                 150                 155                 160

Gln Gly Asn Lys Val Glu Leu Leu Pro Glu Asn Ser Glu Phe Lys Pro
                165                 170                 175

Ile Val Val Asp Leu Arg Gln Gln Ser Phe Thr Ile Glu Gly Leu Ala
            180                 185                 190

Val Gly Val Ile Arg Asn Gly Asp Trp Leu
        195                 200
```

<210> 37
<211> 271

<212> DNA
<213> herpes simplex virus 7

<400> 37

```
atgggcccta aaaagaagcg taaagtcgcc cccccgaccg atgtcagcct gggggacgag      60

ctccacttag acggcgagga cgtggcgatg gcgcatgccg acgcgctaga cgatttcgat     120

ctggacatgt tgggggacgg ggattccccg ggcccgggat ttacccccca cgactccgcc     180

ccctacggcg ctctggatat ggccgacttc gagtttgagc agatgtttac cgatgccctt     240

ggaattgacg agtacggtgg ggaattcccg g                                    271
```

<210> 38
<211> 90
<212> PRT
<213> herpes simplex virus 7

<400> 38

```
Met Gly Pro Lys Lys Lys Arg Lys Val Ala Pro Pro Thr Asp Val Ser
1               5                   10                  15

Leu Gly Asp Glu Leu His Leu Asp Gly Glu Asp Val Ala Met Ala His
            20                  25                  30

Ala Asp Ala Leu Asp Asp Phe Asp Leu Asp Met Leu Gly Asp Gly Asp
        35                  40                  45

Ser Pro Gly Pro Gly Phe Thr Pro His Asp Ser Ala Pro Tyr Gly Ala
    50                  55                  60

Leu Asp Met Ala Asp Phe Glu Phe Glu Gln Met Phe Thr Asp Ala Leu
65                  70                  75                  80

Gly Ile Asp Glu Tyr Gly Gly Glu Phe Pro
                85                  90
```

<210> 39
<211> 307
<212> DNA
<213> Saccharomyces cerevisiae

<400> 39

```
atgggtgctc ctccaaaaaa gaagagaaag gtagctggta tcaataaaga tatcgaggag     60
tgcaatgcca tcattgagca gtttatcgac tacctgcgca ccggacagga gatgccgatg    120
gaaatggcgg atcaggcgat taacgtggtg ccgggcatga cgccgaaaac cattcttcac    180
gccgggccgc cgatccagcc tgactggctg aaatcgaatg gttttcatga aattgaagcg    240
gatgttaacg ataccagcct cttgctgagt ggagatgcct cctaccctta tgatgtgcca    300

gattatg                                                              307
```

<210> 40
<211> 102
<212> PRT
<213> Saccharomyces cerevisiae

<400> 40

```
Met Gly Ala Pro Pro Lys Lys Lys Arg Lys Val Ala Gly Ile Asn Lys
1               5                   10                  15

Asp Ile Glu Glu Cys Asn Ala Ile Ile Glu Gln Phe Ile Asp Tyr Leu
            20                  25                  30

Arg Thr Gly Gln Glu Met Pro Met Glu Met Ala Asp Gln Ala Ile Asn
        35                  40                  45

Val Val Pro Gly Met Thr Pro Lys Thr Ile Leu His Ala Gly Pro Pro
    50                  55                  60

Ile Gln Pro Asp Trp Leu Lys Ser Asn Gly Phe His Glu Ile Glu Ala
65                  70                  75                  80

Asp Val Asn Asp Thr Ser Leu Leu Leu Ser Gly Asp Ala Ser Tyr Pro
                85                  90                  95

Tyr Asp Val Pro Asp Tyr
            100
```

<210> 41
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> GAL4 response element

<400> 41
ggagtactgt cctccgagc 19

<210> 42
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> 2xLexAop response element

<400> 42
ctgctgtata taaaaccagt ggttatatgt acagta 36

<210> 43
<211> 334
<212> PRT
<213> Choristoneura fumiferana

<400> 43

```
Pro Glu Cys Val Val Pro Glu Thr Gln Cys Ala Met Lys Arg Lys Glu
1               5                   10                  15

Lys Lys Ala Gln Lys Glu Lys Asp Lys Leu Pro Val Ser Thr Thr Thr
            20                  25                  30

Val Asp Asp His Met Pro Pro Ile Met Gln Cys Glu Pro Pro Pro Pro
        35                  40                  45

Glu Ala Ala Arg Ile His Glu Val Val Pro Arg Phe Leu Ser Asp Lys
    50                  55                  60

Leu Leu Glu Thr Asn Arg Gln Lys Asn Ile Pro Gln Leu Thr Ala Asn
65                  70                  75                  80

Gln Gln Phe Leu Ile Ala Arg Leu Ile Trp Tyr Gln Asp Gly Tyr Glu
                85                  90                  95

Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile Thr Gln Thr Trp Gln Gln
            100                 105                 110

Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr Pro Phe Arg Gln Ile Thr
        115                 120                 125

Glu Met Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe Ala Lys Gly
    130                 135                 140

Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro Asp Gln Ile Thr Leu Leu
145                 150                 155                 160

Lys Ala Cys Ser Ser Glu Val Met Met Leu Arg Val Ala Arg Arg Tyr
                165                 170                 175

Asp Ala Ala Ser Asp Ser Val Leu Phe Ala Asn Asn Gln Ala Tyr Thr
            180                 185                 190

Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala Tyr Val Ile Glu Asp Leu
        195                 200                 205

Leu His Phe Cys Arg Cys Met Tyr Ser Met Ala Leu Asp Asn Ile His
```

```
    210                 215                 220

Tyr Ala Leu Leu Thr Ala Val Val Ile Phe Ser Asp Arg Pro Gly Leu
225                 230                 235                 240

Glu Gln Pro Gln Leu Val Glu Glu Ile Gln Arg Tyr Tyr Leu Asn Thr
                245                 250                 255

Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser Gly Ser Ala Arg Ser Ser
            260                 265                 270

Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu Ser Glu Leu Arg Thr Leu
        275                 280                 285

Gly Met Gln Asn Ser Asn Met Cys Ile Ser Leu Lys Leu Lys Asn Arg
    290                 295                 300

Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp Asp Val Ala Asp Met Ser
305                 310                 315                 320

His Thr Gln Pro Pro Pro Ile Leu Glu Ser Pro Thr Asn Leu
                325                 330
```

<210> 44
<211> 549
<212> PRT
<213> Drosophila melanogaster

<400> 44

```
Arg Pro Glu Cys Val Val Pro Glu Asn Gln Cys Ala Met Lys Arg Arg
1               5                   10                  15

Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Met Thr Thr Ser Pro Ser
            20                  25                  30

Ser Gln His Gly Gly Asn Gly Ser Leu Ala Ser Gly Gly Gly Gln Asp
        35                  40                  45

Phe Val Lys Lys Glu Ile Leu Asp Leu Met Thr Cys Glu Pro Pro Gln
    50                  55                  60

His Ala Thr Ile Pro Leu Leu Pro Asp Glu Ile Leu Ala Lys Cys Gln
65                  70                  75                  80

Ala Arg Asn Ile Pro Ser Leu Thr Tyr Asn Gln Leu Ala Val Ile Tyr
                85                  90                  95
```

```
Lys Leu Ile Trp Tyr Gln Asp Gly Tyr Glu Gln Pro Ser Glu Glu Asp
            100                 105                 110

Leu Arg Arg Ile Met Ser Gln Pro Asp Glu Asn Glu Ser Gln Thr Asp
        115                 120                 125

Val Ser Phe Arg His Ile Thr Glu Ile Thr Ile Leu Thr Val Gln Leu
    130                 135                 140

Ile Val Glu Phe Ala Lys Gly Leu Pro Ala Phe Thr Lys Ile Pro Gln
145                 150                 155                 160

Glu Asp Gln Ile Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met
                165                 170                 175

Leu Arg Met Ala Arg Arg Tyr Asp His Ser Ser Asp Ser Ile Phe Phe
            180                 185                 190

Ala Asn Asn Arg Ser Tyr Thr Arg Asp Ser Tyr Lys Met Ala Gly Met
        195                 200                 205

Ala Asp Asn Ile Glu Asp Leu Leu His Phe Cys Arg Gln Met Phe Ser
    210                 215                 220

Met Lys Val Asp Asn Val Glu Tyr Ala Leu Leu Thr Ala Ile Val Ile
225                 230                 235                 240

Phe Ser Asp Arg Pro Gly Leu Glu Lys Ala Gln Leu Val Glu Ala Ile
                245                 250                 255

Gln Ser Tyr Tyr Ile Asp Thr Leu Arg Ile Tyr Ile Leu Asn Arg His
            260                 265                 270

Cys Gly Asp Ser Met Ser Leu Val Phe Tyr Ala Lys Leu Leu Ser Ile
        275                 280                 285

Leu Thr Glu Leu Arg Thr Leu Gly Asn Gln Asn Ala Glu Met Cys Phe
    290                 295                 300

Ser Leu Lys Leu Lys Asn Arg Lys Leu Pro Lys Phe Leu Glu Glu Ile
305                 310                 315                 320

Trp Asp Val His Ala Ile Pro Pro Ser Val Gln Ser His Leu Gln Ile
                325                 330                 335
```

```
Thr Gln Glu Glu Asn Glu Arg Leu Glu Arg Ala Glu Arg Met Arg Ala
            340                 345                 350

Ser Val Gly Gly Ala Ile Thr Ala Gly Ile Asp Cys Asp Ser Ala Ser
        355                 360                 365

Thr Ser Ala Ala Ala Ala Ala Ala Gln His Gln Pro Gln Pro Gln Pro
    370                 375                 380

Gln Pro Gln Pro Ser Ser Leu Thr Gln Asn Asp Ser Gln His Gln Thr
385                 390                 395                 400

Gln Pro Gln Leu Gln Pro Gln Leu Pro Pro Gln Leu Gln Gly Gln Leu
                405                 410                 415

Gln Pro Gln Leu Gln Pro Gln Leu Gln Thr Gln Leu Gln Pro Gln Ile
            420                 425                 430

Gln Pro Gln Pro Gln Leu Leu Pro Val Ser Ala Pro Val Pro Ala Ser
        435                 440                 445

Val Thr Ala Pro Gly Ser Leu Ser Ala Val Ser Thr Ser Ser Glu Tyr
    450                 455                 460

Met Gly Gly Ser Ala Ala Ile Gly Pro Ile Thr Pro Ala Thr Thr Ser
465                 470                 475                 480

Ser Ile Thr Ala Ala Val Thr Ala Ser Ser Thr Thr Ser Ala Val Pro
                485                 490                 495

Met Gly Asn Gly Val Gly Val Gly Val Gly Val Gly Gly Asn Val Ser
            500                 505                 510

Met Tyr Ala Asn Ala Gln Thr Ala Met Ala Leu Met Gly Val Ala Leu
        515                 520                 525

His Ser His Gln Glu Gln Leu Ile Gly Gly Val Ala Val Lys Ser Glu
    530                 535                 540

His Ser Thr Thr Ala
545
```

<210> 45
<211> 1288
<212> DNA
<213> Choristoneura fumiferana

<400> 45

```
aagggccctg cgccccgtca gcaagaggaa ctgtgtctgg tatgcgggga cagagcctcc     60
ggataccact acaatgcgct cacgtgtgaa gggtgtaaag ggttcttcag acggagtgtt    120
accaaaaatg cggtttatat ttgtaaattc ggtcacgctt gcgaaatgga catgtacatg    180
cgacggaaat gccaggagtg ccgcctgaag aagtgcttag ctgtaggcat gaggcctgag    240
tgcgtagtac ccgagactca gtgcgccatg aagcggaaag agaagaaagc acagaaggag    300
aaggacaaac tgcctgtcag cacgacgacg gtggacgacc acatgccgcc cattatgcag    360
tgtgaacctc cacctcctga agcagcaagg attcacgaag tggtcccaag gtttctctcc    420
gacaagctgt tggagacaaa ccggcagaaa aacatccccc agttgacagc caaccagcag    480
ttccttatcg ccaggctcat ctggtaccag gacgggtacg agcagccttc tgatgaagat    540
ttgaagagga ttacgcagac gtggcagcaa gcggacgatg aaaacgaaga gtctgacact    600
cccttccgcc agatcacaga gatgactatc ctcacggtcc aacttatcgt ggagttcgcg    660
aagggattgc cagggttcgc caagatctcg cagcctgatc aaattacgct gcttaaggct    720
tgctcaagtg aggtaatgat gctccgagtc gcgcgacgat acgatgcggc ctcagacagt    780
gttctgttcg cgaacaacca agcgtacact cgcgacaact accgcaaggc tggcatggcc    840
tacgtcatcg aggatctact gcacttctgc cggtgcatgt actctatggc gttggacaac    900
atccattacg cgctgctcac ggctgtcgtc atcttttctg accggccagg gttggagcag    960
ccgcaactgg tggaagaaat ccagcggtac tacctgaata cgctccgcat ctatatcctg   1020
aaccagctga gcgggtcggc gcgttcgtcc gtcatatacg gcaagatcct ctcaatcctc   1080
tctgagctac gcacgctcgg catgcaaaac tccaacatgt gcatctccct caagctcaag   1140
aacagaaagc tgccgccttt cctcgaggag atctgggatg tggcggacat gtcgcacacc   1200
caaccgccgc ctatcctcga gtcccccacg aatctctagc ccctgcgcgc acgcatcgcc   1260
gatgccgcgt ccggccgcgc tgctctga                                      1288
```

<210> 46
<211> 309
<212> DNA
<213> Simian virus 40

<400> 46

ggtgtggaaa gtccccaggc tccccagcag gcagaagtat gcaaagcatg catctcaatt 60

agtcagcaac caggtgtgga aagtccccag gctccccagc aggcagaagt atgcaaagca 120

tgcatctcaa ttagtcagca accatagtcc cgcccctaac tccgcccatc ccgcccctaa 180

ctccgcccag ttccgcccat tctccgcccc atggctgact aattttttt atttatgcag 240

aggccgaggc cgcctcggcc tctgagctat tccagaagta gtgaggaggc ttttttggag 300

gcctaggct 309

<210> 47
<211> 789
<212> DNA
<213> Mus musculus

<400> 47

aagcgggaag ctgtgcagga ggagcggcag cggggcaagg accggaatga gaacgaggtg 60

gagtccacca gcagtgccaa cgaggacatg cctgtagaga agattctgga agccgagctt 120

gctgtcgagc ccaagactga gacatacgtg gaggcaaaca tggggctgaa ccccagctca 180

ccaaatgacc ctgttaccaa catctgtcaa gcagcagaca agcagctctt cactcttgtg 240

gagtgggcca agaggatccc acacttttct gagctgcccc tagacgacca ggtcatcctg 300

ctacgggcag gctggaacga gctgctgatc gcctccttct cccaccgctc catagctgtg 360

aaagatggga ttctcctggc caccggcctg cacgtacacc ggaacagcgc tcacagtgct 420

ggggtgggcg ccatctttga cagggtgcta acagagctgg tgtctaagat gcgtgacatg 480

cagatggaca agacggagct gggctgcctg cgagccattg tcctgttcaa ccctgactct 540

aaggggctct caaaccctgc tgaggtggag gcgttgaggg agaaggtgta tgcgtcacta 600

gaagcgtact gcaaacacaa gtaccctgag cagccgggca ggtttgccaa gctgctgctc 660

cgcctgcctg cactgcgttc catcgggctc aagtgcctgg agcacctgtt cttcttcaag 720

ctcatcgggg acacgcccat cgacaccttc ctcatggaga tgctggaggc accacatcaa 780

gccacctag 789

<210> 48
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic E1b minimal promoter

<400> 48

tatataatgg atccccgggt accg 24

<210> 49
<211> 1653
<212> DNA
<213> Artificial Sequence

<220>
<223> luciferase gene

<400> 49

atggaagacg ccaaaaacat aaagaaaggc ccggcgccat tctatcctct agaggatgga 60

accgctggag agcaactgca taaggctatg aagagatacg ccctggttcc tggaacaatt 120

gcttttacag atgcacatat cgaggtgaac atcacgtacg cggaatactt cgaaatgtcc 180

gttcggttgg cagaagctat gaaacgatat gggctgaata caaatcacag aatcgtcgta 240

tgcagtgaaa actctcttca attctttatg ccggtgttgg gcgcgttatt tatcggagtt 300

gcagttgcgc ccgcgaacga catttataat gaacgtgaat tgctcaacag tatgaacatt 360

tcgcagccta ccgtagtgtt tgtttccaaa aaggggttgc aaaaaatttt gaacgtgcaa 420

aaaaaattac caataatcca gaaaattatt atcatggatt ctaaaacgga ttaccaggga 480

tttcagtcga tgtacacgtt cgtcacatct catctacctc ccggttttaa tgaatacgat 540

tttgtaccag agtcctttga tcgtgacaaa acaattgcac tgataatgaa ttcctctgga 600

tctactgggt tacctaaggg tgtggccctt ccgcatagaa ctgcctgcgt cagattctcg 660

catgccagag atcctatttt tggcaatcaa atcattccgg atactgcgat tttaagtgtt 720

gttccattcc atcacggttt tggaatgttt actacactcg gatatttgat atgtggattt 780

cgagtcgtct taatgtatag atttgaagaa gagctgtttt tacgatccct tcaggattac 840

aaaattcaaa gtgcgttgct agtaccaacc ctattttcat tcttcgccaa aagcactctg 900

attgacaaat acgatttatc taatttacac gaaattgctt ctgggggcgc acctctttcg 960

aaagaagtcg gggaagcggt tgcaaaacgc ttccatcttc cagggatacg acaaggatat 1020

gggctcactg agactacatc agctattctg attacacccg agggggatga taaaccgggc 1080

gcggtcggta aagttgttcc atttttgaa gcgaaggttg tggatctgga taccgggaaa 1140

acgctgggcg ttaatcagag aggcgaatta tgtgtcagag gacctatgat tatgtccggt 1200

tatgtaaaca atccggaagc gaccaacgcc ttgattgaca aggatggatg gctacattct 1260

ggagacatag cttactggga cgaagacgaa cacttcttca tagttgaccg cttgaagtct 1320

ttaattaaat acaaaggata tcaggtggcc cccgctgaat tggaatcgat attgttacaa 1380

caccccaaca tcttcgacgc gggcgtggca ggtcttcccg acgatgacgc cggtgaactt 1440

cccgccgccg ttgttgtttt ggagcacgga aagacgatga cggaaaaaga gatcgtggat 1500

tacgtcgcca gtcaagtaac aaccgcgaaa aagttgcgcg gaggagttgt gtttgtggac 1560

gaagtaccga aaggtcttac cggaaaactc gacgcaagaa aaatcagaga gatcctcata 1620

aaggccaaga agggcggaaa gtccaaattg taa 1653

<210> 50
<211> 714
<212> DNA
<213> Mus musculus

<400> 50

```
gccaacgagg acatgcctgt agagaagatt ctggaagccg agcttgctgt cgagcccaag      60
actgagacat acgtggaggc aaacatgggg ctgaacccca gctcaccaaa tgaccctgtt     120
accaacatct gtcaagcagc agacaagcag ctcttcactc ttgtggagtg ggccaagagg     180
atcccacact tttctgagct gcccctagac gaccaggtca tcctgctacg ggcaggctgg     240
aacgagctgc tgatcgcctc cttctcccac cgctccatag ctgtgaaaga tgggattctc     300
ctggccaccg gcctgcacgt acaccggaac agcgctcaca gtgctggggt gggcgccatc     360
tttgacaggg tgctaacaga gctggtgtct aagatgcgtg acatgcagat ggacaagacg     420
gagctgggct gcctgcgagc cattgtcctg ttcaaccctg actctaaggg gctctcaaac     480
cctgctgagg tggaggcgtt gagggagaag gtgtatgcgt cactagaagc gtactgcaaa     540
cacaagtacc ctgagcagcc gggcaggttt gccaagctgc tgctccgcct gcctgcactg     600
cgttccatcg ggctcaagtg cctggagcac ctgttcttct tcaagctcat cggggacacg     660
cccatcgaca ccttcctcat ggagatgctg gaggcaccac atcaagccac ctag           714
```

<210> 51
<211> 867
<212> DNA
<213> Choristoneura fumiferana

<400> 51

```
aagcgagagg cggtgcaaga ggagcgccag aggaatgctc gcggcgcgga ggatgcgcac     60
ccgagtagct cggtgcaggt aagcgatgag ctgtcaatcg agcgcctaac ggagatggag    120
tctttggtgg cagatcccag cgaggagttc cagttcctcc gcgtggggcc tgacagcaac    180
gtgcctccac gttaccgcgc gcccgtctcc tccctctgcc aaataggcaa caagcaaata    240
gcggcgttgg tggtatgggc gcgcgacatc cctcatttcg ggcagctgga gctggacgat    300
caagtggtac tcatcaaggc ctcctggaat gagctgctac tcttcgccat cgcctggcgc    360
tctatggagt atttggaaga tgagagggag aacggggacg gaacgcggag caccactcag    420
ccacaactga tgtgtctcat gcctggcatg acgttgcacc gcaactcggc gcagcaggcg    480
ggcgtgggcg ccatcttcga ccgcgtgctg tccgagctca gtctgaagat gcgcaccttg    540
cgcatggacc aggccgagta cgtcgcgctc aaagccatcg tgctgctcaa ccctgatgtg    600
aaaggactga agaatcggca agaagttgac gttttgcgag aaaaaatgtt ctcttgcctg    660
gacgactact gccggcggtc gcgaagcaac gaggaaggcc ggtttgcgtc cttgctgctg    720
cggctgccag ctctccgctc catctcgctc aagagcttcg aacacctcta cttcttccac    780
ctcgtggccg aaggctccat cagcggatac atacgagagg cgctccgaaa ccacgcgcct    840
ccgatcgacg tcaatgccat gatgtaa                                        867
```

<210> 52
<211> 711
<212> DNA
<213> Locusta migratoria

<400> 52

aagagagaag cagttcagga ggaaaggcag cgaacaaagg agcgtgatca gaatgaagtt 60
gaatcaacaa gcagcctgca tacagacatg cctgttgaac gcatacttga agctgaaaaa 120
cgagtggagt gcaaagcaga aaaccaagtg gaatatgagc tggtggagtg ggctaaacac 180
atcccgcact tcacatccct acctctggag gaccaggttc tcctcctcag agcaggttgg 240
aatgaactgc taattgcagc attttcacat cgatctgtag atgttaaaga tggcatagta 300
cttgccactg gtctcacagt gcatcgaaat tctgcccatc aagctggagt cggcacaata 360
tttgacagag ttttgacaga actggtagca aagatgagag aaatgaaaat ggataaaact 420
gaacttggct gcttgcgatc tgttattctt ttcaatccag aggtgagggg tttgaaatcc 480
gcccaggaag ttgaacttct acgtgaaaaa gtatatgccg ctttggaaga atatactaga 540
acaacacatc ccgatgaacc aggaagattt gcaaaacttt tgcttcgtct gccttcttta 600
cgttccatag gccttaagtg tttggagcat ttgtttttct ttcgccttat tggagatgtt 660
ccaattgata cgttcctgat ggagatgctt gaatcacctt ctgattcata a 711

<210> 53
<211> 1054
<212> DNA
<213> Choristoneura fumiferana

<400> 53

cctgagtgcg tagtacccga gactcagtgc gccatgaagc ggaaagagaa gaaagcacag 60
aaggagaagg acaaactgcc tgtcagcacg acgacggtgg acgaccacat gccgcccatt 120
atgcagtgtg aacctccacc tcctgaagca gcaaggattc acgaagtggt cccaaggttt 180
ctctccgaca agctgttgga gacaaaccgg cagaaaaaca tcccccagtt gacagccaac 240
cagcagttcc ttatcgccag gctcatctgg taccaggacg ggtacgagca gccttctgat 300
gaagatttga agaggattac gcagacgtgg cagcaagcgg acgatgaaaa cgaagagtct 360
gacactccct tccgccagat cacagagatg actatcctca cggtccaact tatcgtggag 420
ttcgcgaagg gattgccagg gttcgccaag atctcgcagc ctgatcaaat tacgctgctt 480
aaggcttgct caagtgaggt aatgatgctc cgagtcgcgc gacgatacga tgcggcctca 540
gacagtgttc tgttcgcgaa caaccaagcg tacactcgcg acaactaccg caaggctggc 600
atggcctacg tcatcgagga tctactgcac ttctgccggt gcatgtactc tatggcgttg 660

gacaacatcc attacgcgct gctcacggct gtcgtcatct tttctgaccg gccagggttg 720
gagcagccgc aactggtgga agaaatccag cggtactacc tgaatacgct ccgcatctat 780
atcctgaacc agctgagcgg gtcggcgcgt tcgtccgtca tatacggcaa gatcctctca 840
atcctctctg agctacgcac gctcggcatg caaaactcca acatgtgcat ctccctcaag 900
ctcaagaaca gaaagctgcc gcctttcctc gaggagatct gggatgtggc ggacatgtcg 960
cacacccaac cgccgcctat cctcgagtcc cccacgaatc tctagcccct gcgcgcacgc 1020
atcgccgatg ccgcgtccgg ccgcgctgct ctga 1054

<210> 54
<211> 1542
<212> DNA
<213> Choristoneura fumiferana

<400> 54

ctggacctga aacacgaagt ggcttaccga ggggtgctcc caggccaggt gaaggccgaa 60
ccgggggtcc acaacggcca ggtcaacggc cacgtgaggg actggatggc aggcggcgct 120
ggtgccaatt cgccgtctcc gggagcggtg gctcaacccc agcctaacaa tgggtattcg 180
tcgccactct cctcgggaag ctacgggccc tacagtccaa atgggaaaat aggccgtgag 240
gaactgtcgc cagcttcaag tataaatggg tgcagtacag atggcgaggc acgacgtcag 300
aagaagggcc ctgcgccccg tcagcaagag gaactgtgtc tggtatgcgg ggacagagcc 360
tccggatacc actacaatgc gctcacgtgt gaagggtgta aagggttctt cagacggagt 420
gttaccaaaa atgcggttta tatttgtaaa ttcggtcacg cttgcgaaat ggacatgtac 480
atgcgacgga aatgccagga gtgccgcctg aagaagtgct tagctgtagg catgaggcct 540
gagtgcgtag tacccgagac tcagtgcgcc atgaagcgga aagagaagaa agcacagaag 600
gagaaggaca aactgcctgt cagcacgacg acggtggacg accacatgcc gcccattatg 660
cagtgtgaac ctccacctcc tgaagcagca aggattcacg aagtggtccc aaggtttctc 720
tccgacaagc tgttggagac aaaccggcag aaaaacatcc cccagttgac agccaaccag 780
cagttcctta tcgccaggct catctggtac caggacgggt acgagcagcc ttctgatgaa 840
gatttgaaga ggattacgca gacgtggcag caagcggacg atgaaaacga agagtctgac 900
actcccttcc gccagatcac agagatgact atcctcacgg tccaacttat cgtggagttc 960
gcgaagggat tgccagggtt cgccaagatc tcgcagcctg atcaaattac gctgcttaag 1020
gcttgctcaa gtgaggtaat gatgctccga gtcgcgcgac gatacgatgc ggcctcagac 1080
agtgttctgt tcgcgaacaa ccaagcgtac actcgcgaca actaccgcaa ggctggcatg 1140
gcctacgtca tcgaggatct actgcacttc tgccggtgca tgtactctat ggcgttggac 1200

aacatccatt acgcgctgct cacggctgtc gtcatctttt ctgaccggcc agggttggag 1260
cagccgcaac tggtggaaga aatccagcgg tactacctga atacgctccg catctatatc 1320
ctgaaccagc tgagcgggtc ggcgcgttcg tccgtcatat acggcaagat cctctcaatc 1380
ctctctgagc tacgcacgct cggcatgcaa aactccaaca tgtgcatctc cctcaagctc 1440
aagaacagaa agctgccgcc tttcctcgag gagatctggg atgtggcgga catgtcgcac 1500
acccaaccgc cgcctatcct cgagtccccc acgaatctct ag 1542

<210> 55
<211> 1110
<212> DNA
<213> Choristoneura fumiferana

<400> 55

```
gcggtttata tttgtaaatt cggtcacgct tgcgaaatgg acatgtacat gcgacggaaa     60
tgccaggagt gccgcctgaa gaagtgctta gctgtaggca tgaggcctga gtgcgtagta    120
cccgagactc agtgcgccat gaagcggaaa gagaagaaag cacagaagga gaaggacaaa    180
ctgcctgtca gcacgacgac ggtggacgac cacatgccgc ccattatgca gtgtgaacct    240
ccacctcctg aagcagcaag gattcacgaa gtggtcccaa ggtttctctc cgacaagctg    300
ttggagacaa accggcagaa aaacatcccc cagttgacag ccaaccagca gttccttatc    360
gccaggctca tctggtacca ggacgggtac gagcagcctt ctgatgaaga tttgaagagg    420
attacgcaga cgtggcagca agcggacgat gaaaacgaag agtctgacac tcccttccgc    480
cagatcacag agatgactat cctcacggtc caacttatcg tggagttcgc gaagggattg    540
ccagggttcg ccaagatctc gcagcctgat caaattacgc tgcttaaggc ttgctcaagt    600
gaggtaatga tgctccgagt cgcgcgacga tacgatgcgg cctcagacag tgttctgttc    660
gcgaacaacc aagcgtacac tcgcgacaac taccgcaagg ctggcatggc ctacgtcatc    720
gaggatctac tgcacttctg ccggtgcatg tactctatgg cgttggacaa catccattac    780
gcgctgctca cggctgtcgt catcttttct gaccggccag ggttggagca gccgcaactg    840
gtggaagaaa tccagcggta ctacctgaat acgctccgca tctatatcct gaaccagctg    900
agcgggtcgg cgcgttcgtc cgtcatatac ggcaagatcc tctcaatcct ctctgagcta    960
cgcacgctcg gcatgcaaaa ctccaacatg tgcatctccc tcaagctcaa gaacagaaag   1020
ctgccgcctt tcctcgagga gatctgggat gtggcggaca tgtcgcacac ccaaccgccg   1080
cctatcctcg agtcccccac gaatctctag                                    1110
```

<210> 56
<211> 798
<212> DNA
<213> Choristoneura fumiferana

<400> 56

```
tcggtgcagg taagcgatga gctgtcaatc gagcgcctaa cggagatgga gtctttggtg     60
gcagatccca gcgaggagtt ccagttcctc cgcgtggggc ctgacagcaa cgtgcctcca    120
cgttaccgcg cgcccgtctc ctccctctgc caaataggca acaagcaaat agcggcgttg    180
gtggtatggg cgcgcgacat ccctcatttc gggcagctgg agctggacga tcaagtggta    240
ctcatcaagg cctcctggaa tgagctgcta ctcttcgcca tcgcctggcg ctctatggag    300
tatttggaag atgagaggga gaacggggac ggaacgcgga gcaccactca gccacaactg    360
atgtgtctca tgcctggcat gacgttgcac cgcaactcgg cgcagcaggc gggcgtgggc    420
gccatcttcg accgcgtgct gtccgagctc agtctgaaga tgcgcacctt gcgcatggac    480
caggccgagt acgtcgcgct caaagccatc gtgctgctca accctgatgt gaaaggactg    540
aagaatcggc aagaagttga cgttttgcga gaaaaaatgt tctcttgcct ggacgactac    600
tgccggcggt cgcgaagcaa cgaggaaggc cggtttgcgt ccttgctgct gcggctgcca    660
gctctccgct ccatctcgct caagagcttc gaacacctct acttcttcca cctcgtggcc    720
gaaggctcca tcagcggata catacgagag gcgctccgaa accacgcgcc tccgatcgac    780
gtcaatgcca tgatgtaa                                                  798
```

<210> 57
<211> 1586
<212> DNA
<213> Bamecia argentifoli

<400> 57

```
gaattcgcgg ccgctcgcaa acttccgtac ctctcacccc ctcgccagga ccccccgcca     60
accagttcac cgtcatctcc tccaatggat actcatcccc catgtcttcg ggcagctacg    120
acccttatag tcccaccaat ggaagaatag ggaaagaaga gctttcgccg gcgaatagtc    180
tgaacgggta caacgtggat agctgcgatg cgtcgcggaa gaagaaggga ggaacgggtc    240
ggcagcagga ggagctgtgt ctcgtctgcg gggaccgcgc ctccggctac cactacaacg    300
ccctcacctg cgaaggctgc aagggcttct tccgtcggag catcaccaag aatgccgtct    360
accagtgtaa atatggaaat aattgtgaaa ttgacatgta catgaggcga aaatgccaag    420
agtgtcgtct caagaagtgt ctcagcgttg gcatgaggcc agaatgtgta gttcccgaat    480
tccagtgtgc tgtgaagcga aaagagaaaa aagcgcaaaa ggacaaagat aaacctaact    540
caacgacgag ttgttctcca gatggaatca aacaagagat agatcctcaa aggctggata    600
cagattcgca gctattgtct gtaaatggag ttaaacccat tactccagag caagaagagc    660
tcatccatag gctagtttat tttcaaaatg aatatgaaca tccatcccca gaggatatca    720
```

```
aaaggatagt taatgctgca ccagaagaag aaaatgtagc tgaagaaagg tttaggcata     780
ttacagaaat tacaattctc actgtacagt taattgtgga attttctaag cgattacctg     840
gttttgacaa actaattcgt gaagatcaaa tagctttatt aaaggcatgt agtagtgaag     900
taatgatgtt tagaatggca aggaggtatg atgctgaaac agattcgata ttgtttgcaa     960
ctaaccagcc gtatacgaga gaatcataca ctgtagctgg catgggtgat actgtggagg    1020
atctgctccg attttgtcga catatgtgtg ccatgaaagt cgataacgca gaatatgctc    1080
ttctcactgc cattgtaatt ttttcagaac gaccatctct aagtgaaggc tggaaggttg    1140
agaagattca agaaatttac atagaagcat taaaagcata tgttgaaaat cgaaggaaac    1200
catatgcaac aaccattttt gctaagttac tatctgtttt aactgaacta cgaacattag    1260
ggaatatgaa ttcagaaaca tgcttctcat tgaagctgaa gaatagaaag gtgccatcct    1320
tcctcgagga gatttgggat gttgtttcat aaacagtctt acctcaattc catgttactt    1380
ttcatatttg atttatctca gcaggtggct cagtacttat cctcacatta ctgagctcac    1440
ggtatgctca tacaattata acttgtaata tcatatcggt gatgacaaat ttgttacaat    1500
attctttgtt accttaacac aatgttgatc tcataatgat gtatgaattt ttctgttttt    1560
gcaaaaaaaa aagcggccgc gaattc                                         1586
```

<210> 58
<211> 1109
<212> DNA
<213> Nephotetix cincticeps

<400> 58

```
caggaggagc tctgcctgtt gtgcggagac cgagcgtcgg gataccacta caacgctctc    60
acctgcgaag gatgcaaggg cttctttcgg aggagtatca ccaaaaacgc agtgtaccag   120
tccaaatacg gcaccaattg tgaaatagac atgtatatgc ggcgcaagtg ccaggagtgc   180
cgactcaaga agtgcctcag tgtagggatg aggccagaat gtgtagtacc tgagtatcaa   240
tgtgccgtaa aaaggaaaga gaaaaaagct caaaaggaca aagataaacc tgtctcttca   300
accaatggct cgcctgaaat gagaatagac caggacaacc gttgtgtggt gttgcagagt   360
gaagacaaca ggtacaactc gagtacgccc agtttcggag tcaaacccct cagtccagaa   420
caagaggagc tcatccacag gctcgtctac ttccagaacg agtacgaaca ccctgccgag   480
gaggatctca agcggatcga gaacctcccc tgtgacgacg atgacccgtg tgatgttcgc   540
tacaaacaca ttacggagat cacaatactc acagtccagc tcatcgtgga gtttgcgaaa   600
aaactgcctg gtttcgacaa actactgaga gaggaccaga tcgtgttgct caaggcgtgt   660
tcgagcgagg tgatgatgct gcggatggcg cggaggtacg acgtccagac agactcgatc   720

ctgttcgcca acaaccagcc gtacacgcga gagtcgtaca cgatggcagg cgtgggggaa   780
gtcatcgaag atctgctgcg gttcggccga ctcatgtgct ccatgaaggt ggacaatgcc   840
gagtatgctc tgctcacggc catcgtcatc ttctccgagc ggccgaacct ggcggaagga   900
tggaaggttg agaagatcca ggagatctac ctggaggcgc tcaagtccta cgtggacaac   960
cgagtgaaac ctcgcagtcc gaccatcttc gccaaactgc tctccgttct caccgagctg  1020
cgaacactcg gcaaccagaa ctccgagatg tgcttctcgt taaactacgc aaccgcaaac  1080
atgccaccgt tcctcgaaga aatctggga                                    1109
```

<210> 59
<211> 401
<212> PRT
<213> Choristoneura fumiferana

<400> 59

```
Cys Leu Val Cys Gly Asp Arg Ala Ser Gly Tyr His Tyr Asn Ala Leu
1               5                   10                  15

Thr Cys Glu Gly Cys Lys Gly Phe Phe Arg Arg Ser Val Thr Lys Asn
            20                  25                  30

Ala Val Tyr Ile Cys Lys Phe Gly His Ala Cys Glu Met Asp Met Tyr
        35                  40                  45

Met Arg Arg Lys Cys Gln Glu Cys Arg Leu Lys Lys Cys Leu Ala Val
    50                  55                  60

Gly Met Arg Pro Glu Cys Val Val Pro Glu Thr Gln Cys Ala Met Lys
65                  70                  75                  80

Arg Lys Glu Lys Lys Ala Gln Lys Glu Lys Asp Lys Leu Pro Val Ser
                85                  90                  95

Thr Thr Thr Val Asp Asp His Met Pro Pro Ile Met Gln Cys Glu Pro
            100                 105                 110

Pro Pro Pro Glu Ala Ala Arg Ile His Glu Val Val Pro Arg Phe Leu
        115                 120                 125

Ser Asp Lys Leu Leu Glu Thr Asn Arg Gln Lys Asn Ile Pro Gln Leu
    130                 135                 140

Thr Ala Asn Gln Gln Phe Leu Ile Ala Arg Leu Ile Trp Tyr Gln Asp
145                 150                 155                 160
```

```
Gly Tyr Glu Gln Pro Ser Asp Glu Asp Leu Lys Arg Ile Thr Gln Thr
                165                 170                 175

Trp Gln Gln Ala Asp Asp Glu Asn Glu Glu Ser Asp Thr Pro Phe Arg
            180                 185                 190

Gln Ile Thr Glu Met Thr Ile Leu Thr Val Gln Leu Ile Val Glu Phe
        195                 200                 205

Ala Lys Gly Leu Pro Gly Phe Ala Lys Ile Ser Gln Pro Asp Gln Ile
    210                 215                 220

Thr Leu Leu Lys Ala Cys Ser Ser Glu Val Met Met Leu Arg Val Ala
225                 230                 235                 240

Arg Arg Tyr Asp Ala Ala Ser Asp Ser Val Leu Phe Ala Asn Asn Gln
                245                 250                 255

Ala Tyr Thr Arg Asp Asn Tyr Arg Lys Ala Gly Met Ala Tyr Val Ile
            260                 265                 270

Glu Asp Leu Leu His Phe Cys Arg Cys Met Tyr Ser Met Ala Leu Asp
        275                 280                 285

Asn Ile His Tyr Ala Leu Leu Thr Ala Val Val Ile Phe Ser Asp Arg
    290                 295                 300

Pro Gly Leu Glu Gln Pro Gln Leu Val Glu Glu Ile Gln Arg Tyr Tyr
305                 310                 315                 320

Leu Asn Thr Leu Arg Ile Tyr Ile Leu Asn Gln Leu Ser Gly Ser Ala
                325                 330                 335

Arg Ser Ser Val Ile Tyr Gly Lys Ile Leu Ser Ile Leu Ser Glu Leu
            340                 345                 350

Arg Thr Leu Gly Met Gln Asn Ser Asn Met Cys Ile Ser Leu Lys Leu
        355                 360                 365

Lys Asn Arg Lys Leu Pro Pro Phe Leu Glu Glu Ile Trp Asp Val Ala
    370                 375                 380

Asp Met Ser His Thr Gln Pro Pro Pro Ile Leu Glu Ser Pro Thr Asn
385                 390                 395                 400
```

Leu

<210> 60
<211> 825
<212> DNA
<213> Drosophila melanogaster

<400> 60

```
gtgtccaggg atttctcgat cgagcgcatc atagaggccg agcagcgagc ggagacccaa      60
tgcggcgatc gtgcactgac gttcctgcgc gttggtccct attccacagt ccagccggac     120
tacaagggtg ccgtgtcggc cctgtgccaa gtggtcaaca aacagctctt ccagatggtc     180
gaatacgcgc gcatgatgcc gcactttgcc caggtgccgc tggacgacca ggtgattctg     240
ctgaaagccg cttggatcga gctgctcatt gcgaacgtgg cctggtgcag catcgtttcg     300
ctggatgacg gcggtgccgg cggcgggggc ggtggactag gccacgatgg ctcctttgag     360
cgacgatcac cgggccttca gccccagcag ctgttcctca accagagctt ctcgtaccat     420
cgcaacagtg cgatcaaagc cggtgtgtca gccatcttcg accgcatatt gtcggagctg     480
agtgtaaaga tgaagcggct gaatctcgac cgacgcgagc tgtcctgctt gaaggccatc     540
atactgtaca acccggacat acgcgggatc aagagccggg cggagatcga gatgtgccgc     600
gagaaggtgt acgcttgcct ggacgagcac tgccgcctgg aacatccggg cgacgatgga     660
cgctttgcgc aactgctgct gcgtctgccc gctttgcgat cgatcagcct gaagtgccag     720
gatcacctgt tcctcttccg cattaccagc gaccggccgc tggaggagct ctttctcgag     780
cagctggagg cgccgccgcc acccggcctg gcgatgaaac tggag                     825
```

Claims

1. A gene expression modulation system comprising:

    a) a first gene expression cassette that is capable of being expressed in a host cell, comprising a polynucleotide that encodes a first hybrid polypeptide comprising:

        i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and
        ii) an ecdysone receptor ligand binding domain; and

    b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide that encodes a second hybrid polypeptide comprising:

        i) a transactivation domain; and
        ii) a non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain.

2. The gene expression modulation system according to claim 1, further comprising a third gene expression cassette comprising:

i) a response element recognized by the DNA-binding domain of the first hybrid polypeptide;
ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and
iii) a gene whose expression is to be modulated.

3. The gene expression modulation system according to claim 1, wherein the ecdysone receptor ligand binding domain (LBD) of the first hybrid polypeptide is selected from the group consisting of a spruce budworm *Choristoneura fumiferana* EcR ("CfEcR") LBD, a beetle *Tenebrio molitor* EcR ("TmEcR") LBD, a *Manduca sexta* EcR ("MsEcR") LBD, a *Heliothies virescens* EcR ("HvEcR") LBD, a midge *Chironomus tentans* EcR ("CtEcR") LBD, a silk moth *Bombyx mori* EcR ("BmEcR") LBD, a fruit fly *Drosophila melanogaster* EcR ("DmEcR") LBD, a mosquito *Aedes aegypti* EcR ("AaBeR") LBD, a blowfly *Lucilia capitata* EcR ("LcEcR") LBD, a blowfly *Lucilia cuprina* EcR ("LucEcR") LBD, a Mediterranean fruit fly *Ceratisis capitata* EcR ("CcEcR") LBD, a locust *Locusta migratoria* EcR ("LmEcR") LBD, an aphid *Mycus persicae* EcR ("MpEcR") LBD, a fiddler crab *Celuca pugilator* EcR ("CpEcR") LBD, an ixodid tick *Amblyomma americanum* EcR ("AmaEcR") LBD, a whitefly *Bamecia argentifoli* EcR ("BaEcR") LBD, and a leafhopper *Nephotetix cincticeps* EcR ("NcEcR") LBD.

4. The gene expression modulation system according to claim 1, wherein the ecdysone receptor ligand binding domain of the first hybrid polypeptide is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53 and SEQ ID NO: 45.

5. The gene expression modulation system according to claim 1, wherein the ecdysone receptor ligand binding domain of the first hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43 and SEQ ID NO: 59.

6. The gene expression modulation system according to claim 1, wherein the invertebrate retinoid X receptor ligand binding domain of the second hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15. SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

7. The gene expression modulation system according to claim 1, wherein the invertebrate retinoid X receptor ligand binding domains of the second hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24. SEQ ID NO: 25, SEQ ID NO: 26. SEQ ID NO: 27, SEQ ID NO: 28; SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

8. The gene expression modulation system according to claim 1, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain selected from the group consisting of a GAL4 DNA-binding domain and a LexA DNA-binding domain, and an ecdysone receptor ligand binding domain.

9. The gene expression, modulation system according to claim 1, wherein the second gene expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain selected from the group consisting of a VP16 transactivation domain and a B42 acidic activator transactivation domain, and an invertebrate retinoid X receptor ligand binding domain.

10. The gene expression modulation system according to claim 1, wherein the second gene expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 37) and a B42 AD (SEQ ID NO: 39), and an invertebrate retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12. SEQ ID NO: 13. SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18. SEQ ID NO: 19, and SEQ ID NO: 20.

11. The gene expression modulation system according to claim 1, wherein the seconde gene expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence selected from the group consisting of a VP16 AD SEQ ID NO: 38) and a B42 AD (SEQ ID NO: 40), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and

SEQ ID NO: 32.

12. A gene expression modulation system comprising:

a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide that encodes a first hybrid polypeptide comprising:

i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and
ii) a non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain; and

b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide that encodes a second hybrid polypeptide comprising:

i) a transactivation domain; and
ii) an ecdysone receptor ligand binding domain.

13. The gene expression modulation system according to claim 12, further comprising a third gene expression cassette comprising:

i) a response element recognized by the DNA-binding domain of the first hybrid polypeptide;
ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and
iii) a gene whose expression is to be modulated.

14. The gene expression modulation system according to claim 12, wherein the invertebrate retinoid X receptor ligand binding domain of the first hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

15. The gene expression modulation system according to claim 12, wherein the invertebrate retinoid X receptor ligand binding domain of the first hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

16. The gene expression modulation system according to claim 12, wherein the ecdysone receptor ligand binding domain of the second hybrid polypeptide is selected from the group consisting of a spruce budworm *Choristoneura fumiferana* EcR ("CfEcR") LBD, a beetle *Tenebrio molitor* EcR("TmEcR") LBD, a *Manduca sexta* EcR ("MsEcR") LBD, a *Heliothies virescens* EcR ("HvEcR") LBD, a midge *Chironomus tentans* EcR ("CtEcR") LBD, a silk moth *Bombyx mori* EcR ("BmEcR") LBD, a fruit fly *Drosophila melanogaster* EcR ("DmEcR") LBD, a mosquito *Aedes aegypti* EcR ("AaEcR") LBD, a blowfly *Lucilia capitata* EcR ("LcEcR") LBD, a blowfly *Lucilia cuprina* EcR ("LucEcR") LBD, a Mediterranean fruit fly *Ceratisis capitala* EcR ("CcEcR") LBD, a locust *Locusta migratoria* EcR ("LmEcR") LBD, an aphid *Mycus persicae* EcR ("MpEcR") LBD, a fiddler crab *Celuca pugilator* EcR ("CpEcR") LBD, an ixodid tick *Amblyomma americanum* EcR ("AmaEcR") LBD, a whitefly *Bamecia argentifoli* EcR ("BaEcR") LBD, and a leafhopper *Nephotetix cincticeps* EcR ("NcEcR") LBD.

17. The gene expression modulation system according to claim 12, wherein the ecdysone receptor ligand binding domain of the second hybrid polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 53 and SEQ ID NO: 45.

18. The gene expression modulation system according to claim 12, wherein the ecdysone receptor ligand binding domain of the second hybrid polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 43, and SEQ ID NO: 59.

19. The gene expression modulation system according to claim 12, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain selected from the group consisting of a GAL4 DNA-binding domain and a LexA DNA-binding domain, and an invertebrate retinoid X receptor ligand binding domain.

20. The gene expression modulation system according to claim 12, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 33) or a LexA DBD (SEQ ID NO: 35) and an invertebrate retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

21. The gene expression modulation system according to claim 12, wherein the first gene expression cassette comprises a polynucleotide that encodes a first hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 34), and a LexA DBD (SEQ ID NO: 36), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

22. The gene expression modulation system according to claim 12, wherein the second gene expression cassette comprises a polynucleotide that encodes a second hybrid polypeptide comprising a transactivation domain selected from the group consisting of a VP16 transactivation domain and a B42 acidic activator transactivation domain, and an ecdysone receptor ligand binding domain.

23. A gene expression cassette comprising a polynucleotide that encodes a hybrid polypeptide comprising a DNA-binding domain and a non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain, wherein the DNA binding domain is from a nuclear receptor other than an invertebrate retinoid X receptor.

24. The gene expression cassette according to claim 23, wherein the DNA-binding domain is a GAL4 DNA-binding domain or a LexA DNA-binding domain.

25. The gene expression cassette according to claim 23, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 33) an a LexA DBD (SEQ ID NO: 35), and an invertebrate retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

26. The gene expression cassette according to claim 23, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a DNA-binding domain comprising an amino acid sequence selected from the group consisting of a GAL4 DBD (SEQ ID NO: 34) and a LexA DBD (SEQ ID NO: 36), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ 10 NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29. SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

27. A gene expression cassette comprising a polynucleotide that encodes a hybrid polypeptide comprising a transactivation domain and a non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain, wherein the transactivation domain is from a nuclear receptor other than an invertebrate retinoid X receptor.

28. The gene expression cassette according to claim 27, wherein the transactivation domain is a VP 16 transactivation domain or a 842 acidic activator transactivation domain.

29. The gene expression cassette according to claim 27, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 37) and a B42 AD (SEQ ID NO: 39), and an invertebrate retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13. SEQ ID NO: 14, SEQ ID NO: 15, SIZE ID NO: 16, SEQ ID NO: 17**,** SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

30. The gene expression cassette according to claim 29, wherein the gene expression cassette comprises a polynucleotide that encodes a hybrid polypeptide comprising a transactivation domain comprising un amino acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 38) and a B42 AD (SEQ ID NO: 40), and an invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence, selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

31. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces ligand binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;
(b) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces steroid binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;
(c) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation reduces non-steroid binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;
(d) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances ligand binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;
(e) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances steroid binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;
(f) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation enhances non-steroid binding activity of the truncated invertebrate retinoid X receptor ligand binding domain;
(g) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation increases ligand sensitivity of the truncated invertebrate retinoid X receptor ligand binding domain; and
(h) a polynucleotide encoding a truncated non-lepidopteran, non-dipteran invertebrate retinoid X receptor ligand binding domain comprising a truncation mutation, wherein the truncation mutation increases ligand sensitivity of a heterodimer, wherein the heterodimer comprises said truncated invertebrate retinoid X receptor ligand binding domain and a dimerization partner.

32. The isolated polynucleotide according to claim 31, wherein the dimerization partner is an ecdysone receptor polypeptide.

33. An isolated polynucleotide encoding a truncated invertebrate retinoid X receptor ligand binding domain, wherein the polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

34. An isolated polypeptide encoded by the isolated polynucleotide according to claim 33.

35. An isolated truncated invertebrate retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28. SEQ ID NO: 29. SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 32.

36. A method of modulating the expression of a gene in a host cell comprising the steps of:

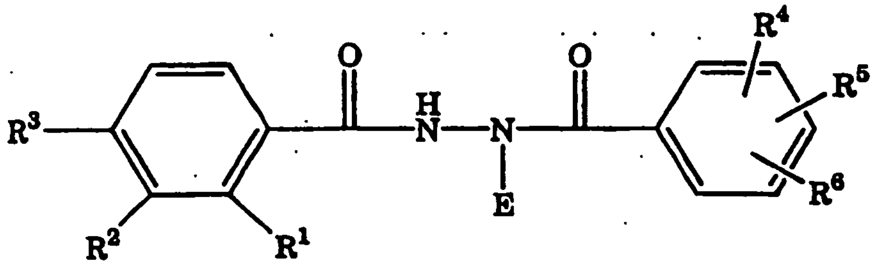
a) introducing into the host cell the gene expression modulation system according to claim 1; and
b) introducing into the host cell a ligand;

wherein the gene to he modulated is a component of a gene expression cassette comprising:

i) a response element recognized by the DNA binding domain from the first hybrid polypeptide:
ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and
iii) a gene whose expression is to be modulated;

whereby upon introduction of the ligand into the host cell, expression of the gene of b)iii) is modulated.

37. The method according to claim 36, wherein the ligand is a compound of the formula:



wherein:

E is a (C_4-C_6)alkyl containing a tertiary carbon or a cyano(C_3-C_5)alkyl containing a tertiary carbon;
R^1 is H, Me, Et, i-Pr, F, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_3Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF_2CF_3, CH=CHCN, allyl, azido, SCN, or $SCHF_2$;
R^2 is H, Me, Et, n-Pr, i-Pr, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_3CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe_2, NEt_2, SMe, SEt, $SOCF_3$, OCF_2CF_2H, GOEt, cyclopropyl, CF_2CF_3, CH=CN, allyl, azido, OCF_3, $OCHF_2$, O-i-Pr, SCN, $SCHF_2$, SOMe, NH-CN, or joined with R^3 and the phenyl carbons to which R^2 and R^3 are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;
R^3 is H, Et, or joined with R^2 and the phenyl carbons to which R^2 and R^3 are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;
R^4, R^5, and R^6 are independently H, Me, Et, F, Cl, Br, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

38. The method according to claim 36, further comprising introducing into the host cell a second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of a retinoic acid.

39. A method of modulating the expression of a gene in a host cell comprising the steps of:

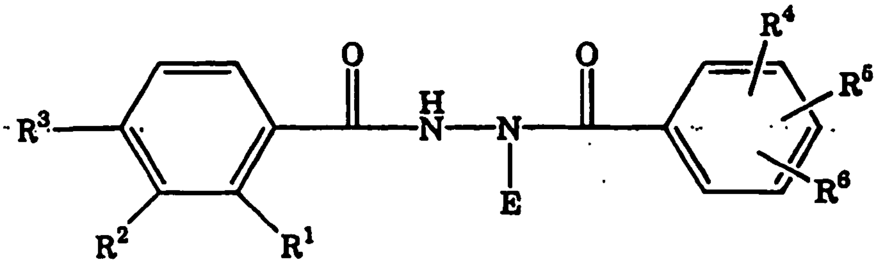
a) introducing into the host cell the gene expression modulation system of claim 12; and
b) introducing into the host cell a ligand;

wherein the gene to be modulated is a component of a gene expression cassette comprising:

i) a response element recognized by the DNA binding domain from the first hybrid polypeptide;
ii) a promoter that is activated by the transactivation domain of the second hybrid polypeptide; and
iii) a gene whose expression is to be modulated;

whereby upon introduction of the ligand into the host cell, expression of the gene of b)iii) is modulated.

40. The method according to claim 39, wherein the ligand is a compound of the formula:



wherein:

E is a (C_4-C_6)alkyl containing a tertiary carbon or a cyano(C_3-C_5)alkyl containing a tertiary carbon;
R^1 is H, Me, Et, i-Pr, F, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF_2CF_3, CH=CHCN, allyl, azido, SCN, or $SCHF_2$;
R^2 is H, Me, Et, n-Pr, i-Pr, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN. C°CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe_2, NEt_2, SMe. SEt, $SOCF_3$, OCF_2CF_2H, COEt, cyclopropyl, CF_2CF_3, CH=CHCN, allyl, azido, OCF_3, $OCHF_2$, O-i-Pr. SCN. $SCHF_2$, SOMe. NH-CN, or joined with R^3 and the phenyl carbons to which R^2 and R^3 are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;
R^3 is H, Et, or joined with R^2 and the phenyl carbons to which R^2 and R^3 are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;
R^4, R^5, and R^6 are independently H, Me, Et, F, Cl, Br, formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CN, C°CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

41. The method according to claim 39, further comprising introducing into the host cell a second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of a retinoic acid.

42. An isolated host cell comprising the gene expression modulation system according to claim 1.

43. The isolated host cell according to claim 42, wherein the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell.

44. The isolated host cell according to claim 43, wherein the mammalian cell is a murine cell or a human cell.

45. An isolated host cell comprising the gene expression modulation system according to claim 12.

46. The isolated host cell according to claim 45, wherein the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, an animal cell, and a mammalian cell.

47. The isolated host cell according to claim 46, wherein the mammalian cell is a murine cell or human cell.

48. A non-human organism comprising the host cell of claim 45.

49. The non-human organism according to claim 48, wherein the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, an animal, and a mammal.

50. The non-human organism according to claim 49, wherein the mammal is selected from the group consisting of a mouse, a rat, a rabbit, a cat, a dog, a bovine, a goat, a pig, a horse, a sheep, a monkey, and a chimpanzee.

51. A non-human organism comprising the host cell of claim 45.

52. The non-human organism according to claim 51, wherein the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, an animal, and a mammal,

53. The non-human organism according to claim 32, wherein the mammal is selected from the group consisting of a mouse, a rat, a rabbit, a cat, a dog, a bovine, a goat, a pig, a horse, a sheep, a monkey, and a chimpanzee.

54. The gene expression modulation system of claim 1, wherein said system exhibits increased ligand sensitivity compared to the equivalent system in which a lepidopteran, dipteran or vertebrate retinoic X receptor ligand binding domain is used.

55. The gene expression modulation system of claim 1, wherein said system exhibits increased sensitivity to non-steroidal ligands compared to the equivalent system in which a lepidopteran, dipteran or vertebrate retinoic X receptor ligand binding domain is used.

56. The gene expression modulation system of claim 12, wherein said system exhibits increased ligand sensitivity compared to the equivalent system in which a lepidopteran, dipteran or vertebrate retinoic X receptor ligand binding domain is used.

57. The gene expression modulation system of claim 12, wherein said system exhibits increased sensitivity to non-steroidal ligands compared to the equivalent system in which a lepidopteran, dipteran or vertebrate retinoic X receptor ligand binding domain is used.

Patentansprüche

1. Genexpressionsmodulationssystem, umfassend:

   a) eine erste Genexpressionskassette, die in einer Wirtszelle exprimiert werden kann und ein Polynukleotid umfasst, das ein erstes hybrides Polypeptid codiert, umfassend:

      i) eine DNA-Bindungsdomäne, die ein Response-Element erkennt, das mit einem Gen in einem Zusammenhang steht, dessen Expression moduliert werden soll, und
      ii) eine Ecdyson-Rezeptor-Ligandenbindungsdomäne und

   b) eine zweite Genexpressionskassette, die in der Wirtszelle exprimiert werden kann und ein Polynukleotid umfasst, das ein zweites hybrides Polypeptid codiert, umfassend:

      i) eine Transaktivierungsdomäne und
      ii) eine Nicht-Lepidoptera-, Nicht-Diptera-Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne.

2. Genexpressionsmodulationssystem gemäß Anspruch 1, welches ferner eine dritte Genexpressionskassette umfasst, welche:

   i) ein Response-Element, das von der DNA-Bindungsdomäne des ersten hybriden Polypeptids erkannt wird,
   ii) einen Promotor, der von der Transaktivierungsdomäne des zweiten hybriden Polypeptids aktiviert wird, und
   iii) ein Gen, dessen Expression moduliert werden soll umfasst.

3. Genexpressionsmodulationssystem gemäß Anspruch 1, wobei die Ecdyson-Rezeptor-Ligandenbindungsdomäne (LBD) des ersten hybriden Polypeptids ausgewählt ist aus der Gruppe bestehend aus einer Larve des Fichtenwicklers *Choristoneura fumiferana* EcR ("CfEcR") LBD, einem Käfer *Tenebrio molitor* EcR ("TmEcR") LBD, einer *Manduca sexta* EcR ("MsEcR") LBD, einem *Heliothies virescens* EcR ("HvEcR") LBD, einer Mücke *Chironomus tentans* EcR ("CtEcR") LBD, einem Seidenspinner *Bombyx mori* EcR ("BmEcR") LBD, einer Fruchtfliege *Drosophila melanogaster* EcR ("DmEcR") LBD, einem Moskito *Aedes aegypti* EcR ("AaEcR") LBD, einer Schmeißfliege *Lucilia capitata* EcR ("LcEcR") LBD, einer Schmeißfliege *Lucilia cuprina* EcR ("LucEcR") LBD, einer Mittelmeerfruchtfliege *Ceratitis capitata* EcR ("CcEcR") LBD, einer Heuschrecke *Locusta migratoria* EcR ("LmEcR") LBD, einer Blattlaus *Myzus persicae* EcR ("MpEcR") LBD, einer Winterkrabbe *Celuca pugilator* EcR ("CpEcR") LBD, einer Ixodes-Zecke *Amblyomma americanum* EcR ("AmaEcR") LBD, einer weißen Fliege *Bamecia argentifoli* EcR (BaEcR") LBD und einer Zwergzikade *Nephotetix cincticeps EcR* ("NcEcR") LBD.

4. Genexpressionsmodulationssystem gemäß Anspruch 1, wobei die Ecdyson-Rezeptor-Ligandenbindungsdomäne des ersten hybriden Polypeptids von einer Nukleinsäuresequenz codiert wird, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 1, SEQ ID NR. 53 und SEQ ID NR. 45.

5. Genexpressionsmodulationssystem gemäß Anspruch 1, wobei die Ecdyson-Rezeptor-Ligandenbindungsdomäne des ersten hybriden Polypeptids eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 5, SEQ ID NR. 43 und SEQ ID NR. 59.

6. Genexpressionsmodulationssystem gemäß Anspruch 1, wobei die Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne des zweiten hybriden Polypeptids von einem Polynukleotid codiert wird, das eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 9, SEQ ID NR. 10, SEQ ID NR. 11, SEQ ID NR. 12, SEQ ID NR. 13, SEQ ID NR. 14, SEQ ID NR. 15, SEQ ID NR. 16, SEQ ID NR. 17, SEQ ID NR. 18, SEQ ID NR. 19 und SEQ ID NR. 20.

7. Genexpressionsmodulationssystem gemäß Anspruch 1, wobei die Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne des zweiten hybriden Polypeptids eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 21, SEQ ID NR. 22, SEQ ID NR. 23, SEQ ID NR. 24, SEQ ID NR. 25, SEQ ID NR. 26, SEQ ID NR. 27, SEQ ID NR. 28, SEQ ID NR. 29, SEQ ID NR. 30, SEQ ID NR. 31 und SEQ ID NR. 32.

8. Genexpressionsmodulationssystem gemäß Anspruch 1, wobei die erste Genexpressionskassette ein Polynukleotid umfasst, das ein erstes hybrides Polypeptid codiert, welches eine DNA-Bindungsdomäne umfasst, die ausgewählt ist aus der Gruppe bestehend aus einer GAL4-DNA-Bindungsdomäne und einer LexA-DNA-Bindungsdomäne und einer Ecdyson-Rezeptor-Ligandenbindungsdomäne.

9. Genexpressionsmodulationssystem gemäß Anspruch 1, wobei die zweite Genexpressionskassette ein Polynukleotid umfasst, das ein zweites hybrides Polypeptid codiert, welches eine Transaktivierungsdomäne umfasst, die ausgewählt ist aus der Gruppe bestehend aus einer VP16-Transaktivierungsdomäne und einer B42-Säure-Aktivator-Transaktivierungsdomäne und einer Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne.

10. Genexpressionsmodulationssystem gemäß Anspruch 1, wobei die zweite Genexpressionskassette ein Polynukleotid umfasst, das ein zweites hybrides Polypeptid codiert, welches eine Transaktivierungsdomäne, die von einem Polynukleotid codiert wird, welches eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus VP16 AD (SEQ ID NR. 37) und B42 AD (SEQ ID NR. 39), und eine Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne, die von einem Polynukleotid codiert wird, das eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 9, SEQ ID NR. 10, SEQ ID NR. 11, SEQ ID NR. 12, SEQ ID NR. 13, SEQ ID NR. 14, SEQ ID NO. 15, SEQ ID NR. 16, SEQ ID NR. 17, SEQ ID NR. 18, SQ ID NR. 19 und SEQ ID NR. 20, umfasst.

11. Genexpressionsmodulationssystem gemäß Anspruch 1, wobei die zweite Genexpressionskassette ein Polynukleotid umfasst, das ein zweites hybrides Polypeptid codiert, welches eine Transaktivierungsdomäne, die eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus VP16 AD (SEQ ID NR. 38) und B42 AD (SEQ ID NR. 40), und eine Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne, die eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 21, SEQ ID NR. 22, SEQ ID NR. 23, SEQ ID NR. 24, SEQ ID NR. 25, SEQ ID NR. 26, SEQ ID NR. 27, SEQ ID NR. 28, SEQ ID NR. 29, SEQ ID NR. 30, SEQ ID NR. 31 und SEQ ID NR. 32, umfasst.

12. Genexpressionsmodulationssystem, umfassend:

a) eine erste Genexpressionskassette, die in einer Wirtszelle exprimiert werden kann und ein Polynukleotid umfasst, das ein erstes hybrides Polypeptid codiert, umfassend:

i) eine DNA-Bindungsdomäne, die ein Response-Element erkennt, das mit einem Gen in einem Zusammenhang steht, dessen Expression moduliert werden soll, und
ii) eine Nicht-Lepidoptera-, Nicht-Diptera-Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne und

b) eine zweite Genexpressionskassette, die in der Wirtszelle exprimiert werden kann und ein Polynukleotid umfasst, das eine zweites hybrides Polypeptid codiert, umfassend:

i) eine Transaktivierungsdomäne und
ii) Ecdyson-Rezeptor-Ligandenbindungsdomäne.

13. Genexpressionsmodulationssystem gemäß Anspruch 12, umfassend ferner eine dritte Genexpressionskassette, welche:

i) ein Response-Element, welches von der DNA-Bindungsdomäne des ersten hybriden Polypeptids erkannt wird,
ii) einen Promotor, der von der Transaktivierungsdomäne des zweiten hybriden Polypeptids aktiviert wird, und
iii) ein Gen, dessen Expression moduliert werden soll,

umfasst.

14. Genexpressionsmodulationssystem gemäß Anspruch 12, wobei die Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne des ersten hybriden Polypeptids von einem Polynukleotid codiert wird, welches eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 9, SEQ ID NR. 10, SEQ ID NR. 11, SEQ ID NR. 12, SEQ ID NR. 13, SEQ ID NR. 14, SEQ ID NR. 15, SEQ ID NR. 16, SEQ ID NR. 17, SEQ ID NR. 18, SEQ ID NR. 19 und SEQ ID NR. 20.

15. Genexpressionsmodulationssystem gemäß Anspruch 12, wobei die Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne des ersten hybriden Polypeptids eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 21, SEQ ID NR. 22, SEQ ID NR. 23, SEQ ID NR. 24, SEQ ID NR. 25, SEQ ID NR. 26, SEQ ID NR. 27, SEQ ID NR. 28, SEQ ID NR. 29, SEQ ID NR. 30, SEQ ID NR. 31 und SEQ ID NR. 32.

16. Genexpressionsmodulationssystem gemäß Anspruch 12, wobei die Ecdyson-Rezeptor-Ligandenbindungsdomäne des zweiten hybriden Polypeptids ausgewählt ist aus der Gruppe bestehend aus einer Larve des Fichtenwicklers *Choristoneura fumiferana* EcR ("CfEcR") LBD, einem Käfer *Tenebrio molitor* EcR ("TmEcR") LBD, einer *Manduca sexta* EcR ("MsEcR") LBD, einem *Heliothies virescens* EcR ("HvEcR") LBD, einer Mücke *Chironomus tentans* EcR ("CtEcR") LBD, einem Seidenspinner *Bombyx mori* EcR ("BmEcR") LBD, einer Fruchtfliege *Drosophila melanogaster* EcR ("DmEcR") LBD, einem Moskito *Aedes aegypti* EcR ("AaEcR") LBD, einer Schmeißfliege *Lucilia capitata* EcR ("LcEcR") LBD, einer Schmeißfliege *Lucilia cuprina* EcR ("LucEcR") LBD, einer Mittelmeerfruchtfliege *Ceratitis capitata* EcR ("CcEcR") LBD, einer Heuschrecke *Locusta migratoria* EcR ("LmEcR") LBD, einer Blattlaus *Myzus persicae* EcR ("MpEcR") LBD, einer Winterkrabbe *Celuca pugilator* EcR ("CpEcR") LBD, einer Ixodes-Zecke *Amblyomma americanum* EcR ("AmaEcR") LBD, einer weißen Fliege *Bamecia argentifoli* EcR (BaEcR") LBD und einer Zwergzikade *Nephotetix cincticeps EcR* ("NcEcR") LBD.

17. Genexpressionsmodulationssystem gemäß Anspruch 12, wobei die Ecdyson-Rezeptor-Ligandenbindungsdomäne des zweiten hybriden Polypeptids von einem Polynukleotid codiert wird, welches eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 1, SEQ ID NR. 53 und SEQ ID NR. 45.

18. Genexpressionsmodulationssystem gemäß Anspruch 12, wobei die Ecdyson-Rezeptor-Ligandenbindungsdomäne des zweiten hybriden Polypeptids eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR.5, SEQ ID NR. 43 und SEQ ID NR. 59.

19. Genexpressionsmodulationssystem gemäß Anspruch 12, wobei die erste Genexpressionskassette ein Polynukleotid umfasst, das ein erstes Polypeptid codiert, welches eine DNA-Bindungsdomäne umfasst, die ausgewählt ist aus der Gruppe bestehend aus einer GAL4-DNA-Bindungsdomäne und einer LexA-DNA-Bindungsdomäne und einer Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne.

20. Genexpressionsmodulationssystem gemäß Anspruch 12, wobei die erste Genexpressionskassette ein Polynukleotid umfasst, das ein erstes hybrides Polypeptid codiert, welches eine DNA-Bindungsdomäne umfasst, die von einem Polynukleotid codiert wird, welches eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus GAL4-DBD (SEQ ID NR. 33) oder LexA-DBD (SEQ ID NR. 35), und eine Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne, die von einem Polynukleotid codiert wird, welches eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 9, SEQ ID NR. 10, SEQ ID NR. 11, SEQ ID NR. 12, SEQ ID NR. 13, SEQ ID NR. 14, SEQ ID NR. 15, SEQ ID NR. 16, SEQ ID NR. 17, SEQ ID NR. 18, SEQ ID NR. 19 und SEQ ID NR. 20.

21. Genexpressionsmodulationssystem gemäß Anspruch 12, wobei die erste Genexpressionskassette ein Polynukleotid umfasst, das ein erstes hybrides Polypeptid codiert, welches eine DNA-Bindungsdomäne umfasst, die eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus GAL4-DBD (SEQ ID NR. 34) und LexA-DBD (SEQ ID NR. 36), und eine Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne, die eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 21, SEQ ID NR. 22, SEQ ID NR. 23, SEQ ID NR. 24, SEQ ID NR. 25, SEQ ID NR. 26, SEQ ID NR. 27, SEQ ID NR. 28, SEQ ID NR. 29, SEQ ID NR. 30, SEQ ID NR. 31 und SEQ ID NR. 32.

22. Genexpressionsmodulationssystem gemäß Anspruch 12, wobei die zweite Genexpressionskassette ein Polynukleotid umfasst, das ein zweites hybrides Polypeptid codiert, welches eine Transaktivierungsdomäne umfasst, die ausgewählt ist aus der Gruppe bestehend aus einer VP16-Transaktivierungsdomäne und einer B42-Säure-Aktivator-Transaktivierungsdomäne und einer Ecdyson-Rezeptor-Ligandenbindungsdomäne.

23. Genexpressionskassette, die ein Polynukleotid umfasst, das ein hybrides Polypeptid codiert, welches eine DNA-

Bindungsdomäne und eine Nicht-Lepidoptera-, Nicht-Diptera-Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne umfasst, wobei die DNA-Bindungsdomäne aus einem anderen nukleären Rezeptor als dem Invertebraten-Retinoid-X-Rezeptor besteht.

24. Genexpressionskassette gemäß Anspruch 23, wobei die DNA-Bindungsdomäne eine GAL4-DNA-Bindungsdomäne oder eine LexA-DNA-Bindungsdomäne ist.

25. Genexpressionskassette gemäß Anspruch 23, wobei die Genexpressionskassette ein Polynukleotid umfasst, das ein hybrides Polypeptid codiert, welches eine DNA-Bindungsdomäne umfasst, die von einem Polynukleotid codiert wird, das eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus GAL4 DBD (SEQ ID NR. 33) und Lex DBD (SEQ ID NR. 35), und eine Invertebraten-Reünoid-X-Rezeptor-Ligandenbindungsdomäne, die von einem Polynukleotid codiert wird, das eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 9, SEQ ID NR. 10, SEQ ID NR. 11, SEQ ID NR. 12, SEQ ID NR. 13, SEQ ID NR. 14, SEQ ID NR. 15, SEQ ID NR. 16, SEQ ID NR. 17, SEQ ID NR. 18, SEQ ID NR. 19 und SEQ ID NR. 20.

26. Genexpressionskassette gemäß Anspruch 23, wobei die Genexpressionskassette ein Polynukleotid umfasst, das ein hybrides Polypeptid codiert, welches eine DNA-Bindungsdomäne umfasst, die eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus GAL4 DBD (SEQ ID NR. 34) und LexA DBD (SEQ ID NR. 36), und eine Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne, die eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 21, SEQ ID NR. 22, SEQ ID NR. 23, SEQ ID NR. 24, SEQ ID NR. 25, SEQ ID NR. 26, SEQ ID NR. 27, SEQ ID NR. 28, SEQ ID NR. 29, SEQ ID NR. 30, SEQ ID NR. 31 und SEQ ID NR. 32.

27. Genexpressionskassette, die ein Polynukleotid umfasst, das ein hybrides Polypeptid codiert, welches eine Transaktivierungsdomäne und eine Nicht-Lepidoptera-, Nicht-Diptera-Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne umfasst, wobei die Transaktivierungsdomäne aus einem anderen nukleären Rezeptor als dem Invertebraten Retinoid-X-Rezeptor besteht.

28. Genexpressionskassette gemäß Anspruch 27, wobei die Transaktivierungsdomäne eine VP16-Transaktivierungsdomäne oder eine B42-Säure-Aktivator-Transaktivierungsdomäne ist.

29. Genexpressionskassette gemäß Anspruch 27, wobei die Genexpressionskassette ein Polynukleotid umfasst, das ein hybrides Polypeptid codiert, welches eine Transaktivierungsdomäne umfasst, die von einem Polynukleotid codiert wird, das eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus VP16 AD (SEQ ID NR. 37) und B42 AD (SEQ ID NR. 39), und eine Invertebraien-Retinoid-X-Rezeptor-Ligandenbindungsdomäne, die von einem Polynukleotid codiert wird, welches eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 9, SEQ ID NR. 10, SEQ ID NR. 11, SEQ ID NR. 12, SEQ ID NR. 13, SEQ ID NR. 14, SEQ ID NR. 15, SEQ ID NR. 16, SEQ ID NR. 17, SEQ ID NR. 18, SEQ ID NR. 19 und SEQ ID NR. 20.

30. Genexpressionskassette gemäß Anspruch 29, wobei die Genexpressionskassette ein Polynukleotid umfasst, das ein hybrides Polypeptid codiert, welches eine Transaktivierungsdomäne umfasst, die eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus VP16 AD (SEQ ID NR. 38) und B42 AD (SEQ ID NR. 40), und eine Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne, die eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 21, SEQ ID NR. 22, SEQ ID NR. 23, SEQ ID NR. 24, SEQ ID NR. 25, SEQ ID NR. 26, SEQ ID NR. 27, SEQ ID NR. 28, SEQ ID NR. 29, SEQ ID NR. 30, SEQ ID NR. 31 und SEQ ID NR. 32.

31. Isoliertes Polynukleotid, das ausgewählt ist aus der Gruppe bestehend aus:

(a) einem Polynukleotid, das eine trunkierte Nicht-Lepidoptera-, Nicht-Diptera-Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne codiert, die eine Trunkierungsmutation umfasst, wobei die Trunkierungsmutation die Ligandenbindungsaktivität der trunkierten Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsriamäne reduziert,
(b) einem Polynukleotid, das eine trunkierte Nicht-Lepidoptera-, Nicht-Diptera-Inveriebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne codiert, die eine Trunkierungsmutation umfasst, wobei die Trunkierungsmutation die Steroidbindungsaktivität der trunkierten Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne reduziert,
(c) einem Polynukleotid, das eine trunkierte Nicht-Lepidoptera-, Nicht-Diptera-Invertebraten-Retinoid-X-Rezep-

tor-Ligandenbindungsdomäne codiert, die eine Trunkierungsmutation umfasst, wobei die Trunkierungsmutation die Nicht-SteroidBindungsaktivität der trunkierten Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne reduziert,
(d) einem Polynukleotid, das eine trunkierte Nicht-Lepidoptera-, Nicht-Diptera-Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne codiert, die eine Trunkierungsmutation umfasst, wobei die Trunkierungsmutation die Ligendenbindungsaktivität der trunkierten Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne erhöht,
(e) einem Polynukleotid, das eine trunkierte Nicht-Lepidoptera-, Nicht-Diptera-Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne codiert, die eine Trunkierungsmutation umfasst, wobei die Trunkierungsmutation die Steroidbindungsaktivität der trunkierten Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne erhöht,
(f) einem Polynukleotid, das eine trunkierte Nicht-Lepidoptera-, Nicht-Diptera-Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne codiert, die eine Trunkierungsmutation umfasst, wobei die Trunkierungsrnutatian die Nicht-SteroidBindungsaktivität der trunkierten Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne erhöht,
(g) einem Polynukleotid, das eine trunkierte Nicht-Lepidoptera-, Nicht-Diptera-Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne codiert, die eine Trunkierungsmutation umfasst, wobei die Trunkierungsmutation die Ligandensensitivität der trunkierten Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne erhöht, und
(h) einem Polynukleotid, das eine trunkierte Nicht-Lepidoptera-, Nicht-Diptera-Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne codiert, die eine Trunkierungsmutation umfasst, wobei die Trunkierungsmutation die Ligandensensitivität eines Heterodimers erhöht, wobei das Heterodimer die trunkierte Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne und einen Dimerisationspartner umfasst.

32. Isoliertes Polynukleotid gemäß Anspruch 31, wobei der Dimerisationspartner ein Ecdyson-Rezeptor-Polypeptid ist.

33. Isoliertes Polynukleotid, das eine trunkierte Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne codiert, wobei das Polynukleotid eine Nukleinsäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NR. 15, SEQ ID NR. 16, SEQ ID NO. 17, SEQ ID NR. 18, SEQ ID NR. 19 und SEQ ID NR. 20.

34. Isoliertes Polypeptid, das von dem isolierten Polynukleotid gemäß Anspruch 33 codiert wird.

35. Isolierte trunkierte Invertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne, die eine Aminosäuresequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID Nr. 27, SEQ ID NR. 28, SEQ ID NR. 29, SEQ ID NR. 30, SEQ ID NR. 31 und SEQ ID NR. 32.

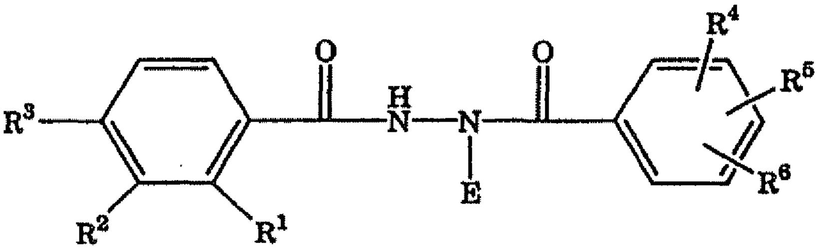
36. Verfahren zur Modulation der Expression eines Gens in einer Wirtszelle, das die folgenden Stufen umfasst:

a) Einbringen des Genexpressionsmodulationssystems gemäß Anspruch 1 in die Wirtszelle, und
b) Einbringen eines Liganden in die Wirtszelle, wobei das zu modulierende Gen Bestandteil einer Genexpressionskassette ist, welche umfasst:

i) ein Response-Element, das von der DNA-Bindungsdomäne von dem ersten hybriden Polypeptid erkannt wird,
ii) einen Promotor, der von der Transaktivierungsdomäne des zweiten hybriden Polypeptids aktiviert wird, und
iii) ein Gen, dessen Expression moduliert werden soll,

wobei nach dem Einbringen des Liganden in die Wirtszelle die Expression des Gens von b) iii) moduliert wird.

37. Verfahren gemäß Anspruch 36, wobei der Ligand eine Verbindung der Formel



ist,
wobei:

E ein (C_4-C_6)-Alkyl ist, das einen tertiären Kohlenstoff oder ein Cyano(C_3-C_5)-Alkyl, das einen tertiären Kohlenstoff enthält, enthält,
R^1 H, Me, Et, i-Pr, F, Formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-Propynyl, 2-Propynyl, Vinyl, OH, OMe, OEt, Cyclopropyl, CF_2CF_3, CH=CHCN, Allyl, Azido, SCN oder $SCHF_2$ ist,
R^2 H, Me, Et, n-Pr, i-Pr, Formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-Propynyl, 2-Propynyl, Vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe_2, NEt_2,SMe, SEt, $SOCF_3$, OCF_2CF_2H, COEt, Cyclopropyl, CF_2CF_3, CH=CHCN, Allyl, Azido, OCF_3, $OCHF_2$, O-i-Pr, SCN, $SCHF_2$, SOMe, NH-CN ist oder mit R^3 und den Phenyl-Kohlenstoffen, an die R^2 und R^3 gebunden sind, zusammengenommen wird, um ein Ethylendioxy, einen Dihydrofuryl-Ring mit dem Sauerstoff neben dem Phenyl-Kohlenstoff zu bilden oder einen Dihydropyryl-Ring mit dem Sauerstoff neben dem Phenyl-Kohlenstoff,
R^3 H, Et ist oder mit R^2 und den Phenyl-Kohlenstoffen zusammengenommen wird, an die R^2 und R^3 gebunden sind, um ein Ethylendioxy, einen Dihydrofuryl-Ring mit dem Sauerstoff neben dem Phenyl-Kohlenstoff zu bildern oder einen Dihydropyryl-Ring mit dem Sauerstoff neben dem Phenyl-Kohlenstoff,
R^4, R^5 und R^6 unabhängig voneinander H, Me, Et, F, Cl, Br, Formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CN, C°CH, 1-Propynyl, 2-Propynyl, Vinyl, OMe, OEt, SMe, oder SEt sind.

38. Verfahren gemäß Anspruch 36, welches ferner das Einbringen eines zweiten Liganden in die Wirtszelle umfasst, wobei der zweite Ligand eine 9-cis-Retinsäure oder ein synthetisches Analogen einer Retinsäure ist.

39. Verfahren zur Modulation der Expression eines Gens in einer Wirtszelle, welches die folgenden Stufen umfasst:

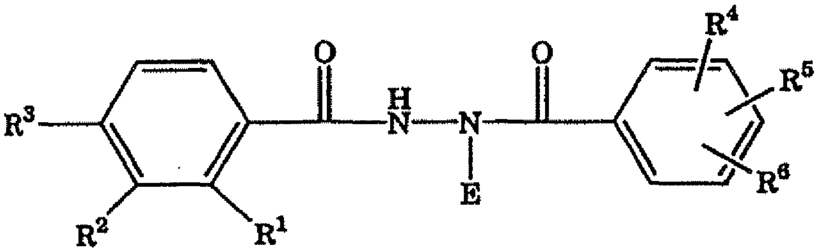
a) Einbringen des Genexpressionsmodulationssystems nach Anspruch 12 in die Wirtszelle und
b) Einbringen eines Liganden in die Wirtszelle,

wobei das zu modulierende Gen Bestandteil einer Genexpressionslcassette ist, welche umfasst:

i) ein Response-Element, das von der DNA-Bindungsdomäne des ersten hybriden Polypeptids erkannt wird,
ii) einen Promotor, der von der Transaktivierungsdomäne des zweiten hybriden Polypeptids aktiviert wird, und
iii) ein Gen, dessen Expression moduliert werden soll,

wobei nach dem Einbringen des Liganden in die Wirtszelle die Expression des Gens von b) iii) moduliert wird.

40. Verfahren gemäß Anspruch 39, wobei der Ligand eine Verbindung der Formel



ist,

wobei:

E ein (C_4-C_6)-Alkyl ist, das einen tertiären Kohlenstoff oder ein Cyano(C_3-C_5)-Alkyl, das einen tertiären Kohlenstoff enthält, enthält,
R^1 H, Me, Et, i-Pr, F, Formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-Propynyl, 2-Propynyl, Vinyl, OH, OMe, OEt, Cyclopropyl, CF_2CF_3, CH=CHCN, Allyl, Azido, SCN oder $SCHF_2$ ist,
R^2 H, Me, Et, n-Pr, i-Pr, Formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-Propynyl, 2-Propynyl, Vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe_2, NEt_2,SMe, SEt, $SOCF_3$, OCF_2CF_2H, COEt, Cyclopropyl, CF_2CF_3, CH=CHCN, Allyl, Azido, OCF_3, $OCHF_2$, O-i-Pr, SCN, $SCHF_2$, SOMe, NH-CN ist oder mit R^3 und den Phenyl-Kohlenstoffen, an die R^2 und R^3 gebunden sind, zusammengenommen wird, um ein Ethylendioxy, einen Dihydrofuryl-Ring mit dem Sauerstoff neben dem Phenyl-Kohlenstoff zu bilden oder einen Dihydropyryl-Ring mit dem Sauerstoff neben dem Phenyl-Kohlenstoff,
R^3 H, Et ist oder mit R^2 und den Phenyl-Kohlenstoffen zusammengenommen wird, an die R^2 und R^3 gebunden sind, um ein Ethylendioxy, einen Dihydrofuryl-Ring mit dem Sauerstoff neben dem Phenyl-Kohlenstoff zu bilden oder einen Dihydropyryl-Ring mit dem Sauerstoff neben dem Phenyl-Kohlenstoff,
R^4, R^5 und R^6 unabhängig voneinander H, Me, Et, F, Cl, Br, Formyl, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CN, C°CH, 1-propinyl, 2-Propynyl, Vinyl, OMe, OEt, SMe, oder SEt sind.

41. Verfahren gemäß Anspruch 39, welches ferner das Einbringen eines zweiten Liganden in die Wirtszelle umfasst, wobei der zweite Ligand eine 9-cis-Retinsäure oder ein synthetisches Analogon einer Retinsäure ist.

42. Isolierte Wirtszeile, die das Genexpressionsmodulationssystem gemäß Anspruch 1 umfasst.

43. Isolierte Wirtszelle gemäß Anspruch 42, wobei die Wirtszelle ausgewählt ist aus der Gruppe bestehend aus einer Bakterienzelle, einer Pilzzelle, einer Hefezelle, einer Tierzelle und einer Säugetierzelle.

44. Isolierte Wirtszelle gemäß Anspruch 43, wobei die Säugetierzelle eine Mäusezelle oder eine menschliche Zelle ist.

45. Isolierte Wirtszelle, die das Genexpressionsmodulationssystem gemäß Anspruch 12 umfasst.

46. Isolierte Wirtszelle gemäß Anspruch 45, wobei die Wirtszelle ausgewählt ist aus der Gruppe bestehend aus einer Bakterienzelle, einer Pilzzelle, einer Hefezelle, einer Tierzelte und einer Säugetierzelle.

47. Isolierte Wirtszelle gemäß Anspruch 46, wobei die Säugetierzelle eine Mäusezelle oder eine menschliche Zelle ist.

48. Nicht-menschlicher Organismus, der die Wirtszelle nach Anspruch 45 umfasst.

49. Nicht-menschlicher Organismus gemäß Anspruch 48, wobei der nichtmenschliche Organismus ausgewählt ist aus der Gruppe bestehend aus einem Bakterium, einem Pilz, einer Hefe, einem Tier und einem Säugetier.

50. Nicht-menschlicher Organismus gemäß Anspruch 49, wobei das Säugetier ausgewählt ist aus der Gruppe bestehend aus einer Maus, einer Ratte, einem Kaninchen, einer Katze, einem Hund, einem Rind, einer Ziege, einem Schwein, einem Pferd, einem Schaf, einem Affen und einem Schimpansen.

51. Nicht-menschlicher Organismus, der die Wirtszelle nach Anspruch 45 umfasst.

52. Nicht-menschlicher Organismus gemäß Anspruch 51, wobei der nichtmenschliche Organismus ausgewählt ist aus der Gruppe bestehend aus einem Bakterium, einem Pilz, einer Hefe, einem Tier und einem Säugetier.

53. Nicht-menschlicher Organismus gemäß Anspruch 52, wobei das Säugetier ausgewählt ist aus der Gruppe bestehend aus einer Maus, einem Kaninchen, einer Katze, einem Hund, einem Rind, einer Ziege, einem Schwein, einem Pferd, einem Schaf, einem Affen und einem Schimpansen.

54. Genexpressionsmodulationssystem nach Anspruch 1, wobei das System im Vergleich zum äquivalenten System, in dem eine Lepidoptera-, Diptera- oder Vertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne verwendet wird, erhöhte Ligandensensitivität zeigt.

55. Genexpressionsmociulationssystem nach Anspruch 1, wobei das System im Vergleich zum äquivalenten System, in dem eine Lepidoptera-, Diptera- oder Vertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne verwendet wird, erhöhte Sensitivität gegenüber nicht-steroidale Liganden zeigt.

56. Genexpressionsmodulationssystem nach Anspruch 12, wobei das System im Vergleich zum äquivalenten System, in dem eine Lepidoptera-, Diptera- oder Vertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne verwendet wird, erhöhte Ligandensensitivität zeigt.

57. Genexpressionsmodulationssystem nach Anspruch 12, wobei das System im Vergleich zum äquivalenten System, in dem eine Lepidoptera-, Diptera- oder Vertebraten-Retinoid-X-Rezeptor-Ligandenbindungsdomäne verwendet wird, erhöhte Sensitivität gegenüber nicht-steroidalen Liganden zeigt.

Revendications

1. Système de modulation de l'expression génique comprenant :

a) une première cassette d'expression génique qui est capable d'être exprimée dans une cellule hôte, comprenant un polynucléotide qui code un premier polypeptide hybride comprenant :

i) un domaine liant l'ADN qui reconnaît un élément de réponse associé avec un gène dont l'expression doit être modulée ; et
ii) un domaine liant un ligand de récepteur d'ecdysone ; et

b) une seconde cassette d'expression génique qui est capable d'être exprimée dans la cellule hôte comprenant un polynucléotide qui code un second polypeptide hybride comprenant :

i) un domaine de transactivation ; et
ii) un domaine liant un ligand de récepteur X des rétinoïdesd'invertébré non diptère non lépidoptère.

2. Système de modulation de l'expression génique selon la revendication 1, comprenant en outre une troisième cassette d'expression génique comprenant :

i) un élément de réponse reconnu par le domaine liant l'ADN du premier polypeptide hybride ;
ii) un promoteur qui est activé par le domaine de transactivation du second polypeptide hybride ; et
iii) un gène dont l'expression doit être modulée.

3. Système de modulation de l'expression génique selon la revendication 1 où le domaine liant un ligand de récepteur d'ecdysone (LBD) du premier polypeptide hybride est choisi dans le groupe consistant en un LBD de EcR de tordeuse des bourgeons de l'épinette *Choristoneura fumiferana* (« CfEcR »), un LBD de EcR de coléoptère *Tenebrio molitor* (« TmEcR »), un LBD de BcR de *Manduca sexta* (« MsEcR »), un LBD de BcR de *Heliothies virescens* (« HvEcR »), un LBD de EcR de chironome *Chironomes tentans* (« CtEcR »), un LBD de EcR de ver à soie *Bombyx mori* (« BmEcR »), un LBD de EcR de mouche des fruits *Drosophila melanogaster* (« DmEcR »), un LBD de EcR de moustique *Aedes aegypti* (« AaEcR »), un LBD de EcR de mouche à viande *Lucilia capitata(«* LcEcR »), un LBD de EcR de mouche à viande *Lucilia cuprina(«* LucEcR »), un LBD de EcR de mouche méditerranéenne des fruits *Ceratitis capitata* (« CcEcR »), un LBD de EcR de criquet *Locusta migratoria* (*«*LmEcR»), un LBD de EcR d'aphide *Myzus persicae(«*MpEcR»), un LBD de EcR de crabe violoniste *Celuca pugilator* (« CpEcR »), un LBD de EcR de tique ixode *Amblyommaamricanum(«* AmaEcR »), un LBD de EcR de mouche blanche *Bamecia argentifoli* (« BaEcR ») et un LBD de EcR de cicadelle *Nephotetix cincticeps.*

4. Système de modulation de l'expression génique selon la revendication 1 où le domaine liant un ligand de récepteur d'ecdysone du premier polypeptide hybride est codé par une séquence d'acide nucléique choisie dans le groupe consistant en SEQ ID NO : 1, SEQ ID NO : 53 et SEQ ID NO : 45.

5. Système de modulation de l'expression génique selon la revendication 1 où le domaine liant un ligand de récepteur d'ecdysone du premier polypeptide hybride comprend une séquence d'aminoacides choisie dans le groupe consistant en SEQ ID NO : 5, SEQ ID NO : 43 et SEQ ID NO : 59.

6. Système de modulation de l'expression génique selon la revendication 1 où le domaine liant un ligand de récepteur

X des rétinoïdes d'invertébré du second polypeptide hybride est codé par un polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe consistant en SEQ ID NO : 9, SEQ ID NO : 10, SEQ ID NO : 11,SEQ ID NO : 12,SEQ ID NO : 13,SEQ ID NO : 14,SEQ ID NO : 15,SEQ ID NO : 16,SEQ ID NO : 17,SEQ ID NO : 18, SEQ ID NO : 19 et SEQ ID NO : 20.

7. Système de modulation de l'expression génique selon la revendication 1 où le domaine liant un ligand de récepteur X des rétinoïdes d'invertébré du second polypeptide hybride comprend une séquence d'aminoacides choisie dans le groupe consistant en SEQ ID NO : 21, SEQ ID NO : 22, SEQ ID NO : 23,SEQ ID NO : 24,SEQ ID NO : 25,SEQ ID NO : 26,SEQ ID NO : 27,SEQ ID NO : 28,SEQ ID NO : 29,SEQ ID NO : 30,SEQ ID NO : 31 et SEQ ID NO : 32.

8. Système de modulation de l'expression génique selon la revendication 1 où la première cassette d'expression génique comprend un polynucléotide qui code un premier polypeptide hybride comprenant un domaine liant l'ADN choisi dans le groupe consistant en un domaine liant l'ADN de GAL4 et un domaine liant l'ADN de LexA, et un domaine liant un ligand de récepteur d'ecdysone.

9. Système de modulation de l'expression génique selon la revendication 1 où la seconde cassette d'expression génique comprend un polynucléotide qui code un second polypeptide hybride comprenant un domaine de transactivation choisi dans le groupe consistant en un domaine de transactivation de VP16 et un domaine de transactivation d'activateur acide de B42, et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré.

10. Système de modulation de l'expression génique selon la revendication 1 où la seconde cassette d'expression génique comprend un polynucléotide qui code un second polypeptide hybride comprenant un domaine de transactivation codé par un polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe consistant en unAD de VP 16 (SEQ ID NO : 37) et unAD de B42 (SEQ ID NO : 39), et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré codé par un polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe consistant enSEQ ID NO : 9, SEQ ID NO: 10, SEQ ID NO : 11,SEQ ID NO : 12,SEQ ID NO: 13,SEQ ID NO: 14,SEQ ID NO : 15,SEQ ID NO : 16,SEQ ID NO : 17,SEQ ID NO : 18, SEQ ID NO : 19 et SEQ ID NO:20.

11. Système de modulation de l'expression génique selon la revendication 1 où la seconde cassette d'expression génique comprend un polynucléotide qui code un second polypeptide hybride comprenant un domaine de transactivation comprenant une séquence d'aminoacides choisie dans le groupe consistant en unAD de VP16 (SEQ ID NO : 38) et unAD de B42 (SEQ ID NO : 40), et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré comprenant une séquence d'aminoacides choisie dans le groupe consistant enSEQ ID NO : 21, SEQ ID NO : 22, SEQ ID NO : 23,SEQ ID NO : 24,SEQ ID NO : 25,SEQ ID NO : 26,SEQ ID NO : 27,SEQ ID NO : 28,SEQ ID NO : 29,SEQ ID NO : 30,SEQ ID NO : 31 et SEQ ID NO : 32.

12. Système de modulation de l'expression génique comprenant :

a) une première cassette d'expression génique qui est capable d'être exprimée dans une cellule hôte, comprenant un polynucléotide qui code un premier polypeptide hybride comprenant :

i) un domaine liant l'ADN qui reconnaît un élément de réponse associé avec un gène dont l'expression doit être modulée ; et
ii) un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré non diptère non lépidoptère ; et

b) une seconde cassette d'expression génique qui est capable d'être exprimée dans la cellule hôte comprenant un polynucléotide qui code un second polypeptide hybride comprenant :

i) un domaine de transactivation ; et
ii) un domaine liant un ligand de récepteur d'ecdysone.

13. Système de modulation de l'expression génique selon la revendication 12, comprenant en outre une troisième cassette d'expression génique comprenant :

i) un élément de réponse reconnu par le domaine liant l'ADN du premier polypeptide hybride ;
ii) un promoteur qui est activé par le domaine de transactivation du second polypeptide hybride ; et
iii) un gène dont l'expression doit être modulée.

14. Système de modulation de l'expression génique selon la revendication 12 où le domaine liant un ligand de récepteur X des rétinoïdes d'invertébré du premier polypeptide hybride est codé par un polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe consistant en SEQ ID NO : 9, SEQ ID NO : 10, SEQ ID NO : 11,SEQ ID NO : 12,SEQ ID NO : 13,SEQ ID NO : 14,SEQ ID NO : 15,SEQ ID NO : 16,SEQ ID NO : 17,SEQ ID NO : 18, SEQ ID NO : 19 et SEQ ID NO : 20.

15. Système de modulation de l'expression génique selon la revendication 12 où le domaine liant un ligand de récepteur X des rétinoïdes d'invertébré du premier polypeptide hybride comprend une séquence d'aminoacides choisie dans le groupe consistant en SEQ ID NO : 21, SEQ ID NO : 22, SEQ ID NO : 23,SEQ ID NO : 24,SEQ ID NO: 25,SEQ ID NO : 26,SEQ ID NO : 27,SEQ ID NO : 28,SEQ ID NO : 29,SEQ ID NO : 30,SEQ ID NO:31 et SEQ ID NO : 32.

16. Système de modulation de l'expression génique selon la revendication 12 où le domaine liant un ligand de récepteur d'ecdysone du second polypeptide hybride est choisi dans le groupe consistant en un LBD de EcR de tordeuse des bourgeons de l'épinette *Choristoneura fumiferana* (« CfEcR »), un LBD de EcR de coléoptère *Tenebrio molitor* (« TmEcR »), un LBD de EcR de *Manduca sexta* (« MsEcR »), un LBD de EcR de *Heliothies virescens* (« HvEcR »), un LBD de EcR de chironome *Chironomus tentans* (« CtEcR »), un LBD de EcR de ver à soie *Bombyx mori* (« BmEcR »), un LBD de EcR de mouche des fruits *Drosophila melanogaster* (« DmEcR »), un LBD de EcR de moustique *Aedes aegypti* (« AaEcR »), un LBD de EcR de mouche à viande *Lucilia capitata(«* LcEcR »), un LBD de EcR de mouche à viande *Lucilia cuphina(«LucEcR»),* un LBD de EcR de mouche méditerranéenne des fruits *Ceratitis capitata(«* CcEcR »), un LBD de EcR de criquet *Locusta migratoria* (« LmEcR »), un LBD de EcR d'aphide *Myzus persicae* (« MpEcR »), un LBD de EcR de crabe violoniste *Celuca pugilator* (« CpEcR »), un LBD de EcR de tique ixode *Amblyommaamericanum(«*AmaEcR»), un LBD de EcR de mouche blanche *Bamecia argentifoli* («BaEcR») et un LBD de EcR de cicadelle *Nephotetix cincticeps.*

17. Système de modulation de l'expression génique selon la revendication 12 où le domaine liant un ligand de récepteur d'ecdysone du second polypeptide hybride est codé par un polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe consistant en SEQ ID NO : 1, SEQ ID NO : 53 et SEQ ID NO : 45.

18. Système de modulation de l'expression génique selon la revendication 12 où le domaine liant un ligand de récepteur d'ecdysone du second polypeptide hybride comprend une séquence d'aminoacides choisie dans le groupe consistant en SEQ ID NO : 5, SEQ ID NO : 43 et SEQ ID NO : 59.

19. Système de modulation de l'expression génique selon la revendication 12 où la première cassette d'expression génique comprend un polynucléotide qui code un premier polypeptide hybride comprenant un domaine liant l'ADN choisi dans le groupe consistant en un domaine liant l'ADN de GAL4 et un domaine liant l'ADN de LexA, et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré.

20. Système de modulation de l'expression génique selon la revendication 12 où la première cassette d'expression génique comprend un polynucléotide qui code un premier polypeptide hybride comprenant un domaine liant l'ADN codé par un polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe consistant en unDBD de GAL4 (SEQ ID NO : 33) et un DBD de LexA (SEQ ID NO : 35),et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré codé par un polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe consistant en SEQ ID NO : 9, SEQ ID NO : 10, SEQ ID NO : 11,SEQ ID NO: 12,SEQ ID NO : 13,SEQ ID NO : 14,SEQ ID NO : 15,SEQ ID NO : 16,SEQ ID NO : 17,SEQ ID NO : 18, SEQ ID NO : 19 et SEQ ID NO : 20.

21. Système de modulation de l'expression génique selon la revendication 12 où la première cassette d'expression génique comprend un polynucléotide qui code un premier polypeptide hybride comprenant un domaine liant l'ADN comprenant une séquence d'aminoacides choisie dans le groupe consistant en un DBD de GAL4 (SEQ ID NO : 34) et un DBD de LexA (SEQ ID NO : 36), et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré comprenant une séquence d'aminoacides choisie dans le groupe consistant en SEQ SEQ ID NO : 21, SEQ ID NO : 22, SEQ ID NO : 23,SEQ ID NO : 24,SEQ ID NO : 25,SEQ ID NO : 26,SEQ ID NO : 27,SEQ ID NO : 28,SEQ ID NO : 29,SEQ ID NO : 30,SEQ ID NO : 31 et SEQ ID NO : 32.

22. Système de modulation de l'expression génique selon la revendication 12 où la seconde cassette d'expression génique comprend un polynucléotide qui code un second polypeptide hybride comprenant un domaine de transactivation choisi dans le groupe consistant en un domaine de transactivation de VP16 et un domaine de transactivation d'activateur acide de B42, et un domaine liant un ligand de récepteur d'ecdysone.

23. Cassette d'expression génique comprenant un polynucléotide qui code un polypeptide hybride comprenant un domaine liant l'ADN et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré non diptère non lépidoptère, où le domaine liant l'ADN provient d'un récepteur nucléaire différent d'un récepteur X des rétinoïdes d'invertébré.

24. Cassette d'expression génique selon la revendication 23 où le domaine liant l'ADN est un domaine liant l'ADN de GAL4 ou un domaine liant l'ADN de LexA.

25. Cassette d'expression génique selon la revendication 23 où la cassette d'expression génique comprend un polynucléotide qui code un polypeptide hybride comprenant un domaine liant l'ADN codé par un polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe consistant en un DBD de GAL4 (SEQ ID NO: 33) et un DBD de LexA (SEQ ID NO : 35), et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré codé par un polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe consistant en SEQ ID NO : 9, SEQ ID NO : 10, SEQ ID NO: 11,SEQ ID NO : 12,SEQ ID NO : 13,SEQ ID NO : 14,SEQ ID NO : 15,SEQ ID NO : 16,SEQ ID NO : 17,SEQ ID NO: 18, SEQ ID NO : 19 et SEQ ID NO : 20.

26. Cassette d'expression génique selon la revendication 23 où la cassette d'expression génique comprend un polynucléotide qui code un polypeptide hybride comprenant un domaine liant l'ADN comprenant une séquence d'aminoacides choisie dans le groupe consistant en un DBD de GAL4 (SEQ ID NO : 34) et un DBD de LexA (SEQ ID NO : 36), et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré comprenant une séquence d'aminoacides choisie dans le groupe consistant en SEQ ID NO : 21, SEQ ID NO: 22, SEQ ID NO: 23,SEQ ID NO : 24,SEQ ID NO : 25,SEQ ID NO: 26,SEQ ID NO : 27,SEQ ID NO : 28,SEQ ID NO : 29,SEQ ID NO : 30,SEQ ID NO: 31 et SEQ ID NO:32.

27. Cassette d'expression génique comprenant un polynucléotide qui code un polypeptide hybride comprenant un domaine de transactivation et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré non diptère non lépidoptère, où le domaine de transactivation provient d'un récepteur nucléaire différent d'un récepteur X des rétinoïdes d'invertébré.

28. Cassette d'expression génique selon la revendication 27 où le domaine de transactivation est un domaine de transactivation de VP16 ou un domaine de transactivation d'activateur acide de B42.

29. Cassette d'expression génique selon la revendication 27 où la cassette d'expression génique comprend un polynucléotide qui code un polypeptide hybride comprenant un domaine de transactivation codé par un polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe consistant en unAD de VP16 (SEQ ID NO : 37) et unAD de B42 (SEQ ID NO : 39), et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré codé par un polynucléotide comprenant une séquence d'acide nucléique choisie dans le groupe consistant en SEQ ID NO : 9, SEQ ID NO : 10,SEQ ID NO : 11,SEQ ID NO : 12,SEQ ID NO : 13,SEQ ID NO : 14,SEQ ID NO : 15,SEQ ID NO : 16,SEQ ID NO : 17,SEQ ID NO:18, SEQ ID NO : 19 et SEQ ID NO : 20.

30. Cassette d'expression génique selon la revendication 29 où la cassette d'expression génique comprend un polynucléotidequi code un polypeptide hybride comprenant un domaine de transactivation comprenant une séquence d'aminoacides choisie dans le groupe consistant en un AD de VP16 (SEQ ID NO : 38) et un AD deB42 (SEQ ID NO : 40), et un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré comprenant une séquence d'aminoacides choisie dans le groupe consistant en SEQ ID NO : 21, SEQ ID NO : 22, SEQ ID NO : 23,SEQ ID NO : 24,SEQ ID NO : 25,SEQ ID NO : 26,SEQ ID NO : 27,SEQ ID NO : 28,SEQ ID NO : 29,SEQ ID NO : 30,SEQ ID NO : 31 et SEQ ID NO : 32.

31. Polynucléotide isolé choisi dans le groupe consistant en :

(a) un polynucléotide codant un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré non diptère non lépidoptère tronqué comprenant une mutation induisant une troncature, où la mutation induisant une troncature réduit l'activité liant les ligands du domaine liant un ligand de récepteur X des rétinoïdes d'invertébré tronqué ;
(b) un polynucléotide codant un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré non diptère non lépidoptère tronqué comprenant une mutation induisant une troncature, où la mutation induisant une troncature réduit l'activité liant les stéroïdes du domaine liant un ligand de récepteur X des rétinoïdes d'invertébré tronqué ;

(c) un polynucléotide codant un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré non diptère non lépidoptère tronqué comprenant une mutation induisant une troncature, où la mutation induisant une troncature réduit l'activité liant les non-stéroïdes du domaine liant un ligand de récepteur X des rétinoïdes d'invertébré tronqué;
(d) un polynucléotide codant un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré non diptère non lépidoptère tronqué comprenant une mutation induisant une troncature, où la mutation induisant une troncature augmente l'activité liant les ligands du domaine liant un ligand de récepteur X des rétinoïdes d'invertébré tronqué ;
(e) un polynucléotide codant un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré non diptère non lépidoptère tronqué comprenant une mutation induisant une troncature, où la mutation induisant une troncature augmente l'activité liant les stéroïdes du domaine liant un ligand de récepteur X des rétinoïdes d'invertébré tronqué ;
(f) un polynucléotide codant un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré non diptère non lépidoptère tronqué comprenant une mutation induisant une troncature, où la mutation induisant une troncature augmente l'activité liant les non-stéroïdes du domaine liant un ligand de récepteur X des rétinoïdes d'invertébré tronqué ;
(g) un polynucléotide codant un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré non diptère non lépidoptère tronqué comprenant une mutation induisant une troncature, où la mutation induisant une troncature augmente la sensibilité aux ligands du domaine liant un ligand de récepteur X des rétinoïdes d'invertébré tronqué ; et
(h) un polynucléotide codant un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré non diptère non lépidoptère tronqué comprenant une mutation induisant une troncature, où la mutation induisant une troncature augmente la sensibilité aux ligands d'un hétérodimére, où l'hétérodimère comprend ledit domaine liant un ligand de récepteur X des rétinoïdes d'invertébré tronqué et un partenaire de dimérisation.

32. Polynucléotide isolé selon la revendication 31 où le partenaire de dimérisation est un polypeptide de récepteur d'ecdysone.

33. Polynucléotide isolé codant un domaine liant un ligand de récepteur X des rétinoïdes d'invertébré tronqué, où le polynucléotide comprend une séquence d'acide nucléique choisie dans le groupe consistant en SEQ ID NO : 15,SEQ ID NO : 16,SEQ ID NO : 17,SEQ ID NO: 18, SEQ ID NO : 19 et SEQ ID NO:20.

34. Polypeptide isolé codé par le polynucléotide isolé selon la revendication 33.

35. Domaine liant un ligand de récepteur X des rétinoïdes d'invertébré tronqué isolé comprenant une séquence d'aminoacides choisie dans le groupe consistant en SEQ ID NO : 27,SEQ ID NO : 28,SEQ ID NO : 29,SEQ ID NO : 30, SEQ ID NO : 31 et SEQ ID NO : 32.

36. Procédé de modulation de l'expression d'un gène dans une cellule hôte comprenant les étapes de :

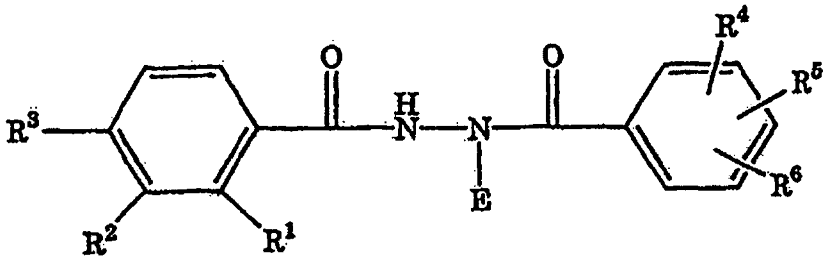
a) introduire dans la cellule hôte le système de modulation de l'expression génique selon la revendication 1 ; et
b) introduire dans la cellule hôte un ligand ;

où le gène à moduler est un composant d'une cassette d'expression génique comprenant :

i) un élément de réponse reconnu par le domaine liant l'ADN provenant du premier polypeptide hybride ;
ii) un promoteur qui est activé par le domaine de transactivation du second polypeptide hybride ; et
iii) un gène dont l'expression doit être modulée ;

où, lors de l'introduction du ligand dans la cellule hôte, l'expression du gène de b)iii) est modulée.

37. Procédé selon la revendication 36 où le ligand est un composé de formule :



où:

E est un (C_4-C_6)alkyle contenant un carbone tertiaire ou un cyano(C_3-C_5)alkyle contenant un carbone tertiaire ;
R^1 est H, Me, Et, i-Pr, F, formyle, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyle, 2-propynyle, vinyle, OH, OMe, OEt, cyclopropyle, CF_2CF_3, CH=CHCN, allyle, azido, SCN ou $SCHF_2$;
R^2 est H, Me, Et, n-Pr, i-Pr, formyle, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyle, 2-propynyle, vinyle, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe_2, NEt_2, SMe, SEt, $SOCF_3$, OCF_2CF_2H, COEt, cyclopropyle, CF_2CF_3, CH=CHCN, allyle, azido, OCF_3, $OCHF_2$, O-i-Pr, SCN, $SCHF_2$, SOMe, NH-CN, ou joint à R^3 et aux carbones de phényle auxquels R^2 et R^3 sont liés pour former un éthylènedioxy, un cycle dihydrofuryle avec l'oxygène adjacent à un carbone de phényle, ou un cycle dihydropyryle avec l'oxygène adjacent à un carbone de phényle ;
R^3 est H, Et, ou joint à R^2 et aux carbones de phényle auxquels R^2 et R^3 sont liés pour former un éthylènedioxy, un cycle dihydrofuryle avec l'oxygène adjacent à un carbone de phényle, ou un cycle dihydropyryle avec l'oxygène adjacent à un carbone de phényle ;
R^4, R^5 et R^6 sont indépendamment H, Me, Et, F, Cl, Br, formyle, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CN, C°CH, 1-propynyle, 2-propynyle, vinyle, OMe, OEt, SMe ou SEt.

38. Procédé selon la revendication 36 comprenant en outre l'introduction dans la cellule hôte d'un second ligand; où le second ligand est l'acide 9-cis-rétinoïque ou un analogue synthétique d'un acide rétinoïque.

39. Procédé de modulation de l'expression d'un gène dans une cellule hôte comprenant les étapes de :

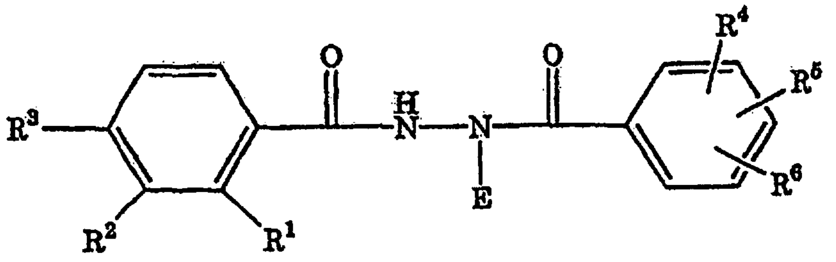
a) introduire dans la cellule hôte le système de modulation de l'expression génique selon la revendication 12 ; et
b) introduire dans la cellule hôte un ligand ;

où le gène à moduler est un composant d'une cassette d'expression génique comprenant :

i) un élément de réponse reconnu par le domaine liant l'ADN provenant du premier polypeptide hybride ;
ii) un promoteur qui est activé par le domaine de transactivation du second polypeptide hybride ; et
iii) un gène dont l'expression doit être modulée ;

où, lors de l'introduction du ligand dans la cellule hôte, l'expression du gène de b)iii) est modulée.

40. Procédé selon la revendication 39 où le ligand est un composé de formule :



où:

E est un (C_4-C_6)alkyle contenant un carbone tertiaire ou un cyano(C_3-C_5)alkyle contenant un carbone tertiaire ;

R^1 est H, Me, Et, i-Pr, F, formyle, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyle, 2-propynyle, vinyle, OH, OMe, OEt, cyclopropyle, CF_2CF_3, CH=CHCN, allyle, azido, SCN ou $SCHF_2$;
R^2 est H, Me, Et, n-Pr, i-Pr, formyle, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CH_2OMe, CH_2CN, CN, C°CH, 1-propynyle, 2-propynyle, vinyle, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe_2, NEt_2, SMe, SEt, $SOCF_3$, OCF_2CF_2H, COEt, cyclopropyle, CF_2CF_3, CH=CHCN, allyle, azido, OCF_3, $OCHF_2$, O-i-Pr, SCN, $SCHF_2$, SOMe, NH-CN, ou joint à R^3 et aux carbones de phényle auxquels R^2 et R^3 sont liés pour former un éthylènedioxy, un cycle dihydrofuryle avec l'oxygène adjacent à un carbone de phényle, ou un cycle dihydropyryle avec l'oxygène adjacent à un carbone de phényle ;
R^3 est H, Et, ou joint à R^2 et aux carbones de phényle auxquels R^2 et R^3 sont liés pour former un éthylènedioxy, un cycle dihydrofuryle avec l'oxygène adjacent à un carbone de phényle, ou un cycle dihydropyryle avec l'oxygène adjacent à un carbone de phényle ;
R^4, R^5 et R^6 sont indépendamment H, Me, Et, F, Cl, Br, formyle, CF_3, CHF_2, $CHCl_2$, CH_2F, CH_2Cl, CH_2OH, CN, C°CH, 1-propynyle, 2-propynyle, vinyle, OMe, OEt, SMe ou SEt.

41. Procédé selon la revendication 39 comprenant en outre l'introduction dans la cellule hôte d'un second ligand, où le second ligand est l'acide 9-cis-rétinoïque ou un analogue synthétique d'un acide rétinoïque.

42. Cellule hôte isolée comprenant le système de modulation de l'expression génique selon la revendication 1.

43. Cellule hôte isolée selon la revendication 42 où la cellule hôte est choisie dans le groupe consistant en une cellule bactérienne, une cellule fongique, une cellule de levure, une cellule animale et une cellule de mammifère.

44. Cellule hôte isolée selon la revendication 43 où la cellule de mammifère est une cellule murine ou une cellule humaine.

45. Cellule hôte isolée comprenant le système de modulation de l'expression génique selon la revendication 12.

46. Cellule hôte isolée selon la revendication 45 où la cellule hôte est choisie dans le groupe consistant en une cellule bactérienne, une cellule fongique, une cellule de levure, une cellule animale et une cellule de mammifère.

47. Cellule hôte isolée selon la revendication 46 où la cellule de mammifère est une cellule murine ou une cellule humaine.

48. Organisme non humain comprenant la cellule hôte selon la revendication 45.

49. Organisme non humain selon la revendication 48 où l'organisme non humain est choisi dans le groupe consistant en une bactérie, un champignon, une levure, un animal et un mammifère.

50. Organisme non humain selon la revendication 49 où le mammifère est choisi dans le groupe consistant en une souris, un rat, un lapin, un chat, un chien, un bovin, une chèvre, un porc, un cheval, un mouton, un singe et un chimpanzé.

51. Organisme non humain comprenant la cellule hôte selon la revendication 45.

52. Organisme non humain selon la revendication 51 où l'organisme non humain est choisi dans le groupe consistant en une bactérie, un champignon, une levure, un animal et un mammifère.

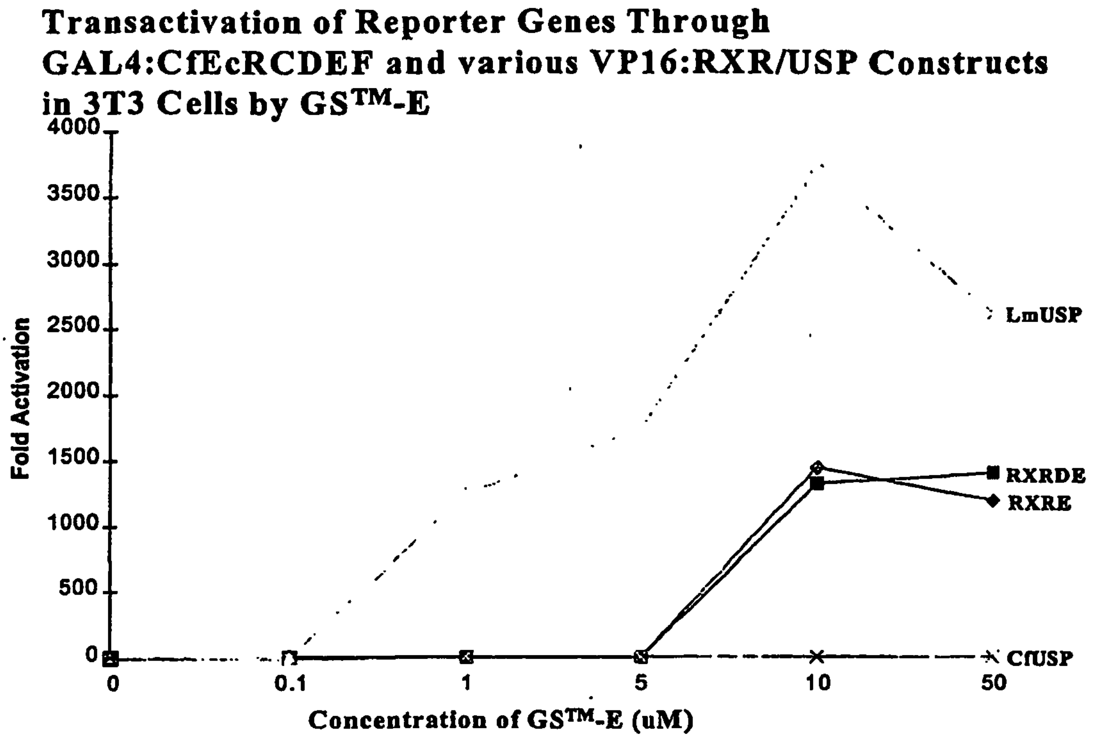
53. Organisme non humain selon la revendication 52 où le mammifère est choisi dans le groupe consistant en une souris, un rat, un lapin, un chat, un chien, un bovin, une chèvre, un porc, un cheval, un mouton, un singe et un chimpanzé.

54. Système de modulation de l'expression génique selon la revendication 1 où ledit système présente une sensibilité aux ligands accrue comparé au système équivalent dans lequel un domaine liant un ligand de récepteur X des rétinoïdes de lépidoptère, de diptère ou de vertébré est utilisé.

55. Système de modulation de l'expression génique selon la revendication 1 où ledit système présente une sensibilité aux ligands non stéroïdiens accrue comparé au système équivalent dans lequel un domaine liant un ligand de récepteur X des rétinoïdes de lépidoptère, de diptère ou de vertébré est utilisé.

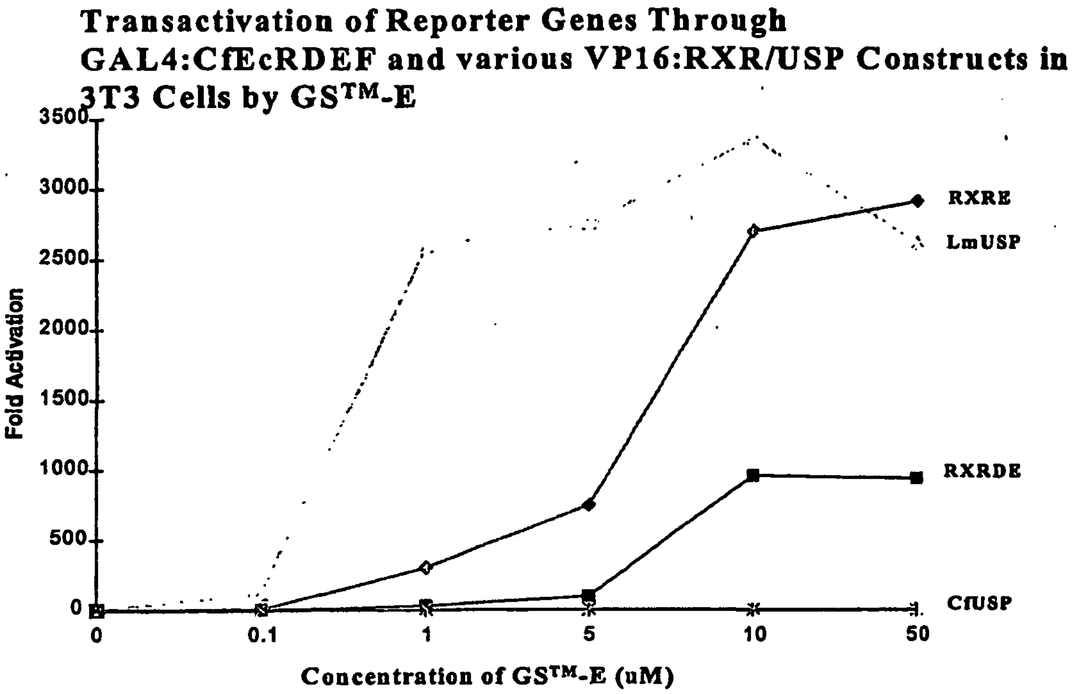
56. Système de modulation de l'expression génique selon la revendication 12 où ledit système présente une sensibilité aux ligands accrue comparé au système équivalent dans lequel un domaine liant un ligand de récepteur X des rétinoïdes de lépidoptère, de diptère ou de vertébré est utilisé.

57. Système de modulation de l'expression génique selon la revendication 12 où ledit système présente une sensibilité aux ligands non stéroidiens accrue comparé au système équivalent dans lequel un domaine liant un ligand de récepteur X des rétinoïdes de lépidoptère, de diptère ou de vertébré est utilisé.



Transactivation of Reporter Genes Through GAL4:CfEcRCDEF and various VP16:RXR/USP Constructs in 3T3 Cells by GS™-E

Figure 1



Transactivation of Reporter Genes Through GAL4:CfEcRDEF and various VP16:RXR/USP Constructs in 3T3 Cells by GS™-E

Figure 2

```
HsRXRbEF  APEEMPVDRILEAELAVEQKSDQGVEGPGGTGGSGSSPNDPVTNICQAADKQLFTLVEWA  60
MmRXRbEF  APEEMPVDRILEAELAVEQKSDQGVEGPGATGGGGSSPNDPVTNICQAADKQLFTLVEWA  60
HsRXRaEF  ANEDMPVERILEAELAVEPKTETYVEAN--MGLNPSSPNDPVTNICQAADKQLFTLVEWA  58
MmRXRaEF  ANEDMPVEKILEAELAVEPKTETYVEAN--MGLNPSSPNDPVTNICQAADKQLFTLVEWA  58
HsRXRgEF  GHEDMPVERILEAELAVEPKTESYGDMN-----MENSTNDPVTNICHAADKQLFTLVEWA  55
MmRXRgEF  SHEDMPVERILEAELAVEPKTESYGDMN-----VENSTNDPVTNICHAADKQLFTLVEWA  55
                    H1                                  H3

                                                              B6
HsRXRbEF  KRIPHFSSLPLDDQVILLRAGWNELLIASFSHRSIDVRDGILLATGLHVHRNSAHSAGVG  120
MmRXRbEF  KRIPHFSSLPLDDQVILLRAGWNELLIASFSHRSIDVRDGILLATGLHVHRNSAHSAGVG  120
HsRXRaEF  KRIPHFSELPLDDQVILLRAGWNELLIASFSHRSIAVKDGILLATGLHVHRNSAHSAGVG  118
MmRXRaEF  KRIPHFSELPLDDQVILLRAGWNELLIASFSHRSIAVKDGILLATGLHVHRNSAHSAGVG  118
HsRXRgEF  KRIPHFSDLTLEDQVILLRAGWNELLIASFSHRSVSVQDGILLATGLHVHRSSAHSAGVG  115
MmRXRgEF  KRIPHFSDLTLEDQVILLRAGWNELLIASFSHRSVSVQDGILLATGLHVHRSSAHSRGVG  115
                         H4            H5          S1       S2      H6

                             B8A1                 B9
HsRXRbEF  AIFDRVLTELVSKMRDMRMDKTELGCLRAIILFNPDAKGLSNPSEVEVLREKVYASLETY  180
MmRXRbEF  AIFDRVLTELVSKMRDMRMDKTELGCLRAIIMFNPDAKGLSNPGEVEILREKVYASLETY  180
HsRXRaEF  AIFDRVLTELVSKMRDMQMDKTELGCLRAIVLFNPDSKGLSNPAEVEALREKVYASLEAY  178
MmRXRaEF  AIFDRVLTELVSKMRDMQMDKTELGCLRAIVLFNPDSKGLSNPAEVEALREKVYASLEAY  178
HsRXRgEF  SIFDRVLTELVSKMKDMQMDKSELGCLRAIVLFNPDAKGLSNPSEVETLREKVYATLEAY  175
MmRXRgEF  SIFDRVLTELVSKMKDMQMDKSELGCLRAIVLFNPDAKGLSNPSEVETLREKVYATLEAY  175
                  H7                 H8                      H9

                    B10                   B11
HsRXRbEF  CKQKYPEQQGRFAKLLLRLPALRSIGLKCLEHLFFFKLIGDTPIDTFLMEMLEAPHQLA  239
MmRXRbEF  CKQKYPEQQGRFAKLLLRLPALRSIGLKCLEHLFFFKLIGDTPIDTFLMEMLEAPHQLA  239
HsRXRaEF  CKHKYPEQPGRFAKLLLRLPALRSIGLKCLEHLFFFKLIGDTPIDTFLMEMLEAPHQMT  237
MmRXRaEF  CKHKYPEQPGRFAKLLLRLPALRSIGLKCLEHLFFFKLIGDTPIDTFLMEMLEAPHQAT  237
HsRXRgEF  TKQKYPEQPGRFAKLLLRLPALRSIGLKCLEHLFFFKLIGDTPIDTFLMEMLETPLQIT  234
MmRXRgEF  TKQKYPEQPGRFAKLLLRLPALRSIGLKCLEHLFFFKLIGDTPIDSFLMEMLETPLQIT  234
                              H10         H11                H12     F
```

Figure 3A

```
LmRXREF   HTDMPVERILEAEKRVECKAENQ----------------------------------VEY 26
AmRXREF   HSDMPIERILEAEKRVECKMEQQ----------------------------------GNY 26
TmRXREF   -AEMPLDRIIEAEKRIECTPAGGSGG------------------------------VGEQ 29
CpRXREF   -SDMPIASIREAELSVDPIDEQPLDQGVRLQVPLAPPDSEKCSFTLPFHPVSEVSCANPL 59
AmaRXR1EF PPEMPLERILEAELRVES-QTGTLSES-----------------------------AQQ- 29
AmaRXR2EF SPDMPLERILEAEMRVEQPAPSVLAQT-----------------------------AASG 31
                     H1

LmRXREF   E----------------LVEWAKHIPHFTSLPLEDQVLLLRAGWNELLIAAFSHRSVDVK 70
AmRXREF   ENAVSHICNATNKQLFQLVAWAKHIPHFTSLPLEDQVLLLRAGWNELLIASFSHRSIDVK 86
TmRXREF   HDGVNNICQATNKQLFQLVQWAKLIPHFTSLPMSDQVLLLRAGWNELLIAAFSHRSIQAQ 89
CpRXREF   QDVVSNICQAADRHLVQLVEWAKHIPHFTDLPIEDQVVLLKAGWNELLIASFSHRSMGVE 119
AmaRXR1EF QDPVSSICQAADRQLHQLVQWAKHIPHFEELPLEDRMVLLKAGWNELLIAAFSHRSVDVR 89
AmaRXR2EF RDPVNSMCQAAP-PLHELVQWARRIPHFEELPIEDRTALLKAGWNELLIAAFSHRSVAVR 90
                          H3                       H4         H5

                          B6                              B8 A1       B9
LmRXREF   DGIVLATGLTVHRNSAHQAGVGTIFDRVLTELVAKMREMKMDKTELGCLRSVILFNPEVR 130
AmRXREF   DGIVLATGITVHRNSAQQAGVGTIFDRVLSELVSKMREMKMDHTELGCLRSIILENPEVR 146
TmRXREF   DAIVLATGLTVNKTSAHAVGVGNIYDRVLSELVNKMKEMKMDKTELGCLRAIILYNPTCR 149
CpRXREF   DGIVLATGLVIHRSSAHQAGVGAIFDRVLSELVAKMKEMKIDKTELGCLRSIVLFNPDAK 179
AmaRXR1EF DGIVLATGLVVQRHSAHGAGVGAIFDRVLTELVAKMREMKMDRTELGCLLAVVLFNPEAK 149
AmaRXR2EF DGIVLATGLVVQRHSAHGAGVGDIFDRVLAELVAKMRDMKMDKTELGCLRAVVLFNPDAK 150
             S1      S2   H6             H7                   H8

                                          B10                 B11
LmRXREF   GLKSAQEVELLREKVYAALEEYTRTTHPDEPGRFAKLLLRLPSLRSIGLKCLEHLFFFRL 190
AmRXREF   GLKSIQEVTLLREKIYGALEGYCRVAWPDDAGRFAKLLLRLPAIRSIGLKCLEYLFFFKM 206
TmRXREF   GIKSVQEVEMLREKIYGVLEEYTRTTHPNEPGRFAKLLLRLPALRSIGLKCSEHLFFFKL 209
CpRXREF   GLNCVNDVEILREKVYAALEEYTRTTYPDEPGRFAKLLLRLPALRSIGLKCLEYLFLFKL 239
AmaRXR1EF GLRTCPSGGPEGESV-SALEEHCRQQYPDQPGRFAKLLLRLPALRSIGLKCLEHLFFFKL 208
AmaRXR2EF GLRNATRVEALREKVYAALEEHCRRHHPDQPGRFGKLLLRLPALRSIGLKCLEHLFFFKL 210
                         H9                         H10         H11

LmRXREF   IGDVPIDTFLMEMLESPSDS-------     210
AmRXREF   IGDVPIDDFLVEMLESRSDP-------     226
TmRXREF   IGDVPIDTFLMEMLESPADA-------     229
CpRXREF   IGDTPLDSYLMKMLVDNPNTSVTPPTS     266
AmaRXR1EF IGDTPIDNFLLSMLEAPSDP-------     228
AmaRXR2EF IGDTPIDSFLLNMLEAPADP-------     230
                     H12   F
```

Figure 3B

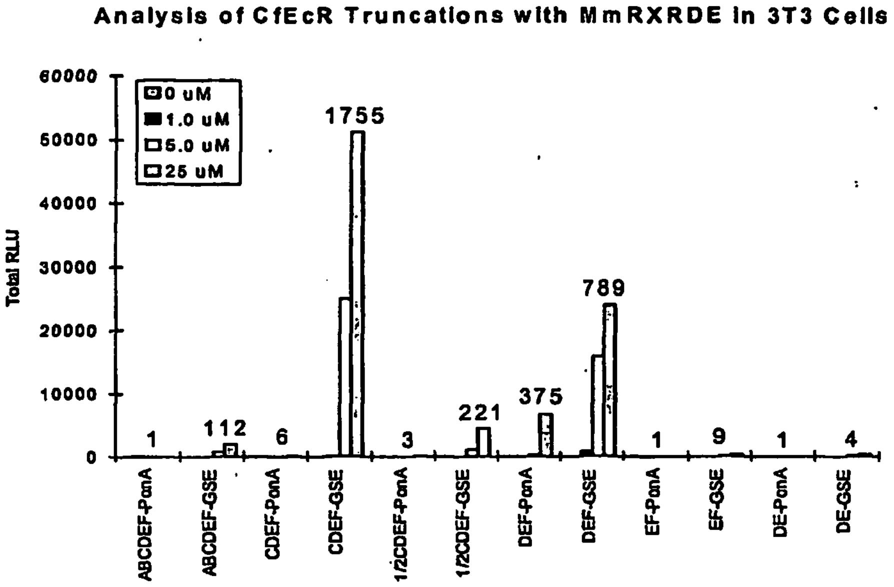


Figure 4

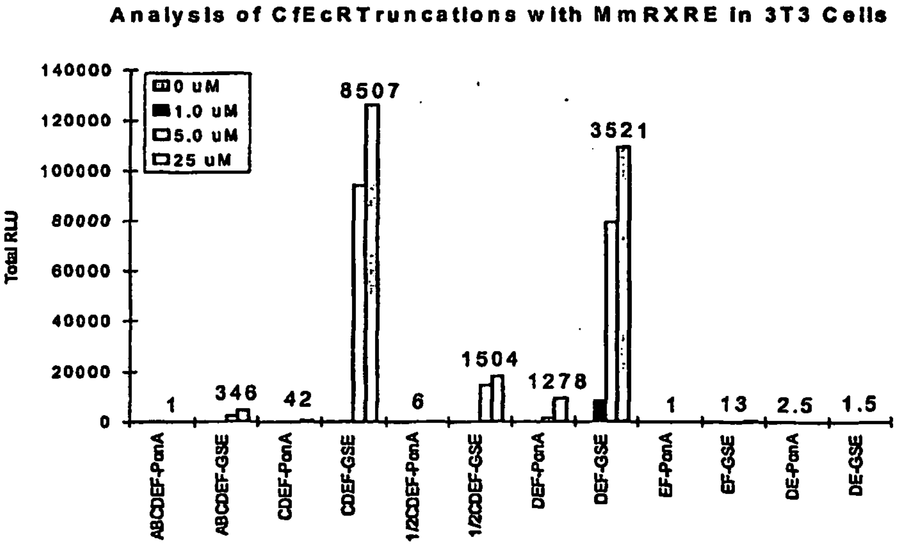


Figure 5

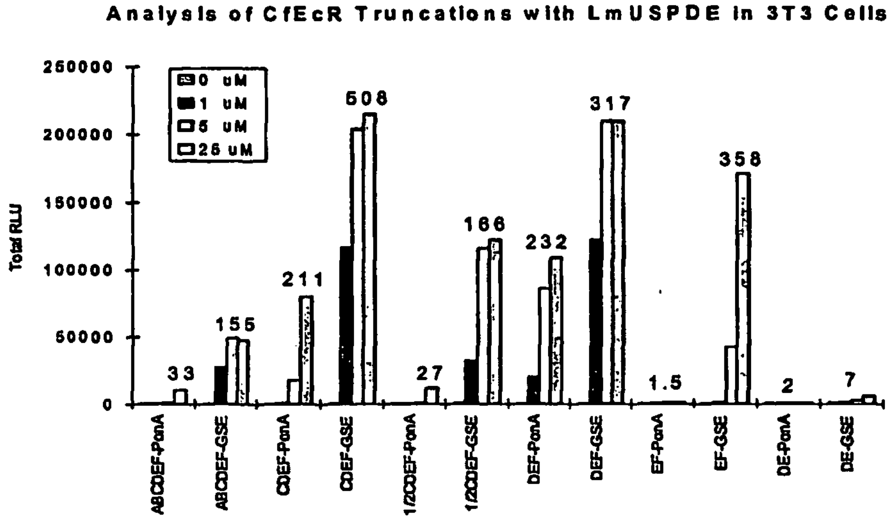


Figure 6

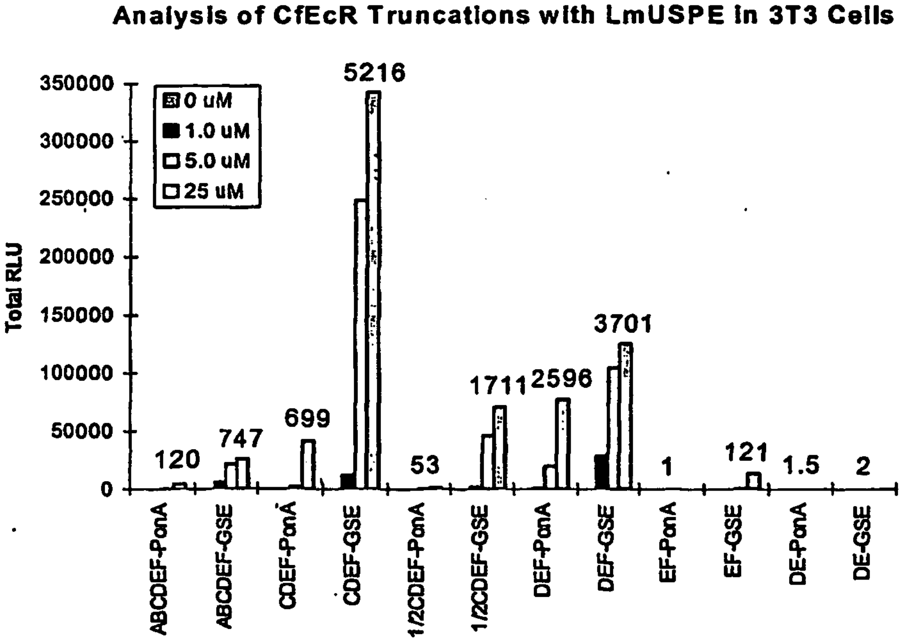


Figure 7

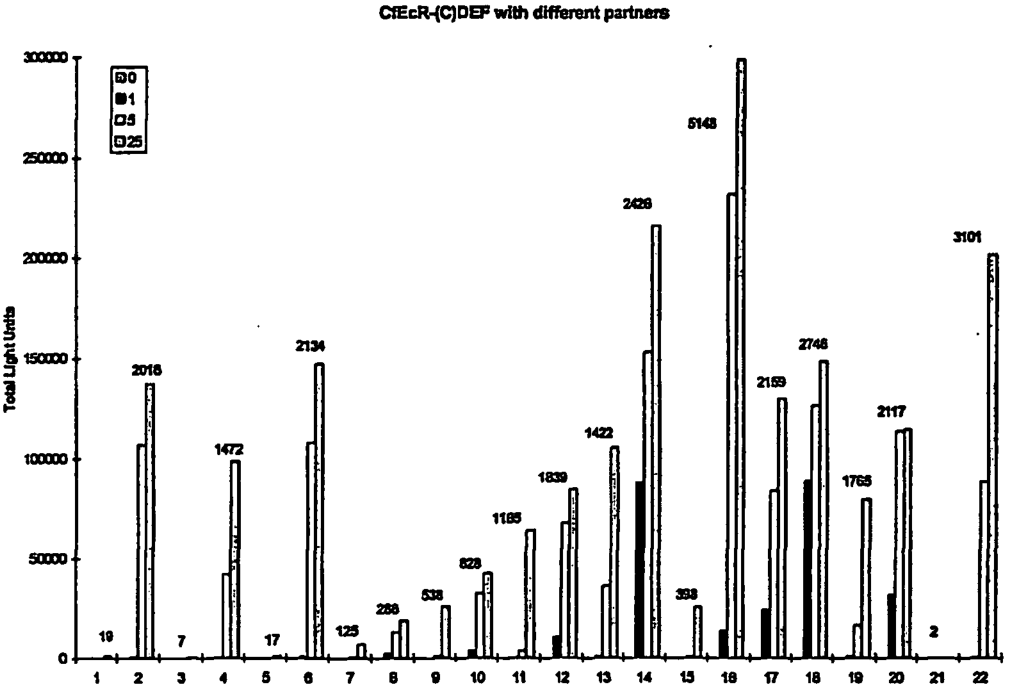

CfEcR-(C)DEF with different partners
Total Light Units
300000
250000
200000
150000
100000
50000
0
0
1
5
25
19
2016
7
1472
17
2134
125
288
538
828
1185
1839
1422
2428
393
5148
2159
2746
1765
2117
2
3101
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22

Figure 8

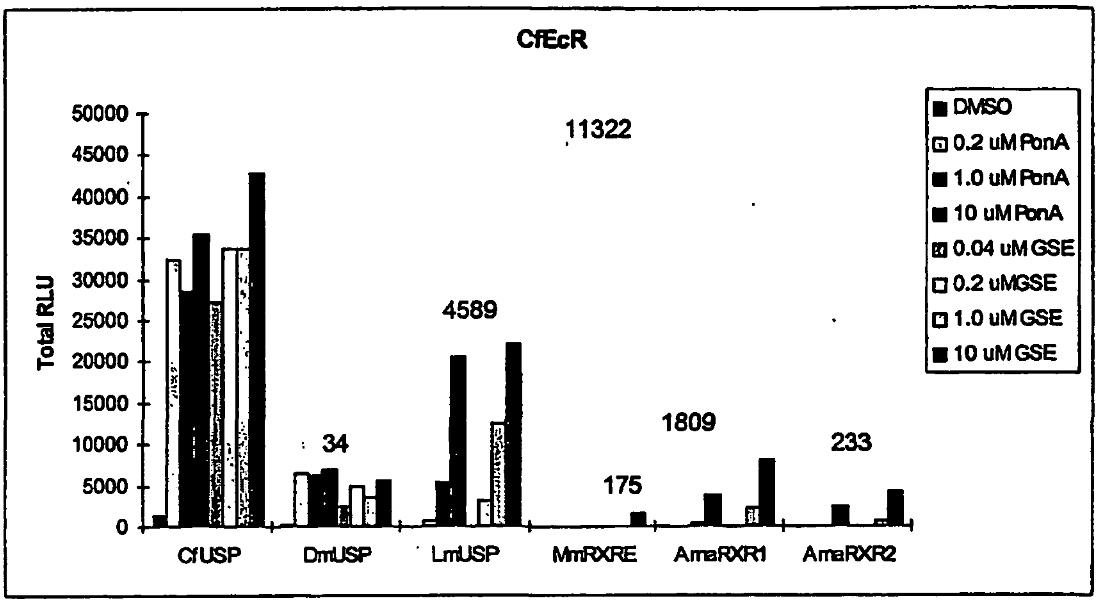

CfEcR
Total RLU
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
11322
4589
34
1809
175
233
CfUSP
DmUSP
LmUSP
MmRXRE
AmaRXR1
AmaRXR2
DMSO
0.2 uM PonA
1.0 uM PonA
10 uM PonA
0.04 uM GSE
0.2 uMGSE
1.0 uM GSE
10 uM GSE

Figure 9

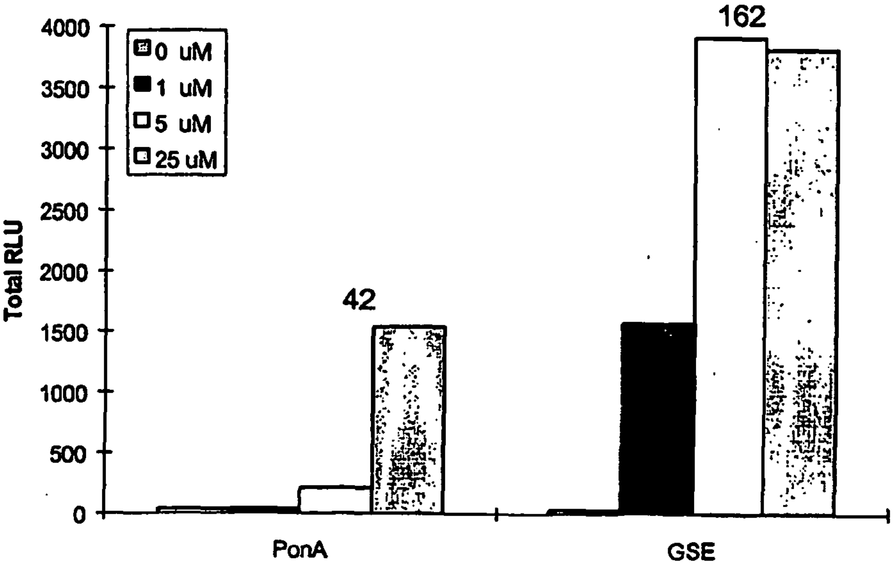

4000
3500
3000
2500
2000
1500
1000
500
0
Total RLU
0 uM
1 uM
5 uM
25 uM
42
162
PonA
GSE

Figure 10

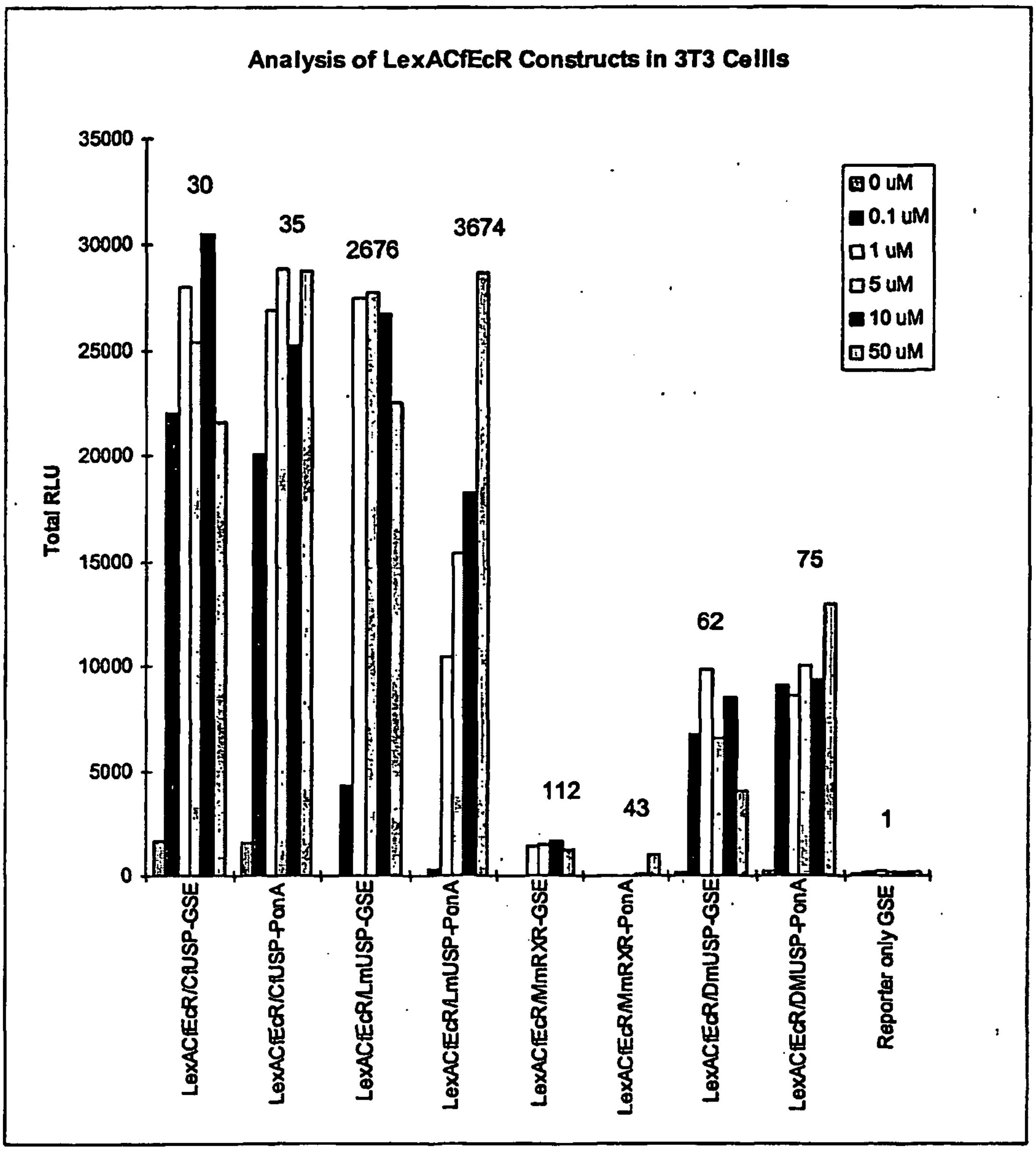

Analysis of LexACfEcR Constructs in 3T3 Cellls
Total RLU
35000
30000
25000
20000
15000
10000
5000
0
0 uM
0.1 uM
1 uM
5 uM
10 uM
50 uM
30
35
2676
3674
112
43
62
75
1
LexACfEcR/CfUSP-GSE
LexACfEcR/CfUSP-PonA
LexACfEcR/LmUSP-GSE
LexACfEcR/LmUSP-PonA
LexACfEcR/MmRXR-GSE
LexACfEcR/MmRXR-PonA
LexACfEcR/DmUSP-GSE
LexACfEcR/DMUSP-PonA
Reporter only GSE

Figure 11

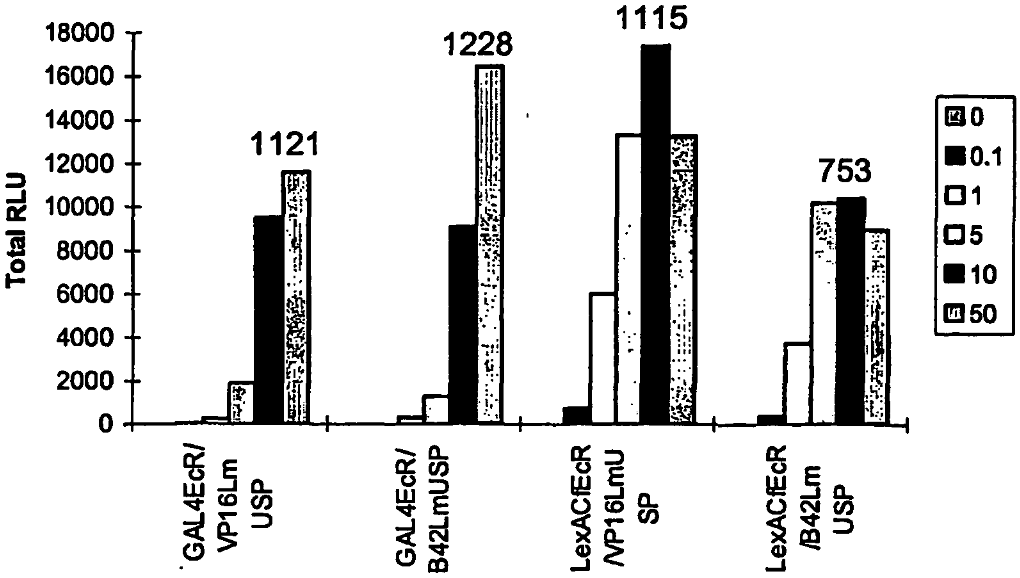


Figure 12

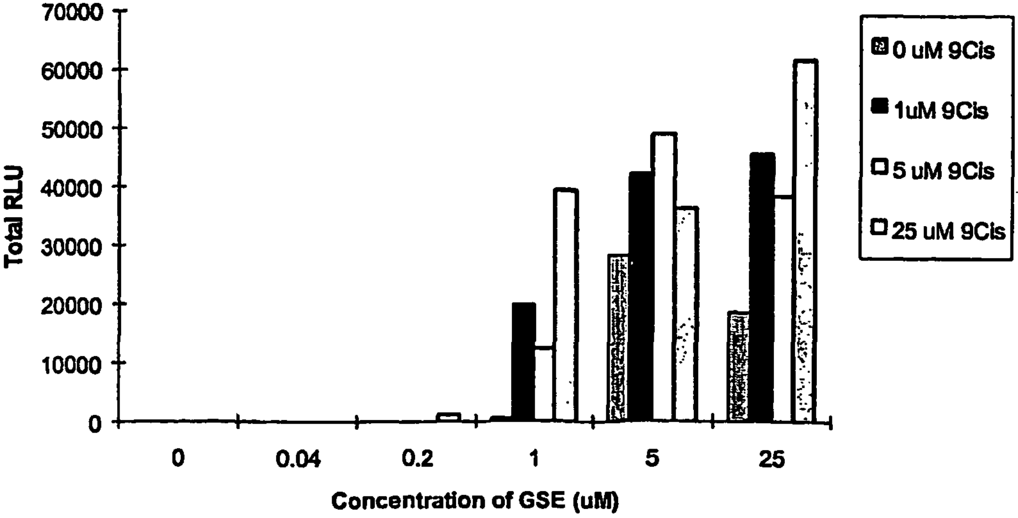


Figure 13

REFERENCES CITED IN THE DESCRIPTION

Patent documents cited in the description

- EP 9600686 PCT **[0010]**
- US 5530028 A **[0010] [0195]**
- US 9705330 W **[0011] [0013]**
- WO 9738117 A **[0011]**
- US 9908381 PCT **[0011]**
- WO 9958155 A **[0011]**
- US 9814215 W **[0011] [0015] [0121]**
- WO 9902683 A **[0011]**
- US 5880333 A **[0012] [0014] [0225]**
- US 9814215 PCT **[0013]**
- US 0109050 W **[0015] [0016] [0125] [0126] [0226]**
- US 9705330 B **[0015]**
- CA 2012311 **[0073]**
- WO 9518863 A **[0074]**
- WO 9617823 A **[0074]**
- US 5459127 A **[0074]**
- WO 9521931 A **[0075]**
- WO 9625508 A **[0075]**
- US 5693622 A **[0076]**
- US 5589466 A **[0076]**
- US 5580859 A **[0076]**
- US 0109050 A **[0121]**
- US 9705330 PCT **[0121]**
- US 5283173 A **[0121]**
- US 4981784 A **[0123]**
- US 6013836 A **[0195]**
- US 5117057 A **[0195]**
- US 5378726 A **[0195]**
- EP 461809 A **[0195]**
- US 5225443 A **[0195]**
- EP 234994 A **[0195]**
- US 4985461 A **[0195]**
- US 60294814 B **[0251]**

Non-patent literature cited in the description

- **WUM et al.** *Proc. Natl. Acad. Sci. USA,* 1986, vol. 83, 5414-5418 **[0006]**
- **ARNHEITER et al.** *Cell,* 1990, vol. 62, 51-61 **[0006]**
- **FILMUS et al.** *Nucleic Acids Research,* 1992, vol. 20, 27550-27560 **[0006]**
- **SPENCER et al.** *Science,* 1993, vol. 262, 1019-24 **[0008]**
- **BELSHAW et al.** *Proc Natl Acad Sci USA,* 1996, vol. 93, 4604-7 **[0008]**
- **DHADIALLA et al.** *Annu. Rev. Entomol.,* 1998, vol. 43, 545-569 **[0010]**
- **KOELLE et al.** *Cell,* 1991, vol. 67, 59-77 **[0010]**
- **SAMBROOK, J. ; FRITSCH, E. F. ; MANIATIS, T.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 **[0048] [0220]**
- **WU et al.** *J. Biol. Chem.,* 1992, vol. 267, 963-967 **[0073]**
- **WU ; WU.** *J. Biol. Chem.,* 1988, vol. 263, 14621-14624 **[0073]**
- **FELGNER et al.** *PNAS,* 1987, vol. 84, 7413 **[0074]**
- **MACKEY et al.** *Proc. Natl. Acad. Sci. U.S.A,* 1988, vol. 85, 8027-8031 **[0074]**
- **ULMER et al.** *Science,* 1993, vol. 259, 1745-1748 **[0074]**
- **FELGNER ; RINGOLD.** *Science,* 1989, vol. 337, 387-388 **[0074]**
- **CURIEL et al.** *Hum. Gene Ther.,* 1992, vol. 3, 147-154 **[0076]**
- **WU ; WU.** *J. Biol. Chem,* 1987, vol. 262, 4429-4432 **[0076]**
- **CHERBAS L.** *Genes Dev.,* 1991, vol. 5, 120-131 **[0087]**
- **D'AVINO PP.** *Mol. Cell. Endocrinol,* 1995, vol. 113, 1-9 **[0087]**
- **ANTONIEWSKI C.** *Mol. Cell Biol.,* 1994, vol. 14, 4465-4474 **[0087]**
- **REECK et al.** *Cell,* 1987, vol. 50, 667 **[0110] [0111]**
- **ALTSCHUL, S. F. et al.** Basic Local Alignment Search Tool. *J. Mol. Biol.,* 1993, vol. 215, 403-410 **[0117]**
- Computational Molecular Biology. Oxford University Press, 1988 **[0118]**
- Biocomputing: Informatics and Genome Projects. Academic Press, 1993 **[0118]**
- Computer Analysis of Sequence Data, Part I. Humana Press, 1994 **[0118]**
- Sequence Analysis in Molecular Biology. Academic Press, 1987 **[0118]**
- Sequence Analysis Primer. Stockton Press, 1991 **[0118]**
- **HIGGINS ; SHARP.** *CABIOS,* 1989, vol. 5, 151-153 **[0118]**
- **ALTSCHUL et al.** *J. Mol. Biol.,* 1990, vol. 215, 403-410 **[0119]**
- **YAO et al.** *Nature,* 1993, vol. 366, 476-479 **[0122]**
- **YAO et al.** *Cell,* 1992, vol. 71, 63-72 **[0122]**

- **GLASS et al.** *Curr. Opin. Cell Biol.,* 1997, vol. 9, 222-232 **[0122]**
- **HORWITZ et al.** *Mol Endocrinol.,* 1996, vol. 10, 1167-1177 **[0122]**
- **EVANS.** *Science,* 1988, vol. 240, 889-895 **[0123]**
- **SADOWSKI et al.** *Nature,* 1988, vol. 335, 563-564 **[0132]**
- **BRENT ; PTASHNE.** *Cell,* 1985, vol. 43, 729-736 **[0132]**
- **KIM et al.** *Proc. Natl. Acad Sci., USA,* 1997, vol. 94, 3616-3620 **[0132]**
- **T. J. SILHAVY ; M. L. BENNAN ; L. W. ENQUIST.** Experiments with Gene Fusions. Cold Spring Harbor Laboratory, 1984 **[0220]**
- **AUSUBEL, F. M. et al.** Current Protocols in Molecular Biology. Greene Publishing Assoc. and Wiley-Interscience, 1987 **[0220]**
- Manual of Methods for General Bacteriology. American Society for Microbiology, 1994 **[0221]**
- **THOMAS D. BROCK.** Biotechnology: A Textbook of Industrial Microbiology. Sinauer Associates, Inc, 1989 **[0221]**
- **GYURIS et al.** *Cell,* 1993, vol. 75, 791-803 **[0245]**