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Proprietor:	Intrexon Corporation
Our Ref.:	INX15393OP
Title:	CHIMERIC RETINOID X RECEPTORS AND THEIR USE IN A NOVEL ECDYSONE RECEPTOR-BASED INDUCIBLE GENE EXPRESSION SYSTEM
Date:	April 8, 2020

New Main Request (marked)

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 sequence 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) a chimeric retinoid X receptor ligand binding domain comprising either
   (A) helices 1-7 of a vertebrate retinoid X receptor and helices 8-12 of an invertebrate RXR, or
   (B) helices 1-8 of a vertebrate retinoid X receptor and helices 9-12 of an invertebrate RXR,
   wherein the invertebrate is a non-dipteran/non-lepidopteran species.

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 either a spruce budworm *Choristoneura fumiferana* EcR ("CfEcR") LBD or a fruit fly *Drosophila*

melanogaster EcR ("DmEcR") 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 polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 65 (CfEcR-DEF), SEQ ID NO: 59 (CfEcR-CDEF) and SEQ ID NO: 67 (DmEcR-DEF).

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: 57 (CfEcR-DEF), SEQ ID NO: 58 (DmEcR-DEF) and SEQ ID NO: 70 (CfEcR-CDEF).

6. The gene expression modulation system according to claim 1, wherein the chimeric 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
   a) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, and
   b) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21.

7. The gene expression modulation system according to claim 1, wherein the chimeric retinoid X receptor ligand binding domain of the second hybrid polypeptide comprises an amino acid sequence selected from the group consisting of
   a) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, and
   b) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21.

8. The gene expression modulation system according to claim 1, wherein the first gene expression cassette comprises a polynucleotide sequence that encodes the 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 the 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 a chimeric 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 the 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: 51) and a B42 AD (SEQ ID NO: 53), and a chimeric retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of
    a) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, and
    b) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21.

11. The gene expression modulation system according to claim 1, wherein the second gene expression cassette comprises a polynucleotide that encodes the second hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 52) and a B42 AD (SEQ ID NO: 54) , and a chimeric retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of
    a) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, and
    b) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21.

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 sequence 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 chimeric retinoid X receptor ligand binding domain comprising either (A) helices 1-7 of a vertebrate retinoid X receptor and helices 8-12 of an invertebrate RXR, or (B) helices 1-8 of a vertebrate retinoid X receptor and helices 9-12 of an invertebrate RXR, wherein the invertebrate is a non-dipteran/non-lepidopteran species; 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.

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

i) a response element that recognizes 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 chimeric 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

a) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, and

b) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21.

15. The gene expression modulation system according to claim 12, wherein the chimeric retinoid X receptor ligand binding domain of the first hybrid polypeptide comprises an amino acid sequence selected from the group consisting of

a) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, and

b) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21.

16. 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: 65 (CfEcR-DEF), SEQ ID NO: 59 (CfEcR-CDEF) and SEQ ID NO: 67 (DmEcR-DEF).

17. 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: 57 (CfEcR-DEF), SEQ ID NO: 58 (DmEcR-DEF) and SEQ ID NO: 70 (CfEcR-CDEF).

18. The gene expression modulation system according to claim 12, wherein the first gene expression cassette comprises a polynucleotide that encodes the 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 a chimeric retinoid X receptor ligand binding domain.

19. The gene expression modulation system according to claim 12, wherein the first gene expression cassette comprises a polynucleotide that encodes the 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: 47) and a LexA DBD (SEQ ID NO: 49), and a chimeric retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of
a) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, and
b) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21.

20. The gene expression modulation system according to claim 12, wherein the first gene expression cassette comprises a polynucleotide that encodes the 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: 48) and a LexA DBD (SEQ ID NO:

50), and a chimeric retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of

a) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, and

b) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21.

21. The gene expression modulation system according to claim 12, wherein the second gene expression cassette comprises a polynucleotide that encodes the second hybrid polypeptide comprising a transactivation domain selected from the group consisting of a VP 16 transactivation domain and a B42 acidic activator transactivation domain, and an ecdysone receptor ligand binding domain.

22. A gene expression cassette comprising a polynucleotide encoding a hybrid polypeptide comprising

a) a DNA-binding domain and

b) a chimeric retinoid X receptor ligand binding domain comprising either

(i) helices 1-7 of a vertebrate retinoid X receptor and helices 8-12 of an invertebrate RXR, or

(ii) helices 1-8 of a vertebrate retinoid X receptor and helices 9-12 of an invertebrate RXR,

wherein the invertebrate is a non-dipteran/non-lepidopteran species.

23. The gene expression cassette according to claim 22, wherein the DNA-binding domain is a GAL4 DNA-binding domain or a LexA DNA-binding domain.

24. The gene expression cassette according to claim 22, wherein the gene expression cassette comprises a polynucleotide encoding 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: 47) and a LexA DBD (SEQ ID NO: 49), and a chimeric retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of

a) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, and

b) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21.

25. The gene expression cassette according to claim 22, wherein the gene expression cassette comprises a polynucleotide encoding 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: 48) and a LexA DBD (SEQ ID NO: 50), and a chimeric retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of

a) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, and

b) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21.

26. A gene expression cassette comprising

a) a polynucleotide encoding a hybrid polypeptide comprising a transactivation domain and

b) a chimeric retinoid X receptor ligand binding domain comprising either

(i) helices 1-7 of a vertebrate retinoid X receptor and helices 8-12 of an invertebrate RXR, or

(ii) helices 1-8 of a vertebrate retinoid X receptor and helices 9-12 of an invertebrate RXR,

wherein the invertebrate is a non-dipteran/non-lepidopteran species.

27. The gene expression cassette according to claim 26, wherein the transactivation domain is a VP16 transactivation domain or a B42 acidic activator transactivation domain.

28. The gene expression cassette according to claim 26, wherein the gene expression cassette comprises a polynucleotide encoding 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: 51) and a B42 AD (SEQ ID NO: 53), and a chimeric retinoid X receptor ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of

a) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, and

b) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21.

29. The gene expression cassette according to claim 28, wherein the gene expression cassette comprises a polynucleotide encoding a hybrid polypeptide comprising a transactivation domain comprising an amino acid sequence selected from the group consisting of a VP16 AD (SEQ ID NO: 52) and a B42 AD (SEQ ID NO: 54), and a chimeric retinoid X receptor ligand binding domain comprising an amino acid sequence selected from the group consisting of

a) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, and

b) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21.

30. An isolated polynucleotide encoding a chimeric retinoid X receptor ligand binding domain, wherein the polynucleotide comprises a nucleic acid sequence selected from the group consisting of

a) nucleotides 1-408 of SEQ ID NO: 13 and nucleotides 337-630 of SEQ ID NO: 21, and

b) nucleotides 1-465 of SEQ ID NO: 13 and nucleotides 403-630 of SEQ ID NO: 21.

31. An isolated polypeptide encoded by the isolated polynucleotide according to claim 30.

32. An isolated chimeric retinoid X receptor polypeptide comprising an amino acid sequence selected from the group consisting of

a) amino acids 1-136 of SEQ ID NO: 13 and amino acids 113-210 of SEQ ID NO: 21, and

b) amino acids 1-155 of SEQ ID NO: 13 and amino acids 135-210 of SEQ ID NO: 21.

33. An *in vitro* method of modulating the expression of a gene in a host cell comprising the gene to be modulated 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 be modulated is a component of a gene expression cassette comprising:

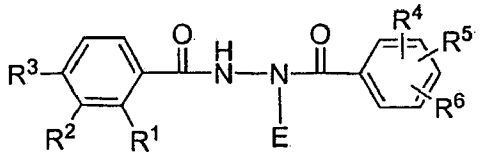
i) a response element recognized by the DNA binding domain from the first hybrid polypeptide;

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.

34. The method according to claim 33, 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, On-Pr, OAc, NMe_2, NEt_2, SMe, SEt, $SOCF_3$ OCF_2CF2H, 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 R6are independently H, Me, Et, F, Cl, Br, formyl, CF_3, CHF2 $CHCl_2$ CH_2F, CH_2Cl, CH_2O, CN, C≡CH, CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

35. The method according to claim 33, 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.

36. An *in vitro* method of modulating the expression of a gene in a host cell comprising the gene to be modulated comprising the steps of:

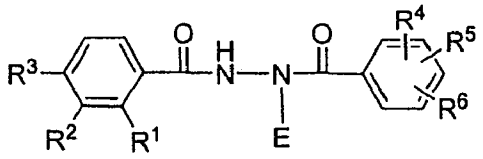
a) introducing into the host cell the gene expression modulation system of claim 12; and b) introducing into the host cell a ligand; wherein the gene to be modulated is a component of a gene expression cassette comprising:

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.

37. The method according to claim 36, wherein the ligand is a compound of the formula:



wherein:

E is a (C4-C6) alkyl containing a tertiary carbon or a cyano (C3-Cs) 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, On-Pr, OAc, NMe_2, NEt_2, SMe, SEt, $SOCF_3$, OCF_2CF2H, COEt, cyclopropyl, CF_2CF3, 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 adjacentto a phenyl carbon;
R^3 is H, Et, or joined with R^2 and the phenyl carbons to which R^2 and R3 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. An isolated host cell comprising the gene expression modulation system according to claim 1.

40. The isolated host cell according to claim 39, 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.

41. The isolated host cell according to claim 40, wherein the mammalian cell is a murine cell or a human cell.

42. An isolated host cell comprising the gene expression modulation system according to claim 12.

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. A non-human organism comprising the host cell of claim 39.~~

~~46. The non-human organism according to claim 45, wherein the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, an animal, and a mammal.~~

~~47. The non-human organism according to claim 46, 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.~~

~~48. A non-human organism comprising the host cell of claim 42.~~

~~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.~~45. The gene expression modulation system of claim 1 or 12, wherein the gene expression modulation system exhibits increased sensitivity to a non-steroid ligand than a gene expression modulation system that contains a vertebrate retinoid X receptor ligand binding domain.

~~52.~~46. The gene expression cassette of claim 22 or 26, wherein the polypeptide exhibits increased sensitivity to a non-steroid ligand than a polypeptide that contains a vertebrate retinoid X receptor ligand binding domain.

~~53.~~47. The gene expression modulation system of any one of claims 1, 12, or ~~51~~45 wherein the system exhibits increased magnitude of gene induction, compared to a gene expression modulation system that contains a vertebrate retinoid X receptor ligand binding domain.

~~54.~~48. The gene expression cassettes of any one of claims 22, 26 or ~~52~~46, wherein the polypeptide exhibits increased magnitude of gene induction, compared to a polypeptide that contains a vertebrate retinoid X receptor ligand binding domain.

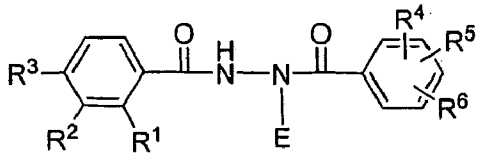
~~55.~~49. Use of a ligand in the manufacture of a medicament for modulating the expression of a gene in a host cell comprising the gene to be modulated; wherein the host cell comprises the gene expression modulation system according to claim 1, and wherein the gene to be modulated is a component of 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.

~~56.~~50. Use according to claim ~~55~~49, 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, CH2CN, 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, On-Pr, OAc, NMe_2, NEt_2, SMe, SEt, $SOCF_3$ OCF_2CF2H, 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_2O, CN, C≡CH, CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt.

~~57.~~51. Use according to claim ~~55~~49, wherein said medicament further comprises a second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of a retinoic acid.

~~58.~~52. Use of a ligand in the manufacture of a medicament for modulating the expression of a gene in a host cell comprising the gene to be modulated; wherein the

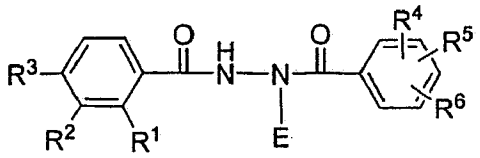
host cell comprises the gene expression modulation system of claim 12; and wherein the gene to be modulated is a component of a gene expression cassette comprising:

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.

~~59~~53. Use according to claim ~~58~~52, wherein the ligand is a compound of the formula:



wherein:

E is a (C4-C6) alkyl containing a tertiary carbon or a cyano (C3-Cs) 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, On-Pr, OAc, NMe_2, NEt_2, SMe, SEt, $SOCF_3$, OCF_2CF2H, 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 R3 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.

~~60.~~54. Use according to claim ~~58~~52, wherein said medicament further comprises a second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of a retinoic acid.