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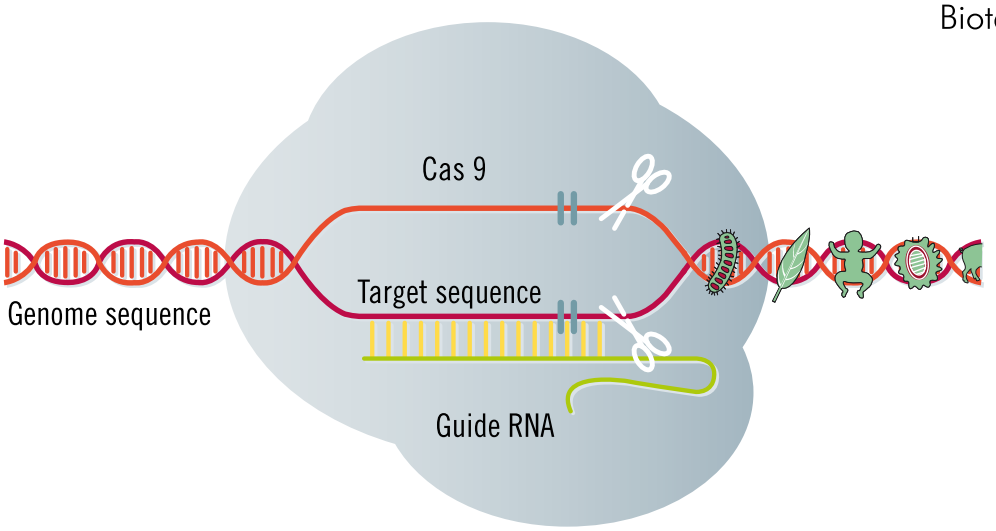

Synthetic gene technologies applied in plants and animals used for food production

Overview on patent applications on new techniques for genetic engineering and risks associated with these methods

Christoph Then 2016

a Testbiotech Report

with support from grassroots foundation

grassroots foundation

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Summary

In the past few years, new methods of genetic engineering have been developed that can also be used in plants and animals for food production. These techniques can be collectively called *gene-editing or synthetic gene technologies*.

This report provides an overview of patents already filed, and of companies active in this field. Further, it gives examples of such plants and animals and the risks associated with their release into the environment or their imminent introduction to the markets. Finally, it discusses some legal and technical aspects based on this overview.

Most of the relevant technological tools are tailored nucleases that allow DNA to be cut at a targeted location and synthetic nucleotides (DNA and RNA) that can be produced in the laboratory (with and without a natural template) and then introduced into the cells and genome of plants and animals. These technologies can be used to:

- insert transgenes
- recombine DNA without inserting DNA from other species (originating from same species or without a natural template)
- replace native DNA by re-synthesised DNA
- remove or silence DNA
- change gene regulation without changing the DNA by manipulating epigenetic mechanisms.

Such technologies might not always give rise to a transgenic organism (because no DNA gets transferred across borders of species), but must, nevertheless, be regarded as methods that result in a genetically engineered organism. To summarise, all these techniques directly interfere with the plant and animal characteristics on the level of the genome by inserting material that was produced outside the cells. Thus, EU regulation (Directive 2001/18) requires these techniques to be regulated. This is made evident in detailed legal analysis.

Regardless, industry and other interested stakeholders are actively lobbying to exempt these techniques from EU regulation (Directive 2001/18) currently applied to genetically engineered organisms. They want these organisms be treated in the same way as plants and animals derived from conventional breeding. The proponents argue that:

- current regulation could become an obstacle for the introduction of the products to the market and therefore negatively impact smaller and medium sized companies.[1]
- the products should be regarded as safe if only minor changes are introduced into plants and animals not giving rise to transgenic plants or animals.

The result could be that products are introduced to the market without previous risk assessment or labelling.

As shown in this overview, current developments in the application of gene editing in plants and animals used for food production are largely driven by patents. Future developments will be massively influenced by the interests of the so-called seed giants, and the introduction of such plants and animals into agriculture will foster market concentration in plant and animal production.

1 www.europarl.europa.eu/committees/en/agri/events.html

There is no doubt that the bigger companies are the ones that will dominate the markets as well as the strategies for research, innovation and product development. This will in turn impact traditional breeders, farmers, food producers and also consumers. In comparison, other factors such as differences in the requirements for market authorisation and labelling cannot be assumed to have a similar major impact on the structure of the seeds market.

In regard to risk regulation, it is shown that gene-editing can lead to wide-ranging off-target effects. These unintended effects might in some cases be the cause of risks and hazards. Therefore, there has to be case-specific risk assessment. In this context, it is not decisive whether new DNA is inserted or parts of the original DNA are removed or the activity of the natural genes is changed by epigenetic effects without changing the structure of the genome.

To assess the actual risks it is necessary to know which techniques were applied for which purposes. The relevant data have to be collected systematically and assessed independently. If these techniques are exempted from regulation, the relevant data will be kept as confidential business information. In this case, neither independent scientists nor authorities will be able to access the data in a way that will enable them to obtain a sufficient overview of the specific techniques, the relevant traits and the associated risks.

Further, with regard to regulatory aspects, it also has to be taken into account that the single steps meant to induce small changes can be applied several times in same organism. This can lead to much more extensive changes in the genome. Moreover, plants and animals showing single or several genetic changes can be crossed with each other. In addition, all the different techniques could possibly be used in combination. All these plants and animals might completely escape the scrutiny of the regulatory bodies if Directive 2011/18 is not applied in a coherent manner.

Therefore, we recommend:

- exemption from patentability of plants and animals used in agriculture and food production
- that plants and animals derived from gene-editing be made subject to EU regulations in Directive 2001/18
- agreement on an international framework for a general ban on genetically engineered organisms that cannot be controlled in their spatio-temporal dimension
- strengthening the precautionary principle.

Zusammenfassung: Synthetische Gentechnik und ihr Einsatz an Pflanzen und Tieren in der Landwirtschaft

In den letzten Jahren wurden neue Gentechnik-Verfahren entwickelt, die auch an Pflanzen und Tieren eingesetzt werden können, die in der Landwirtschaft eingesetzt werden. Diese Verfahren können unter dem Begriff Gene Editing oder Synthetische Gentechnik zusammengefasst werden.

Dieser Bericht gibt einen Überblick über Patente, die in diesem Bereich angemeldet werden und dahinter stehende Firmen. Zudem werden Beispiele für gentechnisch veränderte Pflanzen und Tiere und deren Risiken dargestellt, die in naher Zukunft freigesetzt und vermarktet werden sollen. Schließlich werden rechtliche Fragen und Anforderungen an die Risikobewertung diskutiert.

Eine zentrale Bedeutung haben in diesem Zusammenhang maßgeschneiderte Nukleasen (DNA-Scheren), die es erlauben sollen, die DNA an einer bestimmten Stelle zu verändern. Auch die Möglichkeit, DNA (und RNA) mit und ohne natürliche Vorlagen synthetisch herzustellen, ist für diese Anwendungen entscheidend. Diese Technologien können eingesetzt werden, um

- Transgene in das Erbgut einzuführen,
- Erbgut neu zu kombinieren, ohne dabei Erbgut von einer Spezies auf eine andere zu übertragen (Verwendung von DNA derselben Art oder von Erbgut, das künstlich ohne natürliche Entsprechung synthetisiert wurde),
- natürliche DNA vollständig oder teilweise durch synthetische DNA zu ersetzen,
- Gene zu entfernen oder stillzulegen,
- über epigenetische Mechanismen die Genregulierung zu verändern, ohne die Struktur der DNA zu verändern.

Diese Technologien führen nicht immer dazu, dass transgene Organismen entstehen (welche das Erbgut fremder Arten in sich tragen), sie sind aber immer als gentechnische Verfahren anzusehen: Die Methoden basieren auf einem direkten Eingriff auf der Ebene des Genoms unter Verwendung von Material, das außerhalb der Zellen zubereitet wurde. Daher müssen diese Techniken in der EU auch als Gentechnik reguliert werden. Dies zeigen mehrere rechtliche Gutachten im Detail.

Trotzdem fordern Industrie und andere Protagonisten, dass die neuen Verfahren von der EU-Regulierung für gentechnisch veränderte Organismen ausgenommen werden. Sie verlangen, dass diese neuen Organismen wie Pflanzen und Tiere aus konventioneller Züchtung behandelt werden. Diese Akteure behaupten, dass

- die bestehende Gentechnik-Regulierung zum Problem bei der Markteinführung entsprechender Produkte werden könnte, von dem vor allem kleinere und mittlere Unternehmen betroffen wären[2],
- die Organismen als sicher angesehen werden sollten, weil nur kleinere Veränderungen im Erbgut vorgenommen würden und keine Gene jenseits der Artgrenzen übertragen würden.

Im Ergebnis würden entsprechende Pflanzen und Tiere ohne Risikobewertung und Kennzeichnung auf den Markt gebracht.

2 www.europarl.europa.eu/committees/en/agri/events.html

Diesen Behauptungen der Industrie muss widersprochen werden: Wie dieser Bericht zeigt, wird die Entwicklung derzeit maßgeblich von Patentanmeldungen beeinflusst. Die Konzerne, die schon jetzt als „Seed Giants" gelten, werden auch die weitere Entwicklung ganz wesentlich beeinflussen. Durch die Einführung von entsprechenden Pflanzen und Tieren würde die Marktkonzentration im Bereich der Pflanzen – und Tierzucht noch erheblich verschärft.

Es kann daher keinen Zweifel daran geben, dass die großen Konzerne die Märkte, Forschungsstrategien und die Produktentwicklung bei den neuen Gentechnik-Verfahren dominieren werden. Diese Entwicklung wird traditionelle Züchter, Landwirte, Lebensmittelhersteller und auch die Verbraucher betreffen. Im Vergleich dazu haben Anforderungen an die Marktzulassung und Kennzeichnung weit weniger Einfluss auf die Struktur der Saatgutmärkte.

In Bezug auf die Risikobewertung wurde bereits mehrfach gezeigt, dass auch die angeblich gezielteren Methoden des Gene Editing zu einer großen Anzahl von Nebenwirkungen führen. Diese können mit erheblichen Risiken für Mensch und Umwelt einhergehen. Deswegen müssen die jeweiligen Organismen in jedem Fall auf Risiken geprüft werden. Der Bericht zeigt, dass es dabei nicht entscheidend ist, ob neue Gene eingeführt oder herausgeschnitten, oder die Aktivität von Genen über die Mechanismen der Epigenetik verändert werden.

Um diese Risiken zu bewerten, muss bekannt sein, welche Techniken mit welchen Absichten eingesetzt wurden. Die entsprechenden Daten müssen systematisch gesammelt und unabhängig bewertet werden. Wenn diese Techniken von der Regulierung ausgenommen werden, können relevante Informationen beispielsweise als Geschäftsgeheimnis behandelt werden. In diesem Fall würden weder die Behörden noch unabhängige Wissenschaftler Zugang zu den nötigen Daten bekommen. Es wäre so unmöglich, einen ausreichenden Überblick über die spezifischen Technologien, die relevanten Anwendungen und die damit verbundenen Risiken zu bekommen.

Zudem muss berücksichtigt werden, dass einzelne Schritte, durch die möglicherweise nur kleine Veränderungen im Erbgut ausgelöst werden, mehrere Male nacheinander am selben Organismus durchgeführt werden können. Dabei können multiple und weitreichende Veränderungen am Erbgut herbeigeführt werden. Ähnlich ist das Ergebnis, wenn man Pflanzen und Tiere mit einzelnen Veränderungen miteinander kreuzt. Manche Firmen kombinieren auch verschiedene Verfahren. All diese Pflanzen und Tiere könnten aus dem Rahmen der Risikobewertung vollständig herausfallen, wenn die Richtlinie 2001/18 nicht systematisch angewendet wird.

Daher empfehlen wir,

- Pflanzen und Tiere, die in der Landwirtschaft eingesetzt werden, vollständig von der Patentierung auszunehmen,
- Pflanzen und Tiere, die mit den neuen Gentechnik-Verfahren hergestellt werden, vollständig der EU Regulierung 2001/18 zu unterwerfen,
- sich international darauf zu verständigen, dass die Freisetzung von gentechnisch veränderten Organismen, deren Ausbreitung nicht kontrolliert werden kann, verboten wird und
- das Vorsorgeprinzip zu stärken.

1. Overview: Key tools used in synthetic gene technologies

In the past few years, new methods of genetic engineering have been developed that can also be used to produce plants and animals for food production. These techniques can be collectively called *gene-editing or synthetic gene technologies.*

With the emergence of techniques using synthetic nucleotides (DNA and RNSa), nucleases (DNA scissors) and epigenetic modification, many experts are proclaiming a new era of super genetics. In particular, so-called DNA scissors (nucleases) offer new possibilities of making changes in the genome. Preliminary studies detailing the application of these techniques in plants (such as Arabidopsis, sorghum, rice and wheat), fish, flies, worms, rats, rabbits, frogs, non-human primates and human cells (see Sander & Joung, 2014), and also animals used in food production such as cattle (Tan et al., 2013), sheep (Han et al., 2014) and pigs (Hai et al., 2014) suggest that technologies such as CRISPR and TALEN (for more details see below) are universally applicable across the biological kingdoms. They enable targeted DNA manipulation, even at multiple sites simultaneously (Bortesi & Fischer, 2014, Segal & Meckel, 2013, Baker 2014).

The application of nucleases are synergistic to methods of DNA synthesis that have been developed in recent years: It is no longer necessary to isolate DNA from an organism in order to transfer genes. Knowledge of the specific DNA (or RNA) structure is sufficient to synthesise the nucleotides as needed in the laboratory. Synthesis of nucleotides is not confined to native templates, but can also provide artificial sequences. The new technologies such as DNA scissors can insert synthetic DNA anywhere in the genome and even enable radical changes. The nucleotides can also be used in a transient way, by introducing them into the cells but not into the genome.

Some of the organisms derived from these techniques will have new genetic conditions or biological characteristics that do not exist in natural biodiversity. Other organisms may appear unchanged in their genetic condition even though their genome has been partially or completely replaced by synthetic DNA. For example, in 2010, a microorganism was presented whose DNA was completely re-synthesised in the laboratory (Gibson et al., 2010). In 2014, a publication showed that a whole chromosome in yeast had been replaced by synthetic DNA (Annaluru, et al., 2014).

1.1 CRISPR-Cas

Nucleases are proteins (enzymes) which can be used to splice DNA, hence the use of the term "DNA scissors" or "gene scissors". Some of these tools such as zinc finger nucleases were developed some time ago, but did not play a major role in the genetic engineering of plants and animals used for food production.

In recent years, several new nucleases have been developed that in principle allow for targeted DNA introduction or modification at any chosen site in the genome. The current star of the nuclease family is known as CRISPR-Cas. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and consists of a guide-RNA region, which can match with a targeted DNA sequence. RNA is capable of mirroring and 'recognising' DNA structure, so that the CRISPR-Cas system can be directed to specific sequences in the genome. The Cas enzyme, which is coupled with an RNA to guide it, functions as the 'DNA scissors' and can 'cut' a single DNA strand or both simultaneously. Mutations often occur when the cell's own mechanisms seek to repair the breaks, causing, for example, genes to be silenced. CRISPR-Cas also allows synthesised DNA to be introduced to the site or to cut off larger or smaller parts of DNA. The Cas enzyme can also be used to silence genes without cutting the DNA.

The system is described as being surprisingly simple and efficient to operate. Since the possibilities of the CRISPR-Cas system were first discovered some two or three years ago, the number of publications has grown rapidly and there are already commercial applications for its use in laboratory animals.

The nuclease can be applied as a complex being transiently introduced in the cells to change its genome or gene regulation. It is also possible to insert the DNA which then produces the nuclease into the genome permanently so it will continue to be active in the follow generations.

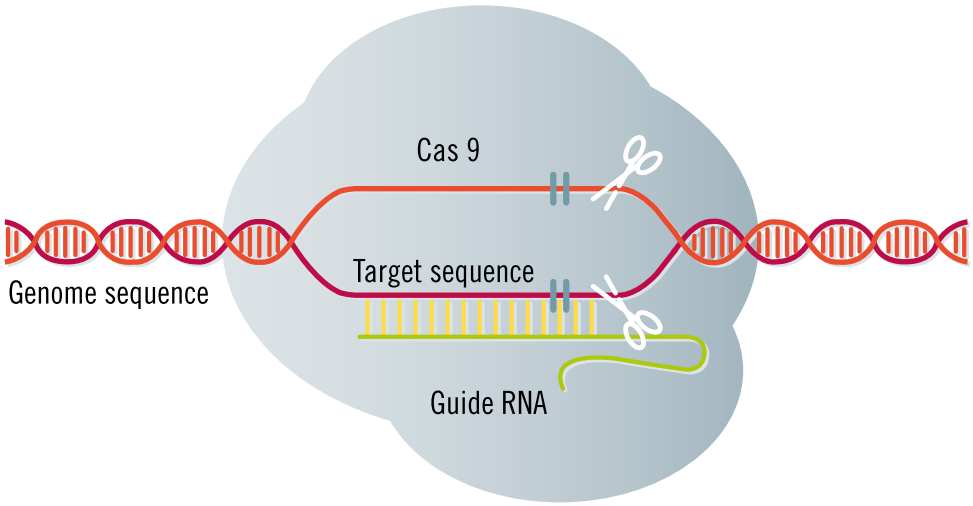

Fig 1: CRISPR-Cas: The nuclease CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) can be tailored to bind to specific DNA sequence that is identified via a guide RNA. It is combined with a protein (enzyme) that can cut the DNA to silence genes or to introduce additional synthetic DNA.

The CRISPR-Cas system can be used for multiple genomic manipulation in one step: The cutting enzyme Cas, after being introduced into the cells, can be combined with a set of guide various RNAs, targeting different sites in the genome.

The nuclease CRISPR-Cas can also be used to establish so-called gene drives. Organisms engineered with gene drives can render organisms that are homozygous in regard to the newly inserted DNA in each generation. This allows the rapid spread of the artificial DNA in the targeted populations (Esvelt et al., 2014).

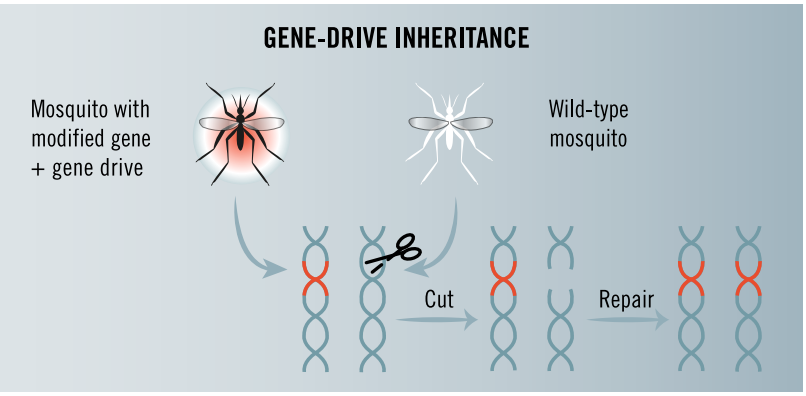

Fig. 2: Gene drive can be based on synthetic gene technology and use of CRISPR enabling a change in the structure of DNA on one chromosome to copy itself to its partner chromosome in every generation. As a result, the newly introduced DNA will speed through a population exponentially faster than normal. Source: Gantz & Bier, 2015, adopted by Testbiotech

Other gene scissors systems such as TALEN (Transcription Activator-Like Effector Nucleases) and mega-nucleases function along similar lines but have proved more difficult to operate. Despite the fact that multiple uses for DNA scissors have been identified, there is as yet no full understanding of how they actually function in detail.

1.2 Nucleotide synthesis

Nucleotides are DNAs and RNAs which are the most relevant in the cells to inherit genetic information and control gene activity by epigenetic mechanisms. The nucleotides can be synthesised in the laboratory to long or short, single or double strands, giving rise to biologically active compounds with or without a natural template.

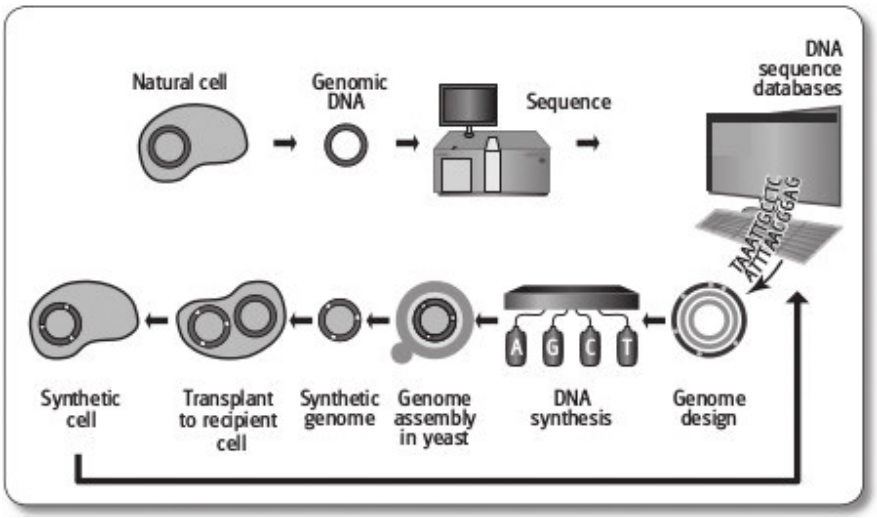

Fig. 3: DNA-sequencing and DNA synthesis go hand in hand. Source: US Presidential Commission for the study of bioethical issues,[3] adopted by Testbiotech

3 www.bioethics.gov/documents/synthetic-biology/PCSBI-Synthetic-Biology-Report-12.16.10.pdf, revised in „Handbuch Agro-Gentechnik", C. Then, Oekom Verlag 2015.

One example for current applications are so-called oligonucleotides, short sequences of DNA that can be introduced into the cells to change the structure of the genome. The short, synthetic DNA components (oligonucleotides) are introduced into cells to prompt the cell to adjust its own DNA to the foreign synthetic nucleotide, thus resulting in changes to the DNA at desired locations in the plant's genome. For example, oligonucleotides are used to make plants resistant to herbicides. The technique can also be used to inactivate an enzyme by disrupting a coding region or to knockout other gene functions.

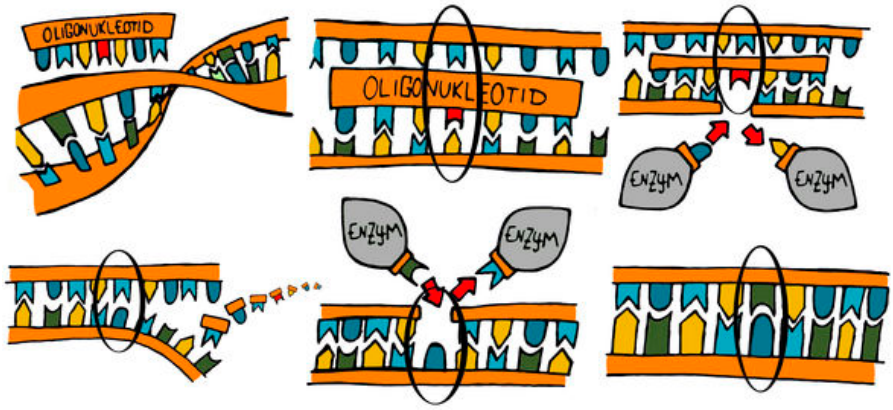

Figure 4. Model of the mode of action of oligonucleotides: 1. The oligonucleotide is inserted into cells 2. The oligonucleotide is fixed at the position with high similarity in the genome. 3. The difference between the plant´s genome and the oligonucleotide induces enzyme repair mechanisms in the cells, one strand of DNA is changed at the relevant position. 4. The oligonucleotide is removed from the plant´s DNA (mechanisms not known). 5. The difference between the two strands of DNA are repaired by the plant's own repair mechanism. 6. The specific alteration within the genome is achieved. Source: http://www.keine-gentechnik.de/dossiers/neue_technologien.html

The technique for introducing short oligonucleotides can also be used to change longer parts of the genome, such as in the case of Multiplex Automated Genome Engineering (MAGE). In this case, multiple changes of the cell's genome are effected either sequentially or simultaneously (Carr et al., 2012). According to a well-known proponent of synthetic biology, George Church, these or similar techniques could even be used stepwise to rewrite the genome of one life form to match that of another (Church & Regis, 2012).

Despite its application in plants and animals, the exact mechanisms for the genome modifications triggered by oligonucleotides are not understood in detail (see for example Lusser et al., 2011).

1.3 RNAi applications

RNAi-techniques, which are used to manipulate gene regulation, are not new as such, but new applications are being developed. The first genetically modified plants approved in the USA in 1994 – the so-called Flavr-Savr tomatoes - were products of RNAi and were genetically modified to block the plant enzyme involved in the degradation of cell walls so that the tomato retains its 'form' for longer. The gene for the enzyme was inserted into the genome in its reoriented form ('antisense') so that it had to be read backwards. The RNA produced by this DNA caused the native gene function to be silenced. Other genetically engineered organisms currently approved on the market include soybeans produced by Pioneer with altered oil quality (Soybean 305423)[4].

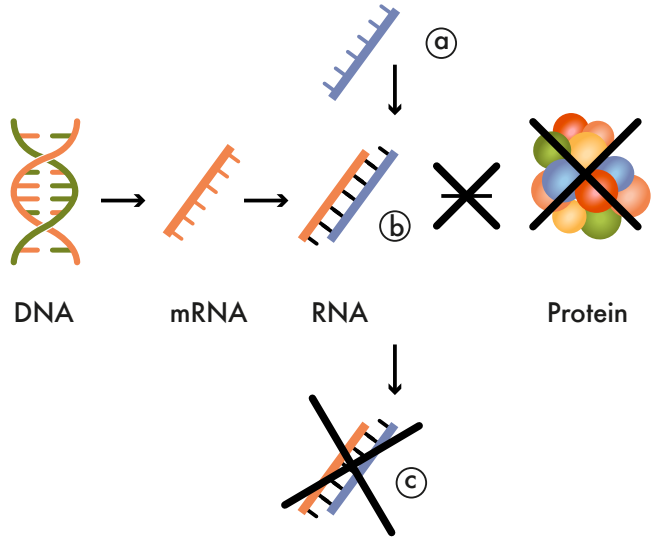

Figure 5: RNA-interference (RNAi) causes destruction of messenger RNA needed for the production of proteins:
a) technically introduced RNA
b) the messenger RNA produced by the cells is bound by the additionally introduced RNA
c) the double stranded RNA is degraded by the cells; as a result the respective protein is not produced.

As has become evident within recent years, RNA interference (RNAi) mechanisms are highly complex instruments of gene regulation that are present in vertebrates, insects, plants and other life forms. Possible applications of RNAi have increased steadily in recent years and synergies. For example, Monsanto wants to introduce genetically engineered maize that produces RNAi as a kind of insecticide: When pest insects feed on the plant, they ingest the additional RNA which then switches off genes that are vital to their survival – a mechanism that is not always successful as tests show performed by Monsanto (Chu et al., 2014).

In this report we will focus on those RNAi applications that are meant to change gene activity in the plants besides those applications with insecticidal impact. Some of these applications require integration of additional DNA in the genome. Some applications are transient, for example, when RNA mixtures are applied to the plants and taken up in the cells where they interfere with the target genes in the crops.

4 www.testbiotech.org/node/1013

2. A landscape shaped by patents

The rise of transgenic plants in the 1980s was concurrent with a change in the landscape of plant breeding. Companies such as Monsanto and DuPont, not previously active in the plant breeding sector, entered the market and expanded their business models based on patents to seed production.

In 1992, for example, an OECD publication (OECD 1992) stated that, within the seeds sector, the main company focus should be on the reorganisation of the seeds market leading to a greater integration and inter-dependency with the agrochemicals sector. Genetic engineering and patents served as a major tool in this context. Any gene sequence introduced into plant material confers patent protection to seeds, plant and progenies, all along the chain of farm and food production up to food and biofuels. Contrary to the IP established under the plant variety protection system, patents can be used to block access to genetic resources needed for further breeding.

Thus, patents became an important factor driving the concentration process. At the same time, many traditional breeders and companies active in genetic engineering were bought up. Consequently, there is still ongoing market concentration, and just a few large companies with an increasingly predominant market position. It is evident that in a breeding sector driven by patents, the bigger companies can easily establish monopolies on traits, plants and breeding material, while the smaller and medium-sized companies lack competitiveness and, in many cases, are either pushed out of the market or bought up by the bigger players. Unlike the traditional breeders and smaller specialised companies, the so-called seed giants such as Monsanto have the resources not only to file single patents, but also file series of strategic applications and still survive costly legal opposition procedures. Moreover, they are also in a position to introduce the products to the market as they have access to the infrastructure needed to reach out to farmers and / or food producers.

Consequently, in most regions where genetically engineered crops are cultivated, there are only a small number of predominant companies who are visibly in control. In comparison, other factors such as differences in the requirements for market authorisation and labelling do not seem to have a major impact on the structure of this specific seed sector.

In the context of the new methods for genetic engineering, this development is likely to be continued: All nucleotides, nucleases, methods, compositions as well as plants and animals will be subjected to the patent regime. 'Patent wars' are already ongoing between those research institutions that discovered CRISPR nucleases. On the one hand, there are the scientists Doudna & Carpentier from the University of California and, on the other hand, Zhang et al at the Broad Institute / MIT (Ledford, 2016). While these institutions are mostly interested in applications of CRISPR-Cas in the medical sector, the relevant patents are also of interest for plant and animal breeding in food production (see below).

In the context of synthetic gene technologies, we have to expect a continuum of the development that started with the first introduction of genetic engineering in the breeding sector. For the foreseeable future, IP-strategies will continue to drive market concentration, the acquisition of smaller companies and the increasingly predominant position of the so-called seed giants.

In 2015, Dow AgroSciences and DuPont/ Pioneer announced a merger that will particularly impact the patent landscape around these new methods.[5] As shown below, these two companies already lead in the number of patent filed in this context.

5 www.dow.com/news/press-releases/dupont%20and%20dow%20to%20combine%20in%20merger%20of%20equals

Furthermore, if the new techniques are applied to farm animals, this would affect a sector which has so far been much less affected by patents than plant breeding. Consequently, we can expect major changes in the animal breeding sector. The introduction of patented animals will especially impact traditional breeders and farmers in areas such as the production of cattle and dairy.

To be able to draw some more specific conclusions, we conducted patent research in this field, focussing on more recent applications, mostly from 2010 to 2015. Further, in order to avoid duplicates, we only selected applications that were filed as WO applications at the World Intellectual Property Organisation (WIPO), leaving aside regional applications at the European Patent Office or the US PTO.

The search criteria chosen were, for example, nucleases such as CRISPR-Cas and zinc finger, the use of synthetic oligonucleotides and RNAi -technologies. In the field of RNAi techniques, we did not take into account applications targeted at pest insects and weeds as they were regarded as too specific to be included in a more general overview. Thus, we mostly selected those RNAi applications concerning gene regulation in plants or animals.

In the area of plant breeding, we paid special attention to the bigger companies and some of their known cooperation partners. In the area of animals used for food production, we mostly focused on the US company Recombinetics, which seems to have the highest number of relevant patent applications.

Patents are only published after a period of 18 months of secrecy and this is reflected in the overview. Thus, in reality, we would expect a much higher number of patents applied for in this field than we are able to give in our overview.

2.1 Companies active in the genetic engineering of plants

In particular, we looked for patent applications filed by companies leading the market in transgenic plants such as Monsanto, DuPont, Syngenta, Bayer and Dow AgroSciences. Further, we were aware of some clusters of cooperation around some of those companies, so we also included some of the companies involved to get a broader picture.

We did not try to determine the overall number of patent applications in the area we examined (nucleases, usage of oligonucleotides and RNAi), since there are many patent applications that are not specific to plant and animal breeding, but more oriented to medical research. We, therefore, left aside patents filed by Broad Institute/MIT and the University of California. The results of our research are shown in Figure 6.

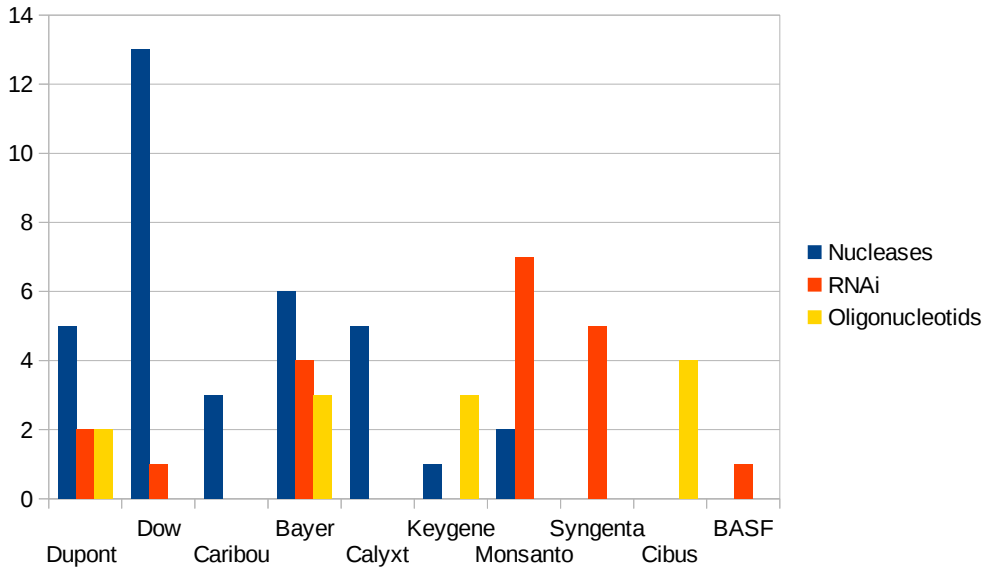

Figure 6: Number of WO Patent applications registered at the World Intellectual Property Organisation (WIPO) claiming new methods of genetic engineering in plants filed by big seed companies and some of their cooperation partners between 2010 and the end of 2015.

From our results presented here we concluded that applications for nucleases are the most relevant (33) followed by RNAi applications (20) and usage of oligonucleotides (12). Further, it is evident that after merging, Dow and DuPont will have a very strong position. Their position will be boosted further by cooperation between DuPont and Caribou (Grushkin, 2016), which is a spin-off from the University of California and pioneered the development of CRISPR nucleases, having applied for patents with considerably broad scope.

There is another relevant cluster around Bayer involving Calyxt (previous Cellectis) and Keygene. In comparison, Monsanto and Syngenta seem to be less active in this field at the moment. However, there might be further cooperation involving these companies which has escaped our attention.

There is one example that can help to exemplify the role of patents in this area: Dow AgroSciences is systematically filing patents on native DNA in plants which are considered suitable for gene insertion guided by nuclease. So even if Dow AgroSiences and DuPont are not the companies that developed the specific techniques, they can hamper or prevent other companies from using it.

2.2 Companies active in the genetic engineering of animals

No big companies appeared to be extensively filing patents on livestock as was the case in plant breeding. However, we identified several smaller companies such as Intrexon (US), Recombinetics (US), Toolgen (Korea) and the Roslin Institute (UK). Further, several patent applications filed by institutions such as the Broad Institute/MIT and the University of California also concern the genetic engineering of animals.

We chose Recombinetics as a typical example for our overview of specific patent applications in this field. This US company is chiefly focussed on the new methods of genetic engineering and their application in large animals, many of them targeted at farm animals. Table 1 gives some examples of the respective patent applications (overall number of WO patent applications filed by Recombinetics up until the end of 2015: 13).

Table 1: Selected patent applications filed by Recombinetics:

Application	Claims
WO2012116274	Usage of nuclease TALEN to increase muscles in cows and pigs
WO2013192316	Usage of nuclease TALEN to increase muscles in cows and pigs and produce cows without horns
WO2014070887	Usage of nuclease TALEN, zinc finger or CRIPSPR to prevent animals from reaching sexual maturation, these animals cannot be used for further breeding.
WO2014110552	Hornless cows by application of methods for gene editing.
WO2014193583	Usage of nucleases to block genes for production of sperm cells.
WO2015168125	Usage of nucleases to create animals with multiple genetic changes.

If patented animals like these are introduced into the farm animal breeding sector, there will be huge consequences: Patent law in the EU (Directive 98/44) would only allow usage of the patented animals to produce milk and meat, but not for further breeding. But already there are big companies poised to take over this business: For example, in the background of the activities of Recombinetics, there is Genus PLC (UK)[6] which is known to be one of the biggest breeding companies.

As we explain below, farm animals such as pigs and cows are not the only relevant species in the area of agriculture. For example, Oxitec (now owned by Intrexon) filed patents on genetically engineered insects that are meant to destroy pest insects. These applications need specific attention when it comes to risk assessment (see below).

6 www.heise.de/tr/artikel/Gentechnik-fuer-den-Kuhstall-2382354.html

3. Risks and uncertainties associated with the new methods

As mentioned above, there are several publications showing that the new techniques such as nucleases can be applied in animals and plants. However, these publications also show that there are substantial technical difficulties leading to low success rates, unintended effects and uncertainties regarding the safety of the plants and animals derived thereof.

In plants, in most cases, protoplast cells are used for the process of genetic engineering. These cells are then selected for insertion of the additional DNA and further developed to what is called a callus, which can then be developed into a plant. If CRISPR nucleases are used, in many cases the DNA for establishing the nuclease is first inserted into the genome by using old fashioned methods such as particle bombardment and random insertion into the genome. It is only the next step, the insertion of new DNA which is more targeted. Each step of the technical process can cause unintended alterations in the genome of the plants or its gene regulation.

In plants, the success rate largely depends on the species. There are several biological reasons for this: For example, the species of Arabidopsis only has a relatively minor genome, while that of maize is much more complex.

In farm animals, in most cases cultures of somatic cells are used for the process of genetic engineering. To develop an embryo, the nucleus of the manipulated cell has to be transferred to an oocyte, a process known as somatic cell nuclear transfer (SCNT) which was developed to give life to the cloned sheep Dolly in 1996. This process still suffers from a low success rate and in many cases causes detrimental side effects in the animals (Tan et al., 2016). Another possibility is the manipulation of an embryo at very early stage. This is a technique that has been used for the last 30 years and still has a low rate of success and a wide range of unintended side effects (Tan et al., 2016). In any case, there are several steps which need to be carried out in the laboratory, all of which can cause unintended alterations in the genome or in gene regulation before a single animal is born.

In general, the results from the application of techniques such as nucleases largely depend on various factors such as the species, the cells used, the kind of nuclease and the intended biological characteristics. A very relevant technical detail, for example, is the specificity of the nuclease. If specificity is not sufficient, the DNA will be cut in several regions of the genome.

In this context it does not matter whether small or larger pieces of DNA are inserted or DNA is just cut or DNA sequences are removed and genes are silenced. Each of these applications is associated with complex technical processes, which are likely to render unintended effects in the genome and/ or gene regulation. Consequently, each plant or animal derived from these techniques has to undergo a detailed risk assessment, taking into account all relevant technical steps of the process.

There are also uncertainties in the applications of isolated nucleotides (DNA or RNA) which are introduced to trigger structural changes in the DNA or interfere with gene regulation. This also requires several steps to be carried out in the laboratory using cell cultures and protoplasts as described above. Unintended side effects will depend on the intended changes, the length of the nucleotide, its biological properties and its selectivity, the cells, the species and the additional steps applied in the laboratory. Consequently, each plant or animal derived from these techniques needs to undergo a detailed risk assessment, taking into account all relevant technical steps of the process.

Below are some examples which show the need for regulation and a case by case risk assessment of plants and animals derived from genome editing.

3.1 Cibus oilseed rape

For a period of several months, starting in mid-2014, the US company, Cibus, had a confidential exchange of emails with the German Federal Office of Consumer Protection and Food Safety (BVL) about its oilseed rape derived from introduction of oligonucleotides. The company wanted permission to release its oilseed rape without the need for detailed risk assessment and was apparently looking for support from an authority sympathetic to its cause. Indeed, the German authority did agree with Cibus that no risk assessment would be needed because they did not consider this plant as derived from process of genetic engineering.[7] However, the process by which the authority came to this conclusion is full of surprising details.

The email correspondence between the BVL and industry was published by civil society organisations in 2015, and shows that the German authorities - before stating that no risk assessment would be necessary – did, in fact, carry out some kind of basic risk assessment and forwarded detailed questions to the company.[8] For example, the authority requested molecular data about the intended or unintended changes in the genome and the plant composition. The data then provided by Cibus showed some significant changes in the plant components (which were not investigated further). Further, the documents show that Cibus did acknowledge that the mechanisms they used to change the plant DNA were not fully understood in detail.

If plants and animals produced by using oligonucleotides were to be exempted from EU regulation, it would not be mandatory to provide any data to the authorities prior to the plants being introduced to the market, and they could be grown without restriction. Consequently, companies could hide all relevant information from the public and prevent any independent risk assessment.

Further, with regard to regulatory aspects, it also has to be taken into account that the single steps meant to induce small changes can be applied several times in the same organism. Moreover, plants and animals showing single or several genetic changes can be crossed with each other. These plants or animals might completely escape the scrutiny of the regulatory bodies if Directive 2011/18 is not applied in a coherent manner.

3.2 DuPont soybeans

As a recent publication shows (Li et al., 2016), DuPont is trying to produce genetically engineered soybeans with the help of nucleases CRISPR-Cas developed by the University of California. In this case, two sites in the genome were targeted to investigate the selectivity of the CRISPR-Cas nuclease. For testing purposes, the aim was to insert an antibiotic resistance marker gene at the two target sites.

To create the soybeans, in a **first step** protoplasts were bombarded with DNA to insert the DNA sequence for the production of the nuclease (the enzyme Cas 9 plus guide RNA) in the genome of the soybeans. Thus, the starting point of the process is a transgenic plant created by random insertion via particle bombardment. Consequently, the cells, callus and plants derived from this process showed numerous unintended changes in the genome such as extra copies of randomly integrated DNA needed for producing the nuclease.

7 www.testbiotech.org/en/node/1176
8 www.testbiotech.org/en/node/1414

In a **second step**, the plant cells were expected to produce the enzyme Cas9 to target the defined region in the genome. The two target sites were expected to be cut, resulting in break of the DNA followed by repair mechanisms organised by the cells. In addition, the DNA construct containing the antibiotic resistance marker gene was to be inserted. This second step was also accompanied by random effects and gave rise to many unintended effects:

- There were substantial differences in the success rate of the targeted sites: One site showed an overall rate of DNA breaks of around 76 percent, the other around 60 percent.
- Around half of the targeted sites showed a break in the two strands, in the other half, just one strand of DNA was cut.
- The deletions observed in the targeted sites varied in length, some of them showing additional unintended insertions of DNA.
- The DNA construct with the antibiotic resistance marker gene was found to be inserted - however, in many cases several copies of the construct were inserted.
- In most cases the DNA of the nuclease was unintentionally co-integrated at the targeted sites.
- Further scrutiny revealed a substantial number of cells with insertions of the additional DNA in other regions of the genome.
- Surprisingly, only a small number of the callus derived from the genetically engineered cells contained the DNA construct as expected. In addition, many of the plants regenerated from the same callus, contained different variations of the targeted sites.
- A small number of plants was finally derived. Most of them revealed unintended changes in the structure of the DNA at the targeted site.
- Further analysis of a few plants that seemed to be successfully transformed revealed that they all contained randomly integrated extra DNA.

In a **third step**, some of the resulting soybeans were crossed further to segregate plants with the intended additional DNA (the antibiotic resistance marker gene) from those with the unintended DNA (the DNA for producing the nuclease). This attempt only was successful in a very small number plants carrying one of the two targeted sites. These remaining plants, were not investigated to determine the overall number of unintended changes in the genome.

In the article, the previous methods are described as suffering from "random gene integration, endogenous gene interruption, multiple gene copies, and often unpredictable gene expression. Hundreds of events must be screened to identify a single copy-integrated gene that does not interrupt any endogenous gene." (Li et al., 2006)

But, in comparison, this example for the application of nuclease CRISPR-Cas in soybeans does not seem to represent a major technical breakthrough. In any case, in the context of risk assessment, each technical step of this process has to be taken into account to assess risks and conclude on safety of the final product.

3.3. Genetically engineered animals

There are several projects currently investigating the use of the new techniques - also in regard to animals used for agriculture and food production. There are also examples showing that applications such as the usage of nucleases can render detrimental effects in the genome:

For example, Semaan et al (2015) reported on pig embryos which were severely damaged by application of nucleases. The experiments were conducted to remove DNA from the pigs' genome originating from viruses. Other publications report on the use of nucleases to enhance the growth of muscle in cattle and sheep (Proudfoot et al., 2015) or pigs (Cyranoski, 2015). In these cases, the application of the nucleases aims to destroy the function of a gene which controls growth in muscles. Similar mutations occur in nature causing oversized muscles in cattle that leads to severe problems during calving. As the publication shows, the applications led to substantial side effects, most animals did not survive. Despite the application of the new methods, the results were hardly predictable and the success rate low. According to Proudfoot et al (2015), in cattle only one single bull survived, which did indeed show a higher muscle growth rate. But it remained a matter of discussion which of the several (intended and unintended) changes in the genome was responsible for this effect.

To summarise these and other similar findings, the outcome of nuclease applications in the genome of mammals is dependent on several details such as the specificity of the targeted sites, the type of nucleases, the amount of nucleases introduced into the cells, the methods chosen to introduce them into the cells, the type of cells, the function of the targeted and / or the inserted gene. According to O´Geen et al (2015), there is no basis for stating that nucleases in general can be regarded as being more targeted and precise than previous methods. Therefore, the side effects and risks have to be assessed in each case.

Other examples show detrimental effects caused by RNAi: For example, Jabed et al. (2012) wanted to produce genetically engineered cows that would produce milk with a reduced content of beta-lacto-globulin (BLG). This protein is known to cause allergies. In a first step, somatic cell cultures were used to introduce the DNA necessary to produce the relevant miRNA that is intended to interfere with protein production and reduce the content of BLG. From these cells, 57 embryos were cloned, but only a single calf was born with the desired genetic information. This calf (called 'Daisy') not only showed a much lower content in beta-lacto-globulin in its milk, but also a general change in the composition of all proteins:

> *"A more detailed analysis of the milk produced by the transgenic calf demonstrated that this change in BLG also affected the levels of all other milk proteins, revealing an intricate balance of milk proteins synthesis."*

It remains doubtful whether milk with such a change in its composition would be suitable for human or animal consumption at all. Further, Daisy does not have a tail. Whether the loss of the tail was due to disturbance of epigenetic mechanisms or the genetic manipulation could not be determined.

There are some specific health risks that go along with this application: It is known that miRNA can pass the gut and enter the blood stream and could then also interfere with cell regulation at the stage

of consumption. It is not unlikely that the artificial miRNA produced in the cows might also be found in the milk in which it case would be consumed together with the milk. Therefore, this is a further risk emerging from the process of genetic manipulation that would require detailed investigation of its impact on human health.

There are more examples showing the uncertainties and risks associated with the application of gene editing in animals used for food production (Then, 2016). It is important to be aware of the plans of companies such as Recombinetics to perform multiple genetic manipulations in the animals (Then, 2016), which also would render multiple side effects and risks to humans, animals and the environment.

3.4. Engineering native populations

As explained above, it is possible to insert the DNA which produces the nuclease CRISPR-Cas into the genome permanently so it will continue to be active in the following generations. This application can be used to establish so-called gene drives. For example, CRISPR-Cas nuclease was inserted in flies enabling a newly inserted DNA to be copied from one chromosome to its partner in every generation. Consequently, the genetic conditions will be homozygous in every generation and speed through a population exponentially faster than normal ('gene drive'). Synthetic DNA therefore could spread through a large native population within a relatively short period of time (Esvelt et al., 2014, Gantz & Bier, 2015).

There are already plans to release genetically engineered insects to eradicate pest insects in agriculture (for overview see: Bauer-Panskus et al, 2015). There are also already patent applications filed by Dow AgroSciences to engineer weed populations to make them more susceptible to herbicides. If these organisms were to be released, the spread of the artificial DNA would not stop at national borders nor could it be kept out of fields with organic agriculture.

Thus, it is not surprising that these applications are fuelling a fiercely controversial debate amongst scientists (Ledford, 2015). The need for new regulation was explicitly addressed in an editorial in Nature magazine of 6 August 2015.[9]

> *"What is new is the advent of CRISPR. This adds an extra dimension to the debate, because it makes gene drives much easier to create and could dramatically accelerate the timeline for a potential release — accidental or intentional. Researchers and funding agencies should take note, and efforts to understand the ecological consequences of a gene drive should be made an urgent priority. Regulators and the wider world need to keep pace with the rapid development of CRISPR technology, and there is little time to waste."*

9 http://www.nature.com/news/driving-test-1.18118

4. Regulatory needs

Within the last few years, industry and interested stakeholders have started several initiatives demanding that the EU Commission and the authorities of EU Member States exempt the techniques used for gene editing from EU regulation 2001/18 (CEO, 2t016). The position of DuPont is made explicit in an article published in 2016 (Grushkin, 2016):

> *"Companies like DuPont hope regulators will view CRISPR no differently than conventional breeding as there is no foreign DNA involved. 'We see it as a breeding technology. With CRISPR the outcomes are the same as you would get from mutagenesis and conventional breeding,' (…)."*

In the US, several plants produced by companies such as Cibus, Dow AgroSciences, Calyxt and Agrivida have already been exempted from regulation (Grushkin, 2016; Camacho et al., 2014).

From a legal perspective, there is no doubt that the techniques used in gene editing are subject to regulation in Directive 2001/18. As has been pointed out in two legal dossiers (Kraemer, 2015; Spranger, 2015), Directive 2001/18 covers all processes where human intervention leads to the insertion of new material into a cell. Only those processes which in 2001 were thought to have a long safety record at the time when the Directive in 2001 was adopted, are exempted. The Directive clearly follows a process-oriented approach and restricts regulation to specific properties of derived products. Further, the precautionary principle was given prominence in the Directive. Thus, if there are any doubts at all about whether a new technique falls under Directive 2001/18 or not, it has to be subjected to the regulation.

As shown in the examples in chapter four, there are many uncertainties and risks associated with the application of the new technologies. So, if plants or animals were to be introduced into the market, the precautionary principle would be the most important approach in dealing with these organisms.

In general, the introduction of synthetic gene technologies makes the need for regulatory action more pressing than ever. In regard to the protection of biodiversity, it can be expected that the number of technically derived organisms being released into the environment within the next few years could increase dramatically. Therefore, the likelihood of negative consequences will also increase - not only stochastically.

It is not possible to assess the risks of these organisms in a way that we can reliably conclude upon long-term safety, especially if there is no spatio-temporal control. And we are not aware of an internationally binding framework that could be used to impose a ban on the release of organisms derived from genetic engineering if spatio-temporal control cannot be established. Such international regulation is urgently required: For example, in the case of a fly inheriting a gene drive, regulatory frameworks of concerned countries could, based on available evidence and/or plausibility, come to decisions that might diverge from country to country – whilst the flies with the gene drive could just simply move across borders.

Thus, within the EU, the precautionary principle has to be substantially strengthened. As shown by the examples above, risk assessment cannot be reduced to the known properties of the final organisms, but has to take the process that was used for its production into account. If the process is ignored, it will be impossible to ask the right questions and develop adequate hypotheses for the process of risk assessment.

Interestingly, even experts in favour of the new technologies being introduced into agriculture are aware of regulatory needs in this field. In a commentary published in Nature Biotechnology in 2016 (Huang et al., 2016) the authors suggest – similarly to DuPont – treating plants derived from genome editing just like conventional breeds:

"Because such genetic stocks could in principle - although generally not in praxis - be generated by conventional breeding or random mutagenesis, they should be considered the same as those used in conventional breeding, which are not regulated."

Nevertheless, they also propose a list steps for risk assessment that are currently not used in conventional breeding. In essence, the approach suggested in the commentary is to

- minimise the risk of escape of the respective organisms from laboratories and fields during the research and development phase;
- demonstrate the absence of foreign sequences, if genome engineering proteins were introduced as DNA construct;
- document DNA sequence changes at the target sites;
- ensure that the primarily targeted site did not suffer unintended secondary editing events, and consider the consequences of potential off-target events on the basis of available reference genome information and whole-genome resequencing technologies.

Looking at these suggestions, it appears that most of the information requested would also be requested under current risk assessment as performed by EFSA. The outcome of the assessment of these data would then frame further steps to be investigated in order to come to conclusions.

Compared to current regulatory system, the most striking difference in the proposal made by Huang et al. (2016) was that they would restrict labelling and transparency to the level of seed registration. Following the authors' advice would require a change of EU legislation, not only limiting risk assessment to a few very basic steps, but also scrapping existing legislation in regard to transparency and labelling. This is relevant on many levels such as consumer choice and protection of organic agriculture.

It should be noted that such a change in the EU framework would fit perfectly into the regulations planned under the free trade agreement of CETA and TTIP. Thus, it seems this proposal (Huang et al., 2016) is presented using scientific language, but is largely influenced by political and economic considerations.

5. Some conclusions and recommendations

As shown in this overview, current developments in using gene editing in plants and animals for food production, is largely driven by patents. Future developments will be greatly influenced by the interests of the so-called seed giants. Introduction of these plants and animals into agriculture will foster market concentration in plant and animal production.

Further, there is no doubt that the bigger companies are the ones that will dominate the markets, the strategies of research, innovation and product development. This development will impact traditional breeders, farmers, food producers and also consumers.

In regard to risk regulation, it is shown that gene-editing can lead to a wide range of off-target effects. These unintended effects might in some cases be the cause of risks and hazards. Therefore, there has to be case-specific risk assessment. In this context, it is not decisive whether new DNA is inserted or parts of the original DNA are removed or the activity of the natural genes are changed by epigenetic effects without changing the structure of the genome. For example, the native DNA from organisms might even be replaced to a great extent by synthetic DNA without obvious changes. There is no doubt that these applications also raise a range of uncertainties and risks that need to be assessed, taking into account the process for their production.

Further, with regard to regulatory aspects, it also has to be taken into account that the single steps meant to induce small changes can be applied several times in same organism. This can lead to much more extensive changes in the genome. Moreover, plants and animals showing single or several genetic changes can be crossed with each other. In addition, all the different techniques could possibly be used in combination. So, if the single steps are exempt from EU regulation then how can the final varieties of the modified organisms be assessed? These plants and animals might completely escape the scrutiny of the regulatory bodies.

To assess the actual risks it is necessary to know which techniques were applied for which purposes. The relevant data have to be collected systematically and assessed independently. If these techniques are exempted from regulation, the relevant data will be kept as confidential business information. In this case, neither independent scientists nor the authorities will be able to access the data in a way that will enable them to obtain a sufficient overview of the specific techniques, the relevant traits and the associated risks.

In the light of these findings, the following recommendations should be used as further guidance.
We recommend:

- exemption from patentability of plants and animals used in agriculture and food production
- that plants and animals derived from gene-editing are subject to EU regulations in Directive 2001/18
- agreement on an international framework for a general ban on genetically engineered organisms that cannot be controlled in their spatio-temporal dimension
- the strengthening the precautionary principle.

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