



# Discussion paper focusing on the scientific relevance of genome editing and on the ethical, legal and societal issues potentially involved

ISSUED BY THE ETHICS COUNCIL OF THE MAX PLANCK SOCIETY

## 1. Introduction and motivation for the discussion paper

*Christiane Walch-Solimena*

As an organization dedicated to fundamental research, the Max Planck Society (MPG) is committed to pursue issues at the very frontiers of current knowledge and bears a special responsibility to critically evaluate novel scientific developments. Such assessment includes both the scientific potential as well as the risks that may be faced if the scientific findings may be put into practice one day in the future. To this end, the MPG Ethics Council has been asked to assemble a working group to outline and discuss questions arising from a revolutionary technology that in recent years has opened up unforeseen opportunities in the manipulation of genes and genomes: the CRISPR-Cas9 gene editing and genome engineering technology. This programmable nuclease represents the most recently developed molecular tool used in genetics and has been harnessed as a versatile, precise and powerful technology that is applied not only to modify genes (e.g. correct mutations, introduce mutations, or cut and paste genetic information in a genome) but also their expression. However, this report will equally address, as far as possible at this early stage, potential risks which may result from a potential implementation of this new technique and the legal as well as ethical considerations concerning this new technology and its potential applications.

While advances in the use of the CRISPR-Cas9 technology have revolutionized the field of genome editing research,

intense controversies have emerged around some of its applications. Thus, first experiments in human embryonic cells have been performed already since 2015 in China<sup>1</sup> intended to correct certain disease-causing mutations. These publications have set off discussions throughout the scientific community and beyond about the ethical and safety implications of this research. An International Summit on Human Genome Editing focused on the future of human genome editing and convened by the US National Academy of Medicine, the UK's Royal Society and the Chinese Academy of Sciences in December 2015 voiced the need for an ongoing global forum. Statements on ethical and societal questions of genome editing, also covering other application areas have followed since then, e.g. by the Berlin-Brandenburg Academy of Sciences and Humanities<sup>2</sup>, the German National Academy of Sciences (Leopoldina)<sup>3</sup>, the US National Academy of Sciences<sup>4</sup>, the Nuffield Council on Bioethics<sup>5</sup> and others. The discourse on the use of genome modification technologies, in humans as well as in other organisms, is complicated by the fact that these are already directly or indirectly regulated while these regulations differ in the scientific and legal cultures concerned.

Humans have, throughout their history, taken action to alter genomes, while domesticating certain animal species and cultivating plants and animals to crops and life stock equipped

<sup>1</sup> Liang P, Xu Y, Zhang X, Ding C, Huang R, Zhang Z, Lv J, Xie X, Chen Y, Li Y, Sun Y, Bai Y, Songyang Z, Ma W, Zhou C, Huang J. CRISPR/Cas9-mediated gene editing in human triploid zygotes. *Protein Cell* 6, 363-372 (2015).

<sup>2</sup> BBAW (Berlin-Brandenburg Academy of Sciences and Humanities). Human genome surgery – towards a responsible evaluation of a new technology (2015). [http://www.gentechnologiebericht.de/bilder/BBAW\\_Human-Genome-Surgery\\_PDF-A1b-1.pdf](http://www.gentechnologiebericht.de/bilder/BBAW_Human-Genome-Surgery_PDF-A1b-1.pdf) (last accessed August 28, 2017).

<sup>3</sup> Nationale Akademie der Wissenschaften Leopoldina, Deutsche Forschungsgemeinschaft, acatech – Deutsche Akademie der Technikwissenschaften, Union der deutschen Akademien der Wissenschaften. The opportunities and limits of genome editing (2015). <https://www.leopoldina.org/de/publikationen/detailansicht/publication/chancen-und-grenzen-des-genome-editing-2015/> (last accessed August 28, 2017).

<sup>4</sup> National Academies of Sciences, National Academy of Medicine, National Academies of Sciences, Engineering, and Medicine, Committee on Human Genome Editing. Human Genome Editing: Science, Ethics, and Governance (2017). <https://www.nap.edu/catalog/24623/human-genome-editing-science-ethics-and-governance> (last accessed August 28, 2017).

<sup>5</sup> Nuffield Council on Bioethics. Genome editing: An ethical review (2016). <http://nuffieldbioethics.org/project/genome-editing/ethical-review-published-september-2016> (last accessed August 28, 2017).



with useful properties to support their own lives and communities. Progress in understanding the mechanisms of heritage and its basis, molecular genetics, has increasingly enabled more deliberate manipulation of genes and allowed the development of gene technology since the 1970s.

In recent years, new molecular tools such as Zinc-Finger Nucleases, TAL effector nucleases (TALENs) and CRISPR-Cas9 have been developed, which now allow altering genes in a precisely targeted way, through a process commonly compared with the editing of text, and therefore called gene or genome editing<sup>6</sup>. The most recently developed technology – CRISPR-Cas9 – is revolutionizing genetic engineering at a fast pace, being applicable to any cell or organism, also beyond the traditional model organisms such as the fruit fly *D. melanogaster*, the nematode *C. elegans* or the mouse *M. musculus*. It is more versatile, precise, easier, faster but also cheaper than previous technologies, and greatly extends the spectrum of feasible experiments. Consequently, the technology is now used successfully in countless laboratories around the world.<sup>7</sup>

Within the MPG, many research teams of the 33 Max Planck Institutes within the Biology & Medicine Section are performing genome editing in a large variety of cells and organisms across all kingdoms of life, in particular with CRISPR-Cas9. According to a questionnaire to the Max Planck Institutes, this technology is generally seen as a great opportunity for faster progress in basic science. Examples for recent publications based on CRISPR-Cas9 are shown below to illustrate such progress in a variety of experimental models (Boxes 1-3). MPG scientists raised potential ethical, legal and societal questions, which were addressed during the deliberations of the Working Group of the Ethics Council.

Genome editing using CRISPR-Cas9 is currently presented in the media not only as a revolutionary research tool, but at the same time new applications are raising high hopes, for example for curing genetic disorders by direct intervention in the human germline. While some of this seems justified by groundbreaking proofs of principle such as the recently published CRISPR-Cas9-mediated genome editing to investigate gene function in human embryogenesis<sup>8</sup>, the scientific community has – already since 2015 – begun to engage in an intense discourse on emerging ethical, legal and societal questions, as genome editing rapidly opens this and several other avenues for the targeted manipulation of genomes.

As a prominent stakeholder of fundamental science the MPG wants to engage in this discourse, explaining its own point of view in terms of the use of genome editing for basic research, pointing out the need for discussion and for renewed framing of ethical, legal and societal issues around genome engineering considering the use of highly efficient tools like the CRISPR-Cas9 technology. There is an urgent need to engage not only with other stakeholders of science, but also with the public at large in assessing the benefits and risks of genome editing in different fields of application. The task at hand is to contribute to such a discourse through more knowledge about the technology itself, its risks, to ensure evidence-based policy advice, and to reflect on the values that are at stake, the interests involved, and visions of the future. It is worthwhile to refer in this context the Nuffield Council on Bioethics, which in its publication on ethical challenges of genome editing<sup>9</sup> lists four reasons why it is mandatory to deal with the ethical questions concerning genome editing namely:

- genome editing is challenging current normative systems;
- greater numbers of users, also outside of traditional institutional settings and academic communities; thus, CRISPR-Cas genome editing presents particular challenges in terms of how ethical reflection and governance systems can engage effectively with technology use;
- differences in speed of development of research and innovation compared to the pace of development of related systems, including normative systems (e.g. changes to the law, to institutional structures, regulatory policies and procedures, and the evolution of public moral consensus) can exacerbate conceptual inconsistencies, increase anxiety and give rise to distrust (between different stakeholders);
- CRISPR-Cas as an enabler of future synthetic biology might become a disruptor of established species specifications (e.g. potentially creating synthetic genes or transgene analogues, complex synthetic organic components or even organisms).

This discourse was initiated through contributions by a working group of the MPG Ethics Council and the Ethic Council itself: Emmanuelle Charpentier and Christina Gross provide an introduction to genome editing technologies highlighting in particular the power of CRISPR-Cas9. A chapter on genome editing in plants by Detlef Weigel is pointing out new opportunities for plant research and crop breeding, and the importance of understanding the biology behind this technology in order to

<sup>6</sup> Nuffield Council on Bioethics (2016) Genome editing: An ethical review; p. 20: "(...) It appears, however, that the metaphor [sc. genome editing] has taken an unshakeable hold. This may owe something to its familiarity, its fertility and the apparent ease with which the metaphor may be extended. The danger of the metaphor lies not in the fact that it is a metaphor, and therefore a non-reducible way of referring to complex realities; it lies in the possibility that the metaphor might either disassemble significant ethical questions through the use of euphemism, or lead reasoning astray by overstretching the power of analogy".

<http://nuffieldbioethics.org/project/genome-editing/ethical-review-published-september-2016> (last accessed September 1, 2017).

<sup>7</sup> Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science* 346, 1258096 (2014). DOI: 10.1126/science.1258096  
Wang F, Qi, L.S. Applications of CRISPR Genome Engineering in Cell Biology. *Trends Cell Biol* 26, 875–888 (2016).  
DOI: <http://dx.doi.org/10.1016/j.tcb.2016.08.004>

<sup>8</sup> Fogarty, N.M.E., McCarthy, A., Snijders, K.E., Powell, B.E., Kubikova, N., Blakeley, P., Leo, R., Elder, K., Wamaitha, S.E., Kim, D., Maciulyte, V., Kleinjung, J., Kim, J.-S., Wells, D., Vallier, L., Bertrero, A., Turner, J.M.A. & Niakan, K.K. Genome editing reveals a role for OCT4 in human embryogenesis. *Nature* 550, 67–73 (2017). doi:10.1038/nature24033

<sup>9</sup> Nuffield Council on Bioethics. Genome editing: An ethical review (2016). <http://nuffieldbioethics.org/project/genome-editing/ethical-review-published-september-2016> (last accessed April 13, 2018).



strengthen ongoing discussions on regulatory aspects thereof. Gene drive, genetically modified viruses and their potential environmental release are explained by Guy Reeves with the intention to differentiate between approaches and their potential applications. Genome editing in stem cell research and also laboratory methods that could be used to advance understanding of biological processes is presented by Hans R. Schöler and Thomas Rauen. Stefan Mundlos and Hans Schöler then discuss new avenues of research and therapy development in humans. The current legal framework of genome editing is the topic of a chapter authored by Silja Vöneky. Finally, the ethics of genome editing together with societal opportunities and challenges are discussed by Klaus Tanner and Christiane Walch-Solimena.

The authors are well aware that many questions raised concerning genome editing are currently unsolved and will need further time and consideration in order to be fully investigated.

In the interest of transparency, and an open public discourse on newly arising research areas, this paper aims to place these questions into the wider context of the law, ethics and society. Discussions in one of these areas, heritable genome editing in humans, has dramatically accelerated since November 2018, when He Jiankui from China announced the birth of twins whose genomes he had altered with CRISPR-Cas9 to generate AIDS resistance.

In a recent publication in *Nature*<sup>10</sup>, a prominent group of 18 scientists and bioethicists has called for a global moratorium on introducing heritable changes into DNA (human in sperm, eggs or embryos) to make genetically modified children. We have taken note of this initiative, but will not comment on it here since it came out after conclusion of the deliberations of our Working Group. We deem further discussions on heritable human genome editing necessary.

## 2. The Development and Impact of Genome Editing Technologies

*Christina Gross and Emmanuelle Charpentier*

Ever since the discovery of DNA as the molecule of inheritance and the elucidation of the genetic code, scientists have sought the means to generate and modify DNA in order to understand the functions of genes and their products. Fundamental research in microbiology endowed scientists with the methods and tools, such as polymerase chain reaction and restriction modification of DNA that gave rise to modern molecular genetics and thus revolutionized the life sciences. Gene targeting technologies that allow the modification of DNA at precise chromosomal locations in cells and living organisms further accelerated biological research.<sup>11</sup> Here, homologous DNA containing a gene or a mutated or repaired variant thereof is integrated at the desired genomic site by exploiting the endogenous homologous recombination machinery. However, the low efficiency of gene targeting curbs its application in many cells and organisms. The observation that site-specific double-stranded breaks in the DNA stimulate homologous recombination suggested a means to improve upon these technologies,<sup>12,13</sup> but for several years this remained little more than theoretical possibility because of the lack of methods to target nucleases to specific genomic sites.

The development of programmable nucleases greatly simplified the modification of genes and ushered in the era of precision genome editing. Zinc finger nucleases (ZFNs)<sup>14</sup> and transcription activator-like effector nucleases (TALENs)<sup>15</sup> were the first programmable nucleases. These chimeric molecules fuse a nuclease domain to a programmable DNA-binding domain to target DNA with high specificity and efficiency. By increasing the efficiency of homologous recombination, they greatly facilitated gene targeting strategies and expanded their application to additional types of cells and organisms.<sup>16</sup> When used alone, ZFNs and TALENs generate double-stranded breaks in the DNA that are subject to non-homologous end joining by the host cell DNA repair machinery. Because this strategy creates small insertions, deletions or point mutations that often disrupt gene function, ZFNs and TALENs allowed the rapid generation of gene knockouts in diverse cells and organisms.

Despite their specificity and efficiency, a notable disadvantage of ZFNs and TALENs is that specificity is encoded by their amino acid sequence, so they must be re-engineered for every DNA target. Research on the bacterial RNA-guided

<sup>10</sup> Lander, E., Baylis, F., Zhang, F., Charpentier, E. et al. Adopt a moratorium on heritable genome editing. *Nature* 567, 165–168 (2019).

<sup>11</sup> Capecchi, M. R. Gene targeting in mice: functional analysis of the mammalian genome for the twenty-first century. *Nat. Rev. Genet.* 6, 507–512 (2005).

<sup>12</sup> Rouet, P., Smih, F. & Jasin, M. Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. *Mol. Cell. Biol.* 14, 8096–8106 (1994).

<sup>13</sup> Rudin, N. & Haber, J. E. Efficient repair of HO-induced chromosomal breaks in *Saccharomyces cerevisiae* by recombination between flanking homologous sequences. *Mol. Cell. Biol.* 8, 3918–3928 (1988).

<sup>14</sup> Kim, Y. G., Cha, J. & Chandrasegaran, S. Hybrid restriction enzymes: zinc finger fusions to Fok I cleavage domain. *Proc. Natl. Acad. Sci. U.S.A.* 93, 1156–1160 (1996).

<sup>15</sup> Miller, J. C. et al. A TALE nuclease architecture for efficient genome editing. *Nat. Biotechnol.* 29, 143–148 (2011).

<sup>16</sup> Gaj, T., Gersbach, C. A. & Barbas, C. F., III. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol.* 31, 397–405 (2013).



CRISPR-Cas (clustered regularly interspaced short palindromic repeats - CRISPR-associated) adaptive immune system that combats mobile genetic elements such as bacteriophages and plasmids hinted at the existence of RNA-guided nucleases.<sup>17,18</sup>

The discovery and repurposing of Cas9 as a programmable, RNA-guided nuclease was heralded as a breakthrough in genome editing.<sup>19</sup> In contrast to earlier technologies, Cas9 and other RNA-guided Cas proteins such as Cas12a/Cpf1<sup>20,21</sup> can be easily programmed to target new DNA sequences by providing the enzyme with an RNA guide complementary to the desired target site. The CRISPR-Cas9 technology not only allows the editing of genes in human cells, animals, and plants, but also makes it feasible to target multiple genes simultaneously. As with ZFNs and TALENs, Cas9 simply cuts the DNA; the actual change in the sequence of the DNA is accomplished by the endogenous DNA repair and recombination machinery. An exception are the newly developed programmable base editors, which fuse a catalytically inactive programmable nuclease (e.g. dCas9) to a base editor (e.g. cytidine deaminase).<sup>22</sup> These enzymes directly change individual nucleotides in the DNA and have the potential to correct disease-associated point mutations. The programmable DNA binding of dCas9

can also be exploited to control gene expression by fusing dCas9 to transcriptional repressors or activators, or to DNA (de-)methylases or histone (de-)acetylases.<sup>23</sup>

Because of its versatility and ease of design as compared to earlier technologies, RNA-programmable Cas9 has rapidly and universally been adopted to engineer or correct mutations, modulate gene expression and mark DNA in a wide variety of cell types and organisms in the three domains of life.<sup>24</sup> This powerful technology has not only revolutionized life science research by broadening genome editing, but is also recognized for its promising and potentially transformative applications in biotechnology, medicine and agriculture that are elaborated upon in the following pages and illustrated with the examples from MPG research projects shown below (Boxes 1-3). Naturally, the ability to easily edit and engineer genomes in humans and other species raises ethical questions that require careful attention and discussion within and beyond the scientific community. What would be, for example, the implications of genome editing in reproductive treatment of human subjects when introducing changes that will be passed on to future generations?

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<sup>17</sup> Barrangou, R. et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science* 315, 1709–1712 (2007).

<sup>18</sup> Deltcheva, E. et al. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature* 471, 602–607 (2011).

<sup>19</sup> Jinek, M. et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337, 816–821 (2012).

<sup>20</sup> Zetsche, B. et al. Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System. *Cell* 163, 759–771 (2015).

<sup>21</sup> Fonfara, I., Richter, H., Bratovič, M., Le Rhun, A. & Charpentier, E. The CRISPR-associated DNA-cleaving enzyme Cpf1 also processes precursor CRISPR RNA. *Nature* 532, 517–521 (2016).

<sup>22</sup> Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A. & Liu, D. R. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 533, 420–424 (2016).

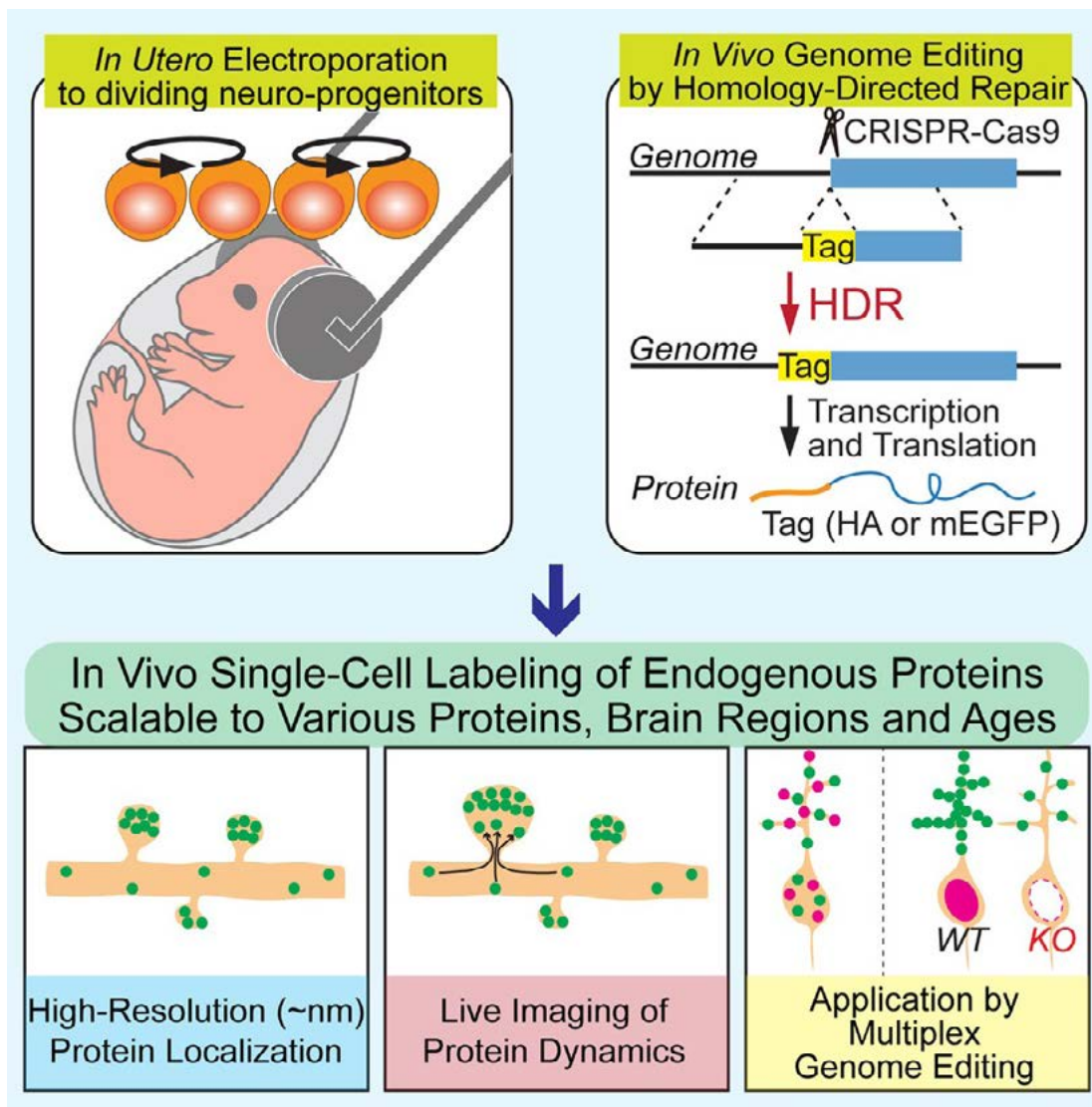
<sup>23</sup> Dominguez, A. A., Lim, W. A. & Qi, L. S. Beyond editing: repurposing CRISPR-Cas9 for precision genome regulation and interrogation. *Nat. Rev. Mol. Cell Biol.* 17, 5–15 (2016).

<sup>24</sup> Doudna, J. A. & Charpentier, E. The new frontier of genome engineering with CRISPR-Cas9. *Science* 346, 1258096–1258096 (2014).



Box 1

## Genome editing in basic research: Protein labeling in the developing mouse brain



Mikuni T et al., *Cell* 165:1803–1817 (2016) © 2016 Elsevier Inc.

In order to better understand cognitive functions of the brain such as learning and memory, scientists need to develop technologies to observe cellular processes in live and behaving animals. Thus far, techniques to observe individual proteins in single neurons have not been available. Scientists at the Max Planck Florida Institute for Neuroscience have now developed such a method named SLENDR (single-cell labeling of endogenous proteins by CRISPR-Cas9-mediated homology-directed repair). For this, genome editing is used to 'knock in' a desired genetic tag precisely at one end of a gene of interest. If di-

viding progenitors of neurons in embryos are targeted by this procedure, a fluorescent protein is generated in the developing neurons. This approach is scalable to various proteins, brain regions and ages and thus allows the observation of the sub-cellular localization of proteins by fluorescence microscopy in the living brain as an important measure for their function.

Source: Mikuni T, Nishiyama J, Sun Y, Kamasawa N and Yasuda R. High throughput, high resolution mapping of protein localization in mammalian brain by in vivo genome editing. *Cell* 165:1803–1817 (2016). Published online 2016 May 12. Doi: 10.1016/j.cell.2016.04.044



### 3. Genome Editing in Plants

Detlef Weigel

Genome editing in plants has been used commercially for over a decade, initially based on oligonucleotide-directed mutagenesis (ODM) and then on zinc finger nucleases and TAL effector nucleases (TALENs). Probably the first commercial example of a genome-edited crop was SU-canola, a canola (rape seed) variety tolerant to sulfonyleurea herbicides. SU-canola is marketed by the US biotech firm Cibus, and has received regulatory approval in the US in 2016. Another biotech start-up firm, Calyxt, has generated several crop varieties with immediate benefit to consumers, such as reduced trans-fat soybean, lower saturated fat canola, or gluten reduced wheat, several of which have already undergone field trials. This is important, because a widespread concern with conventional transgenic crops has been that they have delivered benefits primarily to farmers. Genome editing promises to dramatically change this.

Until the arrival of the CRISPR-Cas9 technology, companies probably used genome editing more often than academics did, because the early methods were cumbersome and expensive. As in biomedical basic research, CRISPR-Cas9 genome editing has taken the plant research community by storm. It allows for the rapid generation of single and multiple mutants in many different genetic backgrounds, and makes non-model organisms, including polyploid ones (those with several sets of near-identical chromosomes), much more accessible to basic research. In addition, an important prospect seems to be field experiments, because the mutations that can be introduced by genome editing can be physiologically more relevant than transgenes. This can help to understand the fitness value of different genes and pathways in the real world. It will, however, depend very much on the legal framework whether field experiments will be easily possible.

Similar to the biomedical arena, all major players in the seed industry have licensed the technology from academic institutions, reflecting the promise that industry sees in the technology.

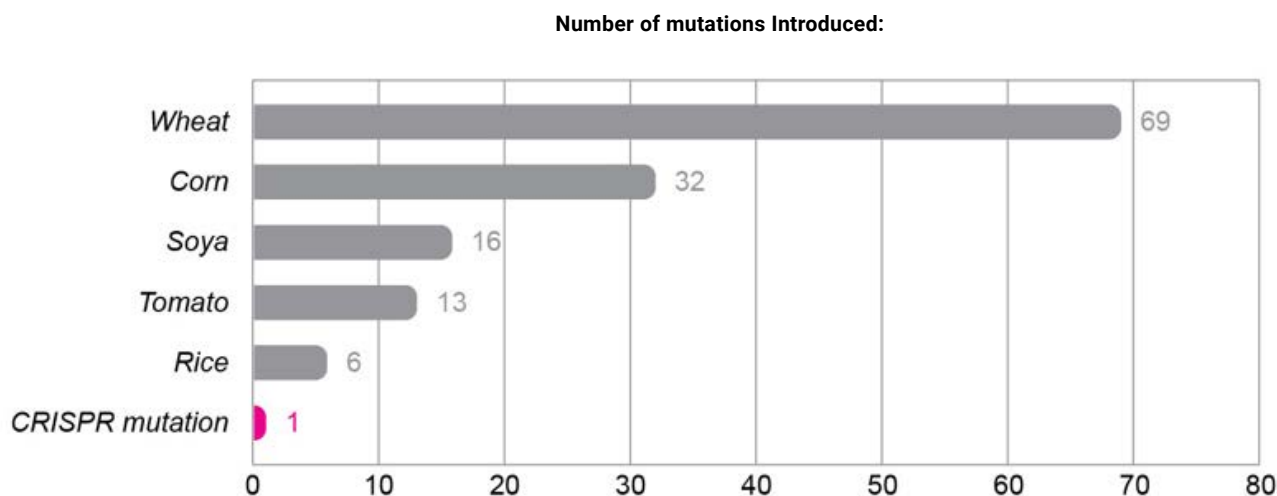
A major reason for the excitement over genome editing in plants is that many desirable traits that have been selected during domestication and breeding are due to knockout mutations, arguably the easiest mutations to engineer and also the ones least likely to encounter regulatory hurdles. Such variants can now be rapidly introduced into the genomes of many different species and varieties. In addition, the replacement of crop genes by the homologous genes from wild relatives will often be desirable as well.

Genome editing may also help to maintain genetic diversity in crops, since it is much easier and faster to introduce a desirable mutation by genome editing into different varieties than by conventional crossing. A further advantage is that one can

recognize much more quickly whether a potentially beneficial mutation has the same, positive effect in different varieties.

Different from human cells, genome editing in plants has typically made use of transgenes that specify expression of the genome editing machinery such as TALENs or Cas9 and its guide RNAs. This poses certain restrictions even if the Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms is amended, such that genome edited plants with minor modifications become exempt from it, or are subject to a lower bar of regulation than conventional genetically modified organisms (GMOs). A first prerequisite for such genome-edited plants to not fall under the same regulatory framework as conventional GMOs would be that the transgenes are removed by crossing. While this is in principle not difficult, proving that there are no longer any foreign DNA sequences in the genome is not trivial. For this reason, several of the major companies are pursuing transgene-free approaches, where Cas9 protein-guide RNA complexes are assembled in vitro and then delivered directly into plant cells (which is the standard approach in biomedical applications). This approach requires, however, considerably more sophisticated technological knowhow. In case Directive 2001/18/EC is amended, it will be important to determine whether regulatory hurdles should differ for genome edited plants obtained via the transgene route and those obtained by direct delivery of protein/RNA complexes. Such differences will impact use by basic researchers (i.e., field experiments) and by smaller breeding companies, neither of which can easily afford the investments required for approval of conventional transgenic plants.

From the perspective of the change in DNA sequence, point mutations introduced by CRISPR-Cas9 genome editing, such as single base pair substitutions, deletions or additions, are often very similar to natural, spontaneous mutations. Indeed, genome editing allows for recreation of mutations that are found naturally in one strain in a different strain, and this is of interest both for basic science and for crop breeding. The rate of such spontaneous mutations is surprisingly high (*Figure 1*); in a genome, the size of humans there are many dozens spontaneous mutations per generation. (Note that many crops have genomes that are considerably larger than those of humans). As with spontaneous mutations, CRISPR-Cas9 merely damages the DNA; the cell's own protein machinery then repairs the damage, which may or may not lead to a mutation. Because of the high background rate of spontaneous mutations, the introduction of individual mutations, particularly point mutations, but also chromosome rearrangements, by CRISPR-Cas9 would barely increase the overall mutation rate, making it very different from random, genome wide mutagenesis by chemicals or irradiation, which typically introduce hundreds, if not thousands of mutations at a time.



**Figure 1: Estimated number of natural spontaneous mutations that occur in every individual plant (grey) compared to a hypothetical single base introduced using CRISPR gene editing (pink).** It is important to note that the indicated number of spontaneous mutations will occur in every individual every generation while the CRISPR mutation will be introduced only once during the development of a commercial or research variety.

Because the current regulatory framework for genetically modified plants in the EU (Directive 2001/18/EC) could not anticipate targeted genome editing; its language appears ambiguous with respect to genome edited plants. It may be reasonably argued, that genome edited plants (and other organisms) do not fall under the Directive, although the European Court of Justice (EUCoJ) ruled otherwise. The Directive states that “genetically modified organism (GMO)” means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.” As discussed above, in terms of the change in DNA sequence,

point mutations as well as structural chromosome rearrangements introduced by targeted genome editing are generally indistinguishable from spontaneous mutations. It seems advisable that Directive 2001/18/EC be updated to either exempt genome-edited plants from the regulations of the Directive, or that at least a lower bar is implemented for allowing release of genome-edited plants, as compared to conventional GMOs.

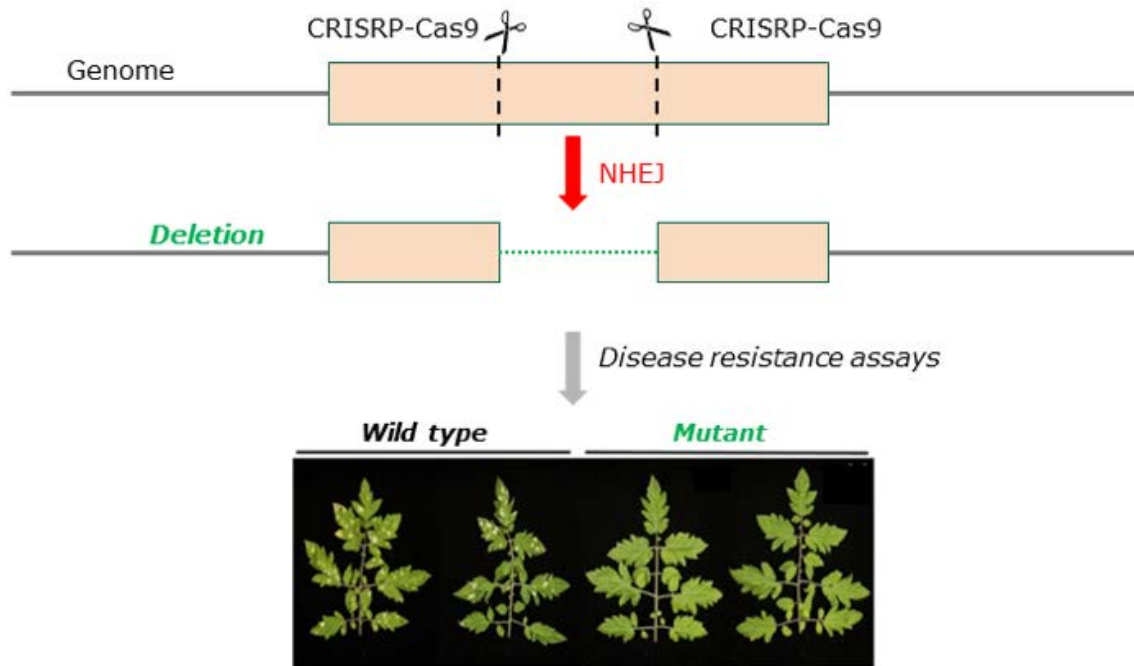
Lastly, regarding the question of whether regulation of genome edited plants should be process or product based, this needs to be handled carefully. We advocate complete transparency and that approval of genome edited varieties requires disclosure of the genetic changes that were made, including the motivation of making these changes as well as how the background knowledge was obtained. In our opinion, this is important to prevent that genetic knowledge acquired in violation of the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity (“Nagoya Protocol”) is illegally exploited.





Box 2

## Genome editing in plant engineering: Transgene-free powdery mildew resistant tomato



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In the face of increasing demand for food of a growing world population and changing growth conditions for crops in the age of climate change, there is a need to generate new plant varieties with valuable traits. Desirable are higher yields, better disease resistance or improved salt and drought tolerance. Scientists at the Sainsbury Laboratory (UK), the Institute of Digital Agriculture (China) and the Max Planck Institute for Developmental Biology have used genome editing with CRISPR-Cas9 to generate a tomato variety resistant to powdery mildew in less than ten months. For this, the wildtype gene responsible for susceptibility to the fungal pathogen was deleted. The components of the CRISPR-Cas9 system

were segregated away in the next plant generation leaving non-transgenic plants with a deletion as it could also occur in nature. The procedure worked with great precision as no mutations were detected in unwanted places of the genome, so-called off-target effects. This technology has great potential to revolutionize plant breeding, since mutations can be introduced into other tomato varieties in less than a year with relatively minimal effort and investment.

Source: Nekrasov V, Wang C, Win J, Lanz C, Weigel D and Kamoun S. Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Scientific Reports* 7:482 (2017). Published online 2017 March 28. Doi: 10.1038/s41598-017-00578-x





## 4. Gene drive and preliminary thoughts about environmental release

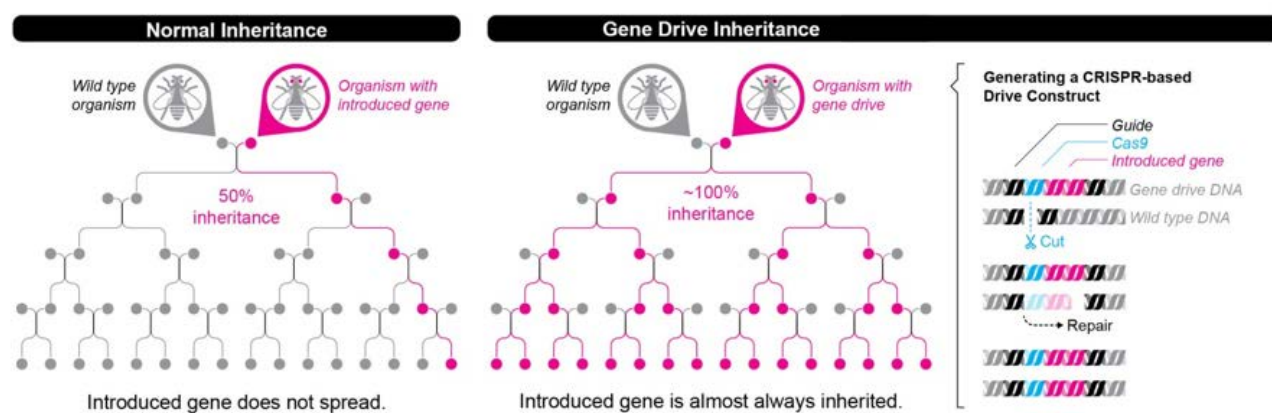
Guy Reeves

Gene drive is a collection of experimental techniques intended to be used to push foreign genes into the chromosomes of wild populations. Though the idea of gene drive can be traced to before the 1970s<sup>25</sup> there have been no attempts to apply gene drive technology in the environment. The principle proposed application of gene drive is to limit the capacity of wild animals to spread disease; a frequently used example is targeting a specific mosquito species to make it unable to spread human malaria. Other applications in crop protection and environmental conservation have also been proposed as gene drive applications.

The original formulation of gene drive was to leave the population size and dynamics of the target population/species unchanged, except for the intentionally introduced genetic change(s). The overriding value of such an approach is that where the genetic change is effective in controlling disease it would be entirely self-sustaining e.g. robust to manipulation. This self-sustaining property could be of enormous value, conceivably even in circumstances where other effective disease

interventions are available. For example, while prophylactic drugs, vaccines, insecticides and bed nets are effective in malaria control, all of them require sustained resources and coordination where established drive systems may not.

As illustrated in *Figure 2*, genetic drive is initiated by the release of individuals with one or multiple drive constructs integrated into their chromosomes. Upon mating between released individuals and wild ones, the drive constructs have the capacity to increase their frequency in wild chromosomes over subsequent generations. Small differences in the many ways drive constructs could be engineered or the nature of the target population/species can result in large differences in key properties of the drive. However, in general, over the course of several generations, the drive constructs can theoretically increase their frequency to the point that all individuals in the target populations possess drive constructs (this could conceivably occur in as few as 5 generations though it will usually take longer).



**Figure 2: How can gene drive work? For an introduced gene (pink) without any driving properties, only a small proportion of descendants are likely to inherit it (left panel). However, for an introduced gene with drive properties a majority of surviving descendants inherit it (middle panel). This is due to an engineered mechanism which favors its transmission to the next generation. Over generations this can lead to all chromosomes in a population possessing the introduced gene.**

If drive is generated using CRISPR editing this can be done through the conversion of heterozygous individuals (one drive construct per pair of chromosomes) into homozygotes (copies of the drive construct on both chromosomes). An illustration of the cut-and-paste approach that may be exploited in a CRISPR drive construct is shown in the right panel.

Drive systems can be usefully thought of having two extremes, based on the relative numbers of individuals that need to be released in order to be successfully initiated.

<sup>25</sup> Curtis, C.F. Possible Use of Translocations to Fix Desirable Genes in Insect Pest Populations. *Nature* 218, 368–369 (1968).



**High threshold drive systems:** Large numbers of individuals need to be initially released, often over several generations to exceed a threshold frequency in the wild target population, e.g. 50%. Only above the threshold frequency will drive construct frequency increase. This means that releases are more resource intensive to initiate but drive is much less likely to spread to other populations or inter-fertile species (or be initiated accidentally).

**Low threshold drive systems:** Only small numbers of individuals need to be released to start the drive process, at its theoretical extreme only a single individual may be sufficient (including accidental release). These drive approaches have the potential to readily jump between populations and to inter-fertile species or subspecies. While some mechanisms have been proposed to limit the geographic and taxonomic spread of such drive elements, if they proved to be ineffective, very large numbers of individuals over wide geographic areas may be impacted. This would increase the potential for genetic resistance to the drive mechanism or the anti-disease effect to evolve, and also make monitoring for such changes more challenging.

In addition, there is a class of what can be thought of as “crippled drive systems” which require continued periodic releases to prevent spontaneous reversal of the population to its original wild state<sup>26</sup>. While not being fully self-sustaining, crippled approaches have been proposed as a prudent and more reversible first-step in the application of this technology.

Prior to the advent of the CRISPR-Cas9 technology, drive was already an area of active research using ZFNs, TALENs, RNAi, and meganucleases. This work was primarily funded by governments and charitable foundations largely in the context of malaria control. Considerable thought had been given to the issues arising from the fact that drive technology can be viewed as not having a realistic personal opt-out. For example, while people in a community can generally elect not to be involved in vaccination or drug treatment programs, this would not be the case where drive constructs had been introduced to a local species. The no opt-out property can also be extended internationally for low-threshold drive systems where the spread between countries is a realistic prospect. It is notable that much of the more recent work exploiting CRISPR-Cas9 tends to be focused on the less predictable low threshold systems, where even the techniques claiming reversibility appear to be designed to leave transgenic drive elements in the wild population (e.g. CRISPR-Cas9 guide RNA constructs<sup>27</sup>).

In common with many other authors we recognize that the gene drive technology is biologically and ethically highly complex. However, this technology does have the potential to

contribute sustainable solutions in circumstances when no attractive alternatives exist. We note however, that if it is perceived that vigorous application of less avant-garde methods have been overlooked, this may contribute to a less positive public view of this experimental technology.

### **Genetically modified virus intentionally released into the environment, preliminary thoughts**

Only genetically modified viruses whose intended application is dependent on their being intentionally released into the environment are considered here. Genetically modified viruses developed for the direct treatment of individuals in clinical or veterinary settings would be covered by other sections of this document. While the focus here is on CRISPR expressing viruses many of the same issues are raised by any genetically modified microorganisms that are intended for dispersal in the environment (e.g. bacteria, fungi or plasmodia).

Genetic modification of many viral species has been possible for over 50 years, due in part to their small and relatively simple genomes. Indeed, experimental field trials of a baculovirus genetically modified to increase its pathogenicity to an insect crop pest occurred in the UK in 1993<sup>28</sup>. Many proposed modifications for use in the environment do not incorporate the expression of CRISPR-Cas9 technology, though viruses with CRISPR activity have been developed for contained use in 2015 (this is not a natural capacity in any known virus). Using CRISPR enabled viruses to alter the genome of a second non-viral species in the environment (e.g. a crop plant or insect disease vector) is an active area of research. Potential applications that might be envisaged include species-specific herbicides or insecticides (to which resistance could be readily managed). While proposals for the use of genetically modified viruses generally share the advantages of both speed and flexibility of action, long standing questions surrounding the controllability of viruses in the environment remain to be addressed.

<sup>26</sup> Gould, F. et al., A Killer–Rescue System for Self-Limiting Gene Drive of Anti-Pathogen Constructs. *Proc. Biol. Sci.* 275, 2823–2829 (2008). doi: <https://doi.org/10.1101/115618>

<sup>27</sup> Min, J. et al., Daisy Quorum Drives for the Genetic Restoration of Wild Populations. (2017).

<sup>28</sup> Cory, J. S. et al., Field Trial of a Genetically Improved Baculovirus Insecticide, *Nature* 370, 138–40, (1994). doi:10.1038/370138a0



**Examples of proposals for applied use of genetically modified viruses in the environment where CRISPR use could be envisaged<sup>29</sup>:**

<i>Wildlife immunization</i>	It has been proposed to use genetically modified viruses to protect wild rabbit populations utilized for hunting from other viruses that were internationally introduced to control pest populations (e.g. myxomatosis and rabbit haemorrhagic disease). A field trial of such a virus was conducted on a remote Spanish Island in 2000.
<i>Insecticidal viruses</i>	As in the baculovirus example given above genetic modifications are made to increase the pathogenicity of viruses to insect pests.
<i>Viral paratransgenesis to limit the capacity of wild insects to spread diseases</i>	The aim is to introduce a genetically modified symbiotic virus into wild populations of insects in an effort to limit the replication or transmission of pathogens that they spread. A possible example is the modification of a virus infecting mosquitoes that spread malaria. <sup>30</sup>
<i>Using insects to spread GM viruses to cause genetic modification of mature plant crops.</i>	A US government agency program to generate genetically modified viruses was initiated in Nov 2016 with a 4-year timetable until "large greenhouse demo". <sup>31</sup> Expression of CRISPR-Cas system is explicitly mentioned in the scientific plan.
<i>Control of bacterial pathogens in agriculture</i>	A US company is currently developing modified viruses to control an emerging bacterial pest of commercial citrus fruit trees. <sup>32</sup> They have a pending application to US regulators for experimental use permission covering approximately 7 million orange trees (USDA 17-044-101r).

<sup>29</sup> Though only one current project explicitly mentions CRISPR enabled viruses in the environment.

<sup>30</sup> Xiaoxia Ren, Egbert Hoiczyk, and Jason L. Rasgon, "Viral Paratransgenesis in the Malaria Vector *Anopheles Gambiae*," *PLOS Pathogens* 4, no. 8 (August 22, 2008): e1000135, doi:10.1371/journal.ppat.1000135.

<sup>31</sup> "Insect Allies - HR001117S0002 (Archived) - Federal Business Opportunities: Opportunities," accessed July 16, 2017, [https://www.fbo.gov/index?s=opportunity&mode=form&id=c1b79c54a1aa8f5990f7b4a3cc7f6576&tab=core&\\_cview=0](https://www.fbo.gov/index?s=opportunity&mode=form&id=c1b79c54a1aa8f5990f7b4a3cc7f6576&tab=core&_cview=0).

<sup>32</sup> Heidi Ledford, "Geneticists Enlist Engineered Virus and CRISPR to Battle Citrus Disease," *Nature News* 545, no. 7654 (May 18, 2017): 277, doi:10.1038/545277a.



## 5. Genome editing (CRISPR-Cas9 system) in stem cell research

Thomas Rauen and Hans R. Schöler

The CRISPR-Cas9 technology has been widely used to produce human disease models in experimental organisms and ranks today amongst the leading molecular biological techniques in basic stem cell research. Combining both technologies, iPSC and CRISPR-Cas9, will not only boost stem cell and genetic research but will also impact personalized medicine.

### The CRISPR-Cas9 technology and basic stem cell research

#### Induced pluripotent stem cells (iPSC)

The ability to induce pluripotency in somatic cells has initiated a new era in the field of regenerative medicine. Induced pluripotent stem cells (iPSCs) specific to individual patients could provide an unlimited source of specialized cell types for replacing diseased or aged tissues. In addition to the prospect of future iPSC-based cell replacement therapies, the ability to derive iPSCs from patients has had a major impact on human disease modeling and has now almost completely replaced previous experimental systems studying human genetic diseases using overexpression studies in cancer cell lines.

Using patient-derived neuronal cells, the most significant progress towards disease modeling was made in the field of neurodegenerative disease because animal models only partially recapitulated these human diseases. Studying dopaminergic neurons differentiated from Parkinson patient-derived iPSCs, Reinhardt et al.<sup>33</sup> provided insights into the molecular causes of the disease and uncovered initial clues for the development of new therapeutics. This is just one example amongst many in the success story of human disease modeling using patient-iPSC derived cells in culture. However, modeling age-related diseases, such as neurodegenerative diseases, appear to be challenging using the iPSC-technology. Patient-specific donor cells fail to maintain age-associated markers, because of the rejuvenating effect of the reprogramming process and, therefore, may not be suitable to model late-onset disease. Consequently, modeling of age-related disease in iPSC-derived lineages often reveal phenotypes that inadequately recapitulate disease.

In contrast to iPSC-derived neurons, induced neurons (iN – generated by a process called direct conversion, transdifferentiation or direct reprogramming of somatic cells into neurons), preserve epigenetic features of their donors age, and thus, enable senescence-associated disease modelling.<sup>34</sup> Hence, the lack of cellular maturity can either be bypassed by the direct conversion of somatic cells into induced neurons (iN) using genetically optimized transcription factor cocktails or genetic modifications to reestablish the age-related gene signature of iPSC-derived cells. Both approaches benefit significantly from the simplicity, the time- and the cost-efficiency of the CRISPR-Cas9-platform in rapidly generating genetic modifications to recapitulate human aging processes in the cell culture dish.

Another important application of CRISPR-Cas9 is its use in generating isogenic iPSC controls to compensate for the inherent variability between individual iPSC lines in their potential to differentiate into functional cells of a given lineage. This variation between cell lines is unpredictable and mostly caused by genetic background differences as well as the reprogramming history.<sup>35</sup> The generation of isogenic pairs of disease-specific, gene-corrected and targeted insertion of the disease causing mutation in patient-iPSCs and iPSCs of healthy donors, has been successfully used to control for line background variation, allowing for the discovery of high-resolution details of the disease causing molecular mechanism.<sup>39</sup> Further development of protocols that directly combine genome editing with reprogramming for the rapid generation of gene-targeted iPSC cell lines would not only accelerate basic research considerably, but would also facilitate transplantation medicine by making gene-corrected cells available to patients in a more timely manner.<sup>36</sup>

#### iPSC-derived Organoids

Recent advances in iPSC-technology have enabled the generation of three-dimensional, self-organizing human in vitro tissues called organoids that have many potential applications in drug discovery and disease research.<sup>37</sup> In combination with precise CRISPR-Cas9 genome editing, the organoid technology allows the generation of in vitro models of neurodegenerative diseases and overcomes the limitations of two-dimensional

<sup>33</sup> Reinhardt P, Schmid B, Burbulla LF, Schondorf DC, Wagner L, Glatza M, Hoing S, Hargus G, Heck SA, Dhingra A, Wu G, Muller S, Brockmann K, Kluba T, Maisel M, Kruger R, Berg D, Tsytsyura Y, Thiel CS, Psathaki OE et al. (2013) Genetic correction of a LRRK2 mutation in human iPSCs links parkinsonian neurodegeneration to ERK-dependent changes in gene expression. *Cell Stem Cell* 12: 354–67.

<sup>34</sup> Mertens J, Paquola AC, Ku M, Hatch E, Bohnke L, Ladjevardi S, McGrath S, Campbell B, Lee H, Herdy JR, Goncalves JT, Toda T, Kim Y, Winkler J, Yao J, Hetzer MW, Gage FH (2015) Directly Reprogrammed Human Neurons Retain Aging-Associated Transcriptomic Signatures and Reveal Age-Related Nucleocytoplasmic Defects. *Cell Stem Cell* 17: 705–18.

<sup>35</sup> Soldner F, Stelzer Y, Shivalila CS, Abraham BJ, Latourelle JC, Barrasa MI, Goldmann J, Myers RH, Young RA, Jaenisch R (2016) Parkinson-associated risk variant in distal enhancer of alpha-synuclein modulates target gene expression. *Nature* 533: 95–9.

<sup>36</sup> Howden SE, Maufort JP, Duffin BM, Elefanty AG, Stanley EG, Thomson JA (2015) Simultaneous Reprogramming and Gene Correction of Patient Fibroblasts. *Stem Cell Reports* 5: 1109–18.

<sup>37</sup> Lancaster MA, Knoblich JA (2014) Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 345: 1247125.



monolayer cultures. iPSC-derived multicellular organoid systems of the human central nervous system (CNS), which bear the hallmarks of organ-like complex cytoarchitecture and function, can be utilized to further study neurodegenerative diseases via single- and multiple genetic knockouts directly in human tissue for the first time. This allows for the creation of both, disease models and basic science-oriented loss-of-function and gain-of-function studies as well as their translation from two-dimensional monolayer systems to the more functional relevant 3D context, which will ensure a multicellular relevant interplay between diverse neuronal cell types.

### Mouse Models and CRISPR-Cas9

The CRISPR-Cas9 system offers significant advantages over other, traditional directed mutagenesis methods, including shorter timelines and the capacity to alter multiple genes simultaneously. CRISPR-Cas9 gene editing is a rapidly growing field in which new techniques and methods are evolving to edit mouse genomes.<sup>38</sup>

New mouse models are easily generated by simply injecting Cas9 mRNA and either one or multiple single guide RNAs (sgRNAs) directly into mouse embryos to generate genomic edits at specific loci. Mice carrying the desired mutation(s) are bred to confirm germline transmission and new mutations can be directly generated in the genetic background of choice. Alternatively, mice can be created from mouse embryonic stem cells that were selected for the desired mutation after CRISPR-Cas9 treatment. Additionally, gene editing using CRISPR-Cas9 can also introduce mutations into specific organs or tissues in postnatal mice by local or systemic injection of separate Cas9 and sgRNA expressing lenti- or adeno-associated viruses. A particularly active area is the use of mouse-CRISPR-gene-editing technologies for generating mouse models to investigate the pathogenic effect of certain human sequence variants or to create mice that carry the same disease-causing mutations as in patients, and can be used as pre-clinical models for developing and validating novel therapies.

A major concern and potential limitation of the CRISPR-Cas9-system is related to the function of Cas9 to cut the DNA thereby initiating the cell's repair mechanism. This usually results in mutations at the target site (desired) but also has the possibility of cleavage and unintended editing at other sites in the genome (off-target sites – see also below). This could not only confuse phenotypic analyses of CRISPR-Cas9-generated mouse mutants, particularly in founder animals (see for review<sup>39</sup>), but would also have implications for other CRISPR-Cas9 editing applications, especially for therapeutic gene treatments and germ line editing (see below).

### Limits of CRISPR-Cas9: Off-Target and unintended On-Target Effects

While CRISPR-Cas9 is able to precisely target and cut specific DNA stretches, it is not yet fully understood how well the RNA-guided Cas9 can discriminate between perfect targets and potential off-targets with mismatches to the intended target sequence. Of particular concern is that there have been, so far, no well-designed efforts to detect genome-wide off-target mutations in an unbiased manner, mostly because the confident detection of rare mutations (such as 1 mutation in a genome with billions of basepairs) is technically extremely challenging. Many of the early efforts focused on improving on-target efficiency, but this will often also increase off-target effects.

Especially for medical applications in humans, minimization of off-target effects but also optimizing the effects at the target site are essential. Fortunately, a series of efforts have been undertaken to enhance CRISPR-Cas9 on-target specificity, by selection of improved Cas9 variants, approaches with defective Cas9 proteins (dCas9 strategy), use of other nucleases, and alterations in the guide RNAs. We agree that understanding the frequency and impact of CRISPR-Cas9 off-target mutations is critical for the development of **clinical applications**.

At the same time, we emphasize that for **basic research**, it seems that the necessary experiments will be readily available to control for CRISPR-Cas9 off-target effects. For example, Hockemeyer and colleagues suggest experiments to adequately address off-target effects should include: (i) the use of several independent guide RNAs to generate a mutant cell line, (ii) complementation of loss-of-function phenotypes, and (iii) secondary editing of the mutant cell line to revert the mutation to a wild-type allele followed by confirmation of phenotypic rescue; and as mentioned above WGS on CRISPR-Cas9-edited cells, organoid-tissues and/or mice.<sup>40</sup>

### CRISPR-Cas9 and animal welfare

*Christiane Walch-Solimena*

The CRISPR-Cas9 mechanism has been adopted as a genetic manipulation tool in a wide variety of model systems due to its ease of use, efficiency and flexibility, raising the question of how this technique might affect the number of animals used in research. While conventional genetics require crossing to select a desired genotype in animals (typically mice), CRISPR-Cas9 enables production and phenotyping of genome-edited animals within a single generation. Therefore, this technology could contribute to the realization of the 3R's principle (Replacement, Reduction, and Refinement)<sup>41</sup> of animal welfare by reducing the number of animals needed for a given experiment as compared to crossing-based genetics in biomedical research (Reduction).

<sup>38</sup> Singh P, Schimenti JC, Bolcun-Filas E (2015) A mouse geneticist's practical guide to CRISPR applications. *Genetics* 199: 1–15.

<sup>39</sup> Wang G, Qi LS. (2016) Applications of CRISPR Genome Engineering in Cell Biology. *Trends Cell Biol.* 26: 875–888.

<sup>40</sup> Hockemeyer D, Jaenisch R (2016) Induced Pluripotent Stem Cells Meet Genome Editing. *Cell Stem Cell* 18: 573–86.

<sup>41</sup> Russell WMS, Burch RL, (1959). *The Principles of Humane Experimental Technique*, Methuen, London.



Other developments, however, having an impact on animal research should be considered. New genome-editing methods open opportunities to address new questions in different areas of biology, from the cell to organism level. In particular, the CRISPR-Cas9 technology enables the genetic manipulation of a much broader species range, not only conventional genetic model systems including *Drosophila*, nematode worms, zebrafish, mice, and cultured mammalian cells, but also nontraditional models where scientists have less experience in terms of experimentation and measures of animal welfare. A continuously expanding array of Cas9 tools provides new avenues to better study the causality between genotype and phenotype, including the study of functional genomics and genomic imaging.<sup>42</sup> Furthermore, the CRISPR-Cas9 toolbox allows creating better models of disease, hopefully even of complex human diseases such as diabetes or neurodegenerative diseases. Again, the versatility of the method has already advanced research in rodent models, but is particularly promising in larger mammals such as pigs.<sup>43</sup> Besides in live animal

models, CRISPR-Cas9 is also successfully used in “diseases in the petri dish”<sup>44</sup> or in patient-derived 3D organoid cultures.<sup>45</sup> This approach can provide data relevant to individual patients and enable replacement of animal experiments.

During a Roundtable on Gene Editing to Modify Animal Genomes of the National Academies’ Institute for Laboratory Animal Research (ILAR) in December 2015, experts in this field discussed the impact of genome editing on the 3Rs. Arguments ranged from “a massive impact on the use of animals in science” to new opportunities through in vitro models as potential surrogates therefore.<sup>46</sup>

While it is still too early to assess the impact of genome editing on the use and welfare of animals in research, these trends have to be closely monitored and the 3Rs principle should be consistently applied, with particular emphasis on highest ethical and scientific standards for new animal models.

## 6. Genome editing in humans

Hans Schöler and Stefan Mundlos

The CRISPR-Cas9 technology can of course also be used to edit the human genome. Many potential applications are currently pursued. A particularly active area of CRISPR related research is the genetic manipulation of patient-derived stem cells to create models for example via organoids for various diseases such as colon cancer, cystic fibrosis, cardiomyopathy, brain malformations, and many others. With CRISPR-Cas9, it is now possible for researchers to correct disease-causing mutations in patient-derived pluripotent stem cells to create isogenic cell lines to differentiate to any cell type of interest for disease research. Generating these isogenic lines is making it possible to unambiguously show the contribution of gene mutations to a disease phenotype. To study the pathogenic effect of human sequence variants is now made much easier by creating similar changes in human cells or in animal models, in particular mice.

The CRISPR-Cas9 technology has a huge potential for therapeutic applications. It is important to distinguish two different approaches and target cells. If the genome is modified in early embryos, all or most of the cells including the germ line, the lineage of the germ cells, will carry the change. This means

that the genomic modification can be passed on to the following generations.

In contrast, the genetic manipulation of somatic cells – all cells besides the germ cells – cannot be inherited by future generations because only cells that are derived from mutated cells will carry the mutation. Genome editing with CRISPR-Cas9 in somatic cells is generally considered to have a large potential in the treatment of congenital genetic diseases and cancer. In contrast to earlier gene therapy approaches that used viral vectors to insert exogenous genes in the genome, CRISPR-Cas9 offers the opportunity to directly change the genome precisely at the desired position. Congenital mutations can, for example, be reverted to the non-mutated state, can be disrupted to inactivate a mutated gene copy, or genomic changes can be introduced that make cells resistant against e.g. a virus. In this case, cells are removed from the body, genetically manipulated, and then returned to the patient. It is expected that this technology will become a routine part of clinical treatment options soon. Ethical considerations involve the existing system of regulatory oversight and ethical norms of somatic cell and gene therapy that is currently in use around the world.

<sup>42</sup> Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. *Science* 346, 1258096 (2014).

DOI: 10.1126/science.1258096 (last accessed 05-09-2017)

Wang G, Qi LS, Applications of CRISPR Genome Engineering in Cell Biology. *Trends Cell Biol* 26: 875-888 (2016).

DOI: <http://dx.doi.org/10.1016/j.tcb.2016.08.004> (last accessed 05-09-2017)

<sup>43</sup> Dow LE. Modeling disease in vivo with CRISPR/Cas9. *Trends Mol Med* 21: 609-621 (2015).

DOI: 10.1016/j.molmed.2015.07.006 (last accessed 05-09-2017)

<sup>44</sup> Marx V. Stem cells: disease models that show and tell. *Nature Methods* 12: 111-114 (2015).

doi:10.1038/nmeth.3263 (last accessed 05-09-2017)

<sup>45</sup> Dow LE. Modeling disease in vivo with CRISPR/Cas9. *Trends Mol Med* 21: 609-621 (2015).

DOI: 10.1016/j.molmed.2015.07.006 (last accessed 05-09-2017) and references therein

<sup>46</sup> ILAR Roundtable ,Gene Editing to Modify Animal Genomes for Research – Scientific and Ethical Considerations’, December 7-8, 2015. <http://nas-sites.org/ilar-roundtable/roundtable-activities/gene-editing-to-modify-animal-genomes-for-research/webcast/> (last accessed 05-09-2017)



Many different approaches are currently under way. These include first clinical trials to treat cancer by removing T cells, to genetically modify the T cells so that, infused back into a patient, they can target and destroy tumor cells. Other approaches target cells of the hematopoietic system, in hemophilia or to treat genetic eye diseases such as Leber congenital amaurosis. In contrast to the usual CRISPR method of extracting cells and re-injecting them into the patient other approaches attempt to attack the human viruses (e.g. Human Papillomavirus or HPV) inside the human body by a non-invasive treatment with the aim to disable the tumor growth mechanism in HPV cells. This CRISPR-based treatment went into clinical trial to combat HPV, which impacts millions of people worldwide. Many more clinical trial CRISPR studies can be expected to occur soon.

One of the major challenges in CRISPR-Cas9 based gene therapy is in the delivery of the components (guide-RNA, Cas9) necessary to initiate the editing process. In cultured mammalian cells various methods of transfection can be used to transiently express Cas9 and gRNAs. Lentiviral vectors have also been used to constitutively express Cas9 and/or gRNAs in cultured human and mouse cells with higher efficiency. However, for gene therapy other approaches are needed. Viral vectors like rAAV and adenovirus, which have robust expression albeit only in the short term, have been suggested and are currently used.

One problem that is encountered in the use of CRISPR-Cas9 is that the system recognizes specific genomic sequences and induces DNA double-strand breaks, which can be repaired by a non-homologous end-joining (NHEJ) repair pathway or by homology-directed repair (HDR). Repair by NHEJ is error prone, a property that can be used e.g. to inactivate a gene, but it is unsuitable for correcting mutations for therapeutic purposes as it may introduce additional mutations ultimately resulting in mixed alleles and cells with different sets of DNA sequences (mosaicism). One approach is therefore to increase HDR vs NHEJ repair to increase precision and/or to select cells with the correct genotype prior to treatment. A further concern are off-target mutations (which can result from editing components binding to sequences with high similarity elsewhere in the genome). Their presence or absence is difficult to prove, as this would involve high precision whole genome sequencing including the detection of structural variations such as deletions, duplications, or translocations.

In contrast to the genetic manipulation of somatic cells, editing the human germ line (includes primordial germ cells, gamete progenitors, gametes, zygotes and embryos) is highly controversial. Heritable genome editing has been discussed for parents known to be at risk of passing on a serious genetic disease to their offspring. Thousands of such diseases that are caused by single gene mutations are known. While individually rare, collectively they affect a sizable fraction of the population and because many of these conditions are severe, they can cause a major burden for affected families. However, approximately 75 % of genetic disease occurs in couples with no family history of the disease in question (i.e. mutations arise newly in the sperm or eggs of the parents).

For the minority of parents with an indicator of familial risk current elective options to prevent transmission of inherited genetic diseases include deciding not to have children, adopting a child, or the use of donated sperm or eggs. Alternatively, in vitro fertilization combined with preimplantation diagnostics can be used to select non-affected embryos. In most situations 50-75% of screened embryos will be unaffected and given a sufficient number of available embryos most couples can identify unaffected embryos (this is not the case for mitochondrial disease). Where a couple has only a small number of embryos available, genome editing in affected embryos theoretically offers the opportunity to augment the preimplantation diagnostic approach to correct existing mutations.

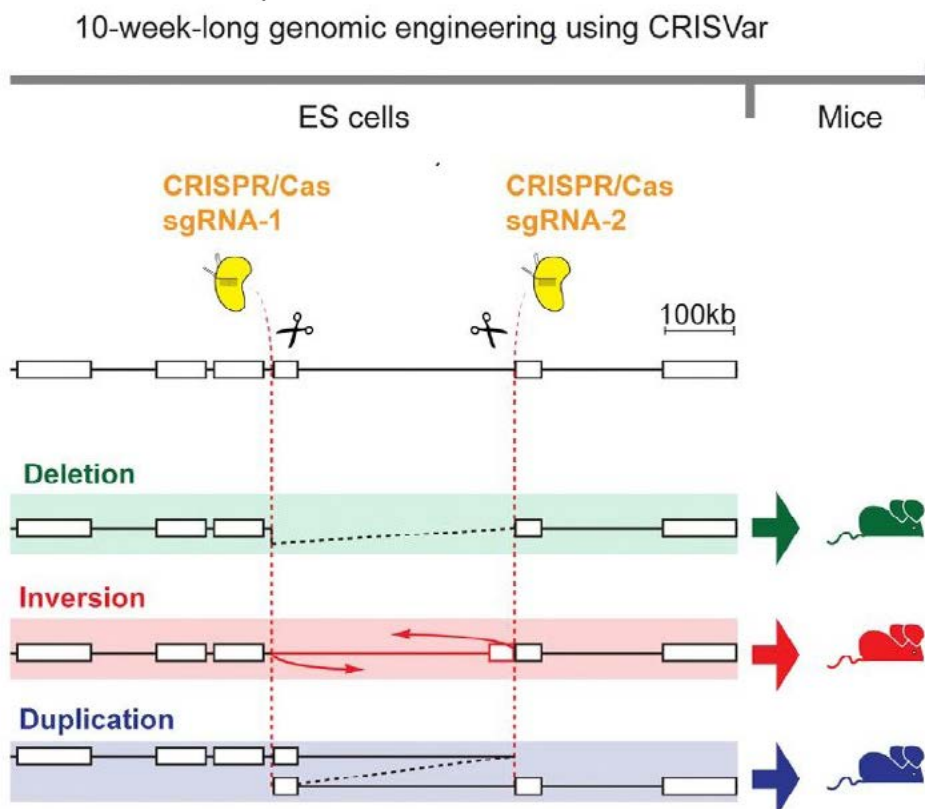
However, multiple concerns responding to perceived risks exist: First, human germline genetic modification is banned in Germany and in 13 other European countries. Safety issues with this new technology are, in particular in regard to off-target effects, not solved. Considering the possible risks, the restriction to preventing a serious disease and the absence of reasonable alternatives would be an absolute requirement. Furthermore, comprehensive long-term multigenerational follow-up would be needed to exclude side effects, a requirement that seems difficult to impossible to fulfill. Under these conditions CRISPR germline interventions are currently out of question.





Box 3

## Genome editing in mouse genetics: Engineering of structural variants for human disease models



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Genetic disorders in humans are caused by different types of mutations. These can be changes in the letter sequence of the genetic code or affect DNA structure in a way that the number or position of letters is altered across large segments of the DNA molecule, resulting in duplications, deletions, inversions or translocations affecting sometimes large parts of chromosomes. In animal models of genetic diseases, thus far it has been impossible or at least labor-intensive and time-consuming to recapitulate these so-called structural variations causing cancer or rare diseases. Scientists from the Max Planck Institute for Molecular Genetics in collaboration with colleagues from Charité - Universitätsmedizin Berlin have recently developed a technique named CRISVar (CRISPR-Cas9 induced structural variants) using CRISPR-Cas9 to engineer such vari-

ations of long stretches of DNA (up to over one megabase) in embryonal stem cells to generate mice in a 10-week protocol. Using this approach they were able to recapitulate a human bone malformation syndrome (Nievergelt-like syndrome) through a large disease-associated genome deletion in an in vivo mouse model. This technique will allow studying the complex molecular pathology of this and other genetic diseases caused by structural variation.

Source: Kraft K, Geuer S, Will AJ, Chan WL, Paliou C, Borschiwer M, Harabula I, Wittler L, Franke, M, Ibrahim DM, Kragestein BK, Spielmann M, Mundlos S, Lupiáñez DG and Andrey G. Deletions, Inversions, Duplications: Engineering of Structural Variants using CRISPR/Cas in Mice. Cell Reports 10:833–839 (2015).  
Published online 2015 February 5. doi.org/10.1016/j.celrep.2015.01.016



## 7. Legal framework

Silja Vöneky

The legal implications of genome editing differ with regard to the above-mentioned fields; besides, the legal implications depend on the types of the new technique and where the research is conducted.

### Legal framework for genome editing in plants and in other living organisms

It is not entirely clear, and will be discussed in the following, whether and to what extent genome editing is governed by existing rules of public international law, European law and national (esp. German) law.

As there are no special rules of customary international law that governs genome editing or genetic engineering there is no specific international law regime that binds all States with regard to the techniques mentioned above.

At the universal level the 2000 Cartagena Protocol on Biosafety to the Convention on Biological Diversity is the decisive international treaty which entails binding rules for living modified organisms (LMOs) that may have adverse effects on biological diversity.<sup>47</sup> Its primary aim is „to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology (...)“.<sup>48</sup> This aim is in accordance with the precautionary principle - as legal principle - which states that where “there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation.”<sup>49</sup> Having 171 parties<sup>50</sup>, 170 States (including Ger-

many) and the EU,<sup>51</sup> the Cartagena Protocol is an important international agreement for the regulation of living modified organisms even though relevant State actors have not signed or ratified the treaty.<sup>52</sup>

However, with regard to the new genome editing techniques in plants and in other living organisms described above, there is – up to now – no intense debate regarding whether organisms derived from genome editing are “living modified organisms” as established by the Protocol.<sup>53</sup> The definition of the term “living modified organism” in Art. 3 Cartagena Protocol<sup>54</sup> is broader than its respective definition in European law and German Law on genetic engineering, which will be discussed below.<sup>55</sup> Whether this definition captures all forms of genome editing is unclear as the scope of the treaty is limited to living modified organisms resulting from modern biotechnology. Here it is questionable whether the development of point mutations overcomes “natural physiological reproductive or recombinant barriers” as is required by the definition of the notion “modern biotechnology” that is part of the treaty.<sup>56</sup> It is not disputed, however, that the Cartagena Protocol is applicable if foreign DNA is integrated into the target organism’s genome.

Further, as a general exemption, the Protocol does not apply to the transboundary movement of living modified organisms in the form of pharmaceuticals for humans which are addressed by other relevant international agreements or organizations.<sup>57</sup> If there is a conflict between the Cartagena Protocol and international law governing trade relations, such as GATT and SPS, it is argued that the Cartagena Protocol, as being *lex specialis* and *lex posterior*, will prevail.<sup>58</sup>

<sup>47</sup> Adopted on 29 January 2000; entered into force on 11 September 2003.

<sup>48</sup> Art. 1 Cartagena Protocol: “In accordance with the precautionary approach contained in Principle 15 of the Rio Declaration on Environment and Development, the objective of this Protocol is to contribute to ensuring an adequate level of protection in the field of the safe transfer, handling and use of living modified organisms resulting from modern biotechnology that may have adverse effects on the conservation and sustainable use of biological diversity, taking also into account risks to human health, and specifically focusing on transboundary movements.”

<sup>49</sup> See Principle 15 of the Rio Declaration on Environment and Development. There are, however, different definitions of the precautionary principle as an *ethical principle*, and it is disputed which scenarios should be governed by a non-legal precautionary principle, see for instance Cass R. Sunstein, *Laws of Fear – Beyond the Precautionary Principle*, 2005, p. 109 et seq., and Daniel Steel, *Philosophy and the Precautionary Principle – Science, Evidence, and Environmental Policy*, 2015, p. 44 et seq.

<sup>50</sup> Cf. list of Parties, available at: <http://bch.cbd.int/protocol/parties/>.

<sup>51</sup> Accession of the EC in 2002; cf. Council Decision 2002/628/EC.

<sup>52</sup> Most relevantly the U.S. is not a State Party of the Protocol.

<sup>53</sup> See for instance Araki/Nojima/Ishii, 32 (2014), *Trends in Biotechnology*, 234

<sup>54</sup> Art. 3 Cartagena Protocol: (...) (g) “Living modified organism” means any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology; (h) “Living organism” means any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids; (...). For an interpretation cf. Mackenzie/Burhenne–Guilmin et al, in IUCN (ed.), *An Explanatory Guide to the Cartagena Protocol on Biosafety*, 2003, 46, para. 212 (available at: <https://portals.iucn.org/library/sites/library/files/documents/EPLP-046.pdf>).

<sup>55</sup> Cf. Mackenzie/Burhenne–Guilmin et al, in IUCN (ed.), *An Explanatory Guide to the Cartagena Protocol on Biosafety*, 2003, 46, para. 214 (available at: <https://portals.iucn.org/library/sites/library/files/documents/EPLP-046.pdf>).

<sup>56</sup> Art. 3 Cartagena Protocol: “(...) (i) “Modern biotechnology” means the application of: a. In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or b. Fusion of cells beyond the taxonomic family, *that overcome natural physiological reproductive or recombination barriers* and that are not techniques used in traditional breeding and selection; (...)” (*italics added*).

<sup>57</sup> Art. 5 Cartagena Protocol.

<sup>58</sup> Cf. Böckenförde, *Biological Safety, MPEPIL*, para. 19 et seq.



As far as it is unclear whether the scope of the 2000 Cartagena Protocol includes new techniques of genome editing the scope of the *2010 Nagoya – Kuala Lumpur Supplementary Protocol to the Cartagena Protocol on Biosafety* is not settled.<sup>59</sup> After entering into force at 5 March 2018<sup>60</sup> the Kuala Lumpur Supplementary Protocol will bind State Parties with regard to questions of liability and redress if the transboundary movement of living modified organisms has caused<sup>61</sup> damage.<sup>62</sup>

The European Union legislation on genetic engineering is laid down in various legal acts. Most relevant is Directive 2001/18/EC<sup>63</sup> which entails the decisive definition of the term “genetically modified organism” (GMO).<sup>64</sup> The applicability of the EU GMO legislation depends on the interpretation of this definition, as the remaining legal acts refer either to this Directive<sup>65</sup> or use mostly identical definitions.<sup>66</sup>

The debate about the applicability of existing European law to organisms derived from genome editing was controversial and complex. The legally relevant arguments referred to different elements of the definition that is part of the Directive as well as to the interpretation of its Annexes.<sup>67</sup>

The definition of the term “genetically modified organism” in Art. 2 of the Directive (“in which the genetic material has been altered in a way that does not occur naturally...”) creates substantial interpretational leeway, rendering – on the one hand – a broader process related interpretation and – on the other hand – a narrower process and product related interpretation justifiable.<sup>68</sup> Not convincing seems a very narrow, purely product related interpretation of the definition since the Directive refers to different techniques whose use does or does not produce GMOs.<sup>69</sup>

Some authors argue, on the contrary, that the definition of GMOs only refers to the process of making the alteration:<sup>70</sup> They bring forward that since point mutations cannot be produced deliberately through natural processes, the Directive is applicable. According to this view all non-natural alterations of an organism’s genome have the effect that there exists a GMO regulated by EU legislation.

Interpreting the definition as process- and product-based others authors bring forward that organisms with point mutations induced by genome editing techniques will “normally not be within the scope of GMO definition”. The main argument is that organisms with point mutations induced by genome editing techniques also occur through mating or natural recombination or – at least – that those organisms “could have come into existence naturally” by mating, natural recombination or traditional breeding methods.<sup>71</sup>

The discussion became more complex as Annex I A Part 1 of the Directive lists processes which are expressly included in the Directive’s scope of application and it was, inter alia, debated whether genome editing processes count as recombinant nucleic acid techniques or as direct injection of heritable material as laid down by the Annex.<sup>72</sup>

Even more importantly, Annex I B of the Directive lists certain processes, which are expressly excluded from the Directive’s scope of application according to Art. 3 (1) Directive. Here, it was discussed whether the production of point mutations through genome editing count as “mutagenesis” as laid down by the Annex. Some argue that chemicals used for conventional mutagenesis processes often interact with the DNA and create mismatches, which are repaired in a similar way as

<sup>59</sup> Art. 3 Nagoya – Kuala Lumpur Supplementary Protocol on Liability and Redress to the Cartagena Protocol on Biosafety.

<sup>60</sup> The protocol was adopted on 15 October 2010 and entered into force on the ninetieth day after the date of deposit of the 40th instrument of ratification, acceptance, approval or accession, cf. Art. 18 Nagoya – Kuala Lumpur Supplementary Protocol. Cf. list of Parties, available at: <https://bch.cbd.int/protocol/supplementary/>.

<sup>61</sup> For the condition of causation cf. Art. 4 Nagoya – Kuala Lumpur Supplementary Protocol: “A causal link shall be established between the damage and the living modified organism in question in accordance with domestic law.”

<sup>62</sup> What constitutes damage is defined in Art. 2 (2) lit. b. For exemptions cf. Art. 6 Nagoya – Kuala Lumpur Supplementary Protocol.

<sup>63</sup> Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms.

<sup>64</sup> Art. 2 (2) Directive 2001/18/EC: “genetically modified organism (GMO), means an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination; (...)”

<sup>65</sup> Cf. Art. 3 (2) Regulation (EC) No 1946/2003 of the European Parliament and of the Council of 15 July 2003 on transboundary movements of genetically modified organisms: ““genetically modified organism”, or “GMO”, means genetically modified organism as defined in Article 2(2) of Directive 2001/18/EC, excluding organisms obtained through the techniques of genetic modification listed in Annex IB to Directive 2001/18/EC.”

<sup>66</sup> Cf. Art. 2 lit. b Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms: In the English version, the definition is identical to that of Directive 2001/18/EC, while the French and German versions have minor differences.

<sup>67</sup> Art. 2 (2) Directive 2001/18/EC: “Within the terms of this definition: (a) genetic modification occurs at least through the use of the techniques listed in Annex I A, part 1; (b) the techniques listed in Annex I A, part 2, are not considered to result in genetic modification; (...)”

<sup>68</sup> Cf. Dederer, in Nakanishi (ed.), *Contemporary Issues in Environmental Law* (2016), 139, 140 et seq.; Wolt/Wang/Yang, *The Regulatory Status of genome edited Crops 14* (2016), *Plant biotechnology journal* 510; Breyer/Herman et al., 8 (2009) *Environmental biosafety research* 57, 61; Kahrman/Bömeke/Leggewie, *EurUP* 2, 2017, 179.

<sup>69</sup> See as well Kahrman/Bömeke/Leggewie, *EurUP* 2, 2017, 179.

<sup>70</sup> Because of the wording “altered in a way” Art. 2 (2) Directive 2001/18/EC; cf. Spranger, *Legal Analysis of the applicability of Directive 2001/18/EC*, October 2015, 26, 41 et seq.; Herdegen/Dederer, *Richtlinie 2001/18/EG*, in Herdegen/Dederer (ed), *Internationales Biotechnologierecht*, November 2015, Vol. I, Part 3, para. 40.

<sup>71</sup> Kahrman/Bömeke/Leggewie, *EurUP* 2, 2017, 180; Ostertag, *GVO-Spuren und Gentechnikrecht*, 2006, 161. For another view see Spranger, *ibid.*, p. 17; Callebaut, *New developments in modern biotechnology*, Master Thesis 2015, p. 56.

<sup>72</sup> Cf. Spranger, *ibid.*, p. 51; Callebaut, *ibid.*, p. 59.



some genome editing techniques.<sup>73</sup> Others criticize that the term “mutagenesis” should be interpreted in light of the historical context of the norm and the precautionary principle, which is why the exemption cannot include the newest technological developments that do not have a sufficient safety record.<sup>74</sup>

Looking at the different competent authorities, EU organs and national organs, the scope of the Directive also did not become much clearer until the decision of the the European Court of Justice (ECJ) in 2018. The European Commission did not publish a final statement but seems to argue that the definition of GMO in the EU legislation is referring both to the characteristics of the organism obtained and to the techniques used.<sup>75</sup> The ECJ had to decide on a case concerning the current questions, especially the interpretation of Annex I B of the Directive and the precautionary principle.<sup>76</sup> For the ECJ Advocate General Bobek delivered an opinion on January 2018.<sup>77</sup> According to this „an organism obtained by mutagenesis can be a GMO under Article 2(2) (of Directive 2001/18/EC) if it fulfils the substantive criteria laid down in that provision.”<sup>78</sup> He stresses, that “Article 2(2) clearly does not require the insertion of foreign DNA in an organism in order for the latter to be characterised as a GMO. It merely says that the genetic material has been altered in a way that does not occur naturally.”<sup>79</sup> And he states that “on the textual level alone, it is already quite clear that it is incorrect to state that under the GMO Directive, there would be a straightforward and unqualified exemption for any and all mutagenesis techniques”.<sup>80</sup> According to his interpretation “a generic category labelled ‘mutagenesis’ should logically encompass all those techniques that are, at the given moment relevant for the case in question, understood as forming part of that category, including

any new ones.”<sup>81</sup> However, he concludes with regard to the understanding of the mutagenesis exemption that “the EU legislature did not make any statement about its safety” and the “exclusion simply meant that the EU legislature did not wish to regulate that matter at EU level.” Hence, his result is that “Member States have the competence to regulate organisms obtained through mutagenesis provided that they comply with their overall EU law obligations”.<sup>82</sup>

Those legal scholars and scientists who argued that the ECJ should decide accordingly were disappointed by the final ruling of the court in July 2018, when the Court decided that organisms whose genetic material has been modified by targeted mutagenesis are subject to the EU Release Directive 2001/18.<sup>83</sup> In line with the Advocate General, the Court first held that organisms produced by mutagenesis qualify as GMOs within the meaning of Directive 2001/18. In the view of the ECJ there is no difference between conventional mutagenesis methods and genome editing as both methods “alter the genetic material of an organism in a way that does not occur naturally, within the meaning of that provision”.<sup>84</sup> The Court deemed this interpretation to be supported by the general scheme of the directive, “which is made clear by a distinction between techniques the use of which results in genetic modification and techniques which are not considered to result in such genetic modification.”<sup>85</sup> However, contrary to the Advocate General’s view, the Court decided that organisms modified with targeted mutagenesis techniques based on genome editing are not exempted from the scope of Directive 2001/18. Essentially relying on recital 17 of that Directive, the Court held that the exemption for organisms obtained from “mutagenesis” only applies to “organisms obtained by techniques/methods

<sup>73</sup> Kahrman/Bömeke/Leggewie, EurUP 2, 2017, 181.

<sup>74</sup> Spranger, *ibid.*, p. 24 et seq., 32.

<sup>75</sup> 17 October 2014, answer of the Commission to the parliamentary question of 3 September 2014, E-006525/2014. “Answer given by Mr Borg on behalf of the Commission: 1. The decision to include or exclude a technique from the scope of Directives 2001/18/EC and 2009/41/EC depends on the interpretation of the definition of Genetically Modified Organisms/Genetically Modified Microorganisms and of the conditions for exemption provided for in the two Directives. This evaluation is complex, because the definition of GMO in the EU legislation is referring both to the characteristics of the organism obtained and to the techniques used. Furthermore, the legislation was drafted when a number of new plant breeding techniques were at an initial stage of development or application and therefore were not specifically addressed. 2. The opinion of the European Food Safety Authority (EFSA) which was requested by the Commission relates the risk assessment of cisgenic and intragenic plants. It does not relate to the legal interpretation of the definition of GMO as laid down in Directive 2001/18/EC which the Commission is carrying out. 3. The Commission intends to complete the legal analysis of new plant breeding techniques within the forthcoming months.” (Available at <http://www.europarl.europa.eu/sides/getAllAnswers.do?reference=E-2014-006525&language=IT>).

<sup>76</sup> Case C-528/16; Request for a preliminary ruling from the Conseil d’État (France) lodged on 17 October 2016; Questions include: “1. Do organisms obtained by mutagenesis constitute genetically modified organisms within the meaning of Article 2 of Directive [2001/18/EC] of 12 March 2001, although they are exempt under Article 3 of and Annex IB to the directive from the obligations laid down for release and placing on the market of genetically modified organisms? In particular, may mutagenesis techniques, in particular new directed mutagenesis techniques implementing genetic engineering processes, be regarded as techniques listed in Annex IA, to which Article 2 refers? Consequently, must Articles 2 and 3 of and Annexes IA and IB to Directive 2001/18 of 12 March 2001 be interpreted as meaning that they exempt from precautionary, impact assessment and traceability measures all organisms and seeds obtained by mutagenesis, or only organisms obtained by conventional random mutagenesis methods by ionising radiation or exposure to mutagenic chemical agents existing before those measures were adopted? (...)”

<sup>77</sup> Opinion of Advocate General Michael Bobek delivered on 18 January 2018, Case C-528/16, available at: <http://curia.europa.eu/juris/document/document.jsf?text=&docid=198532&pageIndex=0&doclang=EN&mode=lst&dir=&occ=first&part=1&cid=135991>.

<sup>78</sup> *Ibid.*, para. 65.

<sup>79</sup> *Ibid.*, para. 61.

<sup>80</sup> *Ibid.*, para. 82.

<sup>81</sup> *Ibid.*, para. 101.

<sup>82</sup> *Ibid.*, para. 123, cf as well 117 et seq.; besides 153-167 with regard to Directive 2002/53.

<sup>83</sup> Court of Justice of the European Union, Case C-528/16 – Confédération paysanne et al. ./ Premier ministre et al., Judgment of 25 July 2018, ECLI:EU:C:2018:583.

<sup>84</sup> *Ibid.*, para. 29

<sup>85</sup> *Ibid.*, para. 32.



ods of mutagenesis which have conventionally been used in a number of applications and have a long safety record”.<sup>86</sup>

In the view of the Court, the “risks associated with techniques of directed mutagenesis involving the use of genetic engineering” and which have been developed since the adoption of Directive 2001/18 “have not thus far been established with certainty”.<sup>87</sup> Furthermore, the development of those new techniques made it possible to produce genetically modified varieties at a rate and in quantities quite unlike those resulting from the application of conventional methods of random mutagenesis.<sup>88</sup> Also referring to the precautionary principle, the ECJ concluded that the exemption of “mutagenesis” techniques from the scope of Directive 2001/18 did not apply to “organisms obtained by means of new techniques/methods of mutagenesis which have appeared or have been mostly developed since Directive 2001/18 was adopted”.<sup>89</sup>

German law on genetic engineering largely corresponds with the above-mentioned EU law. Because of the high degree of harmonization, an interpretation of the German law on genetic engineering that does not correspond with European law would be incoherent with the EU law principle of *effet utile*. Adhering to the aforementioned ECJ judgment, the German Federal Office of Consumer Protection and Food Safety (BVL) repealed an earlier administrative act in which it had decided that a rapeseed strain (SU Canola) developed by a private company using targeted mutagenesis (ODM) did not fall under the current German Genetic Engineering Law (GenTG).<sup>90</sup> BVL stated that this decision could not be upheld because the type of mutagenesis used to breed the strain “has only recently been used in plant breeding” and thus was not covered by the exemption of organisms derived from “mutagenesis” from GMO regulation pursuant to Sect. 3 para. 3b GenTG.<sup>91</sup>

Finally, it seems important to note that it is not disputed that the Directive and other elements of the EU GMO legislation, as well as German law implementing these provisions, are clearly applicable if foreign DNA is integrated into the target organism’s genome. In this case EU GMO legislation is applicable with regard to modern genome editing techniques even if an author supports the (narrower) process- and product-based interpretation of the Directive.<sup>92</sup>

States that are neither members of the EU nor Parties to the Cartagena Protocol are not governed by these specific international legal standards if foreign DNA is integrated into the target organism’s genome.

### Legal framework for gene-drives in plants and in other living organisms

In the case of gene drives in plants and in other living organisms, as insects, foreign DNA is integrated into the target organism’s genome, hence the EU GMO legislation and the Cartagena Protocol are applicable. In accordance with this finding, the German Central Committee on Biological Safety (ZKBS) agrees that organisms that were provided with a gene drive-system are genetically modified organisms in the sense of the relevant EU Directive and the German law on genetic engineering.<sup>93</sup>

However, if States that are parties of the Cartagena Protocol want to release gene drive insects there are legal questions that are not answered by the Protocol itself. A major question is whether the consent of the individuals who live in the area where the modified insects are released. To answer this question is not only of theoretical relevance but has important practical implications, as in 2018 it was reported that genetically engineered mosquitoes in Africa will be released for the first time.<sup>94</sup>

The experiments were one reason for a debate at the Conference of the Parties to the CBD in 2018 about whether there should be a (legally non-binding) moratorium that should stop these experiments and that should bind at least those States that are party of the CBD. However, no consensus was reached by the States parties of this Convention for such a moratorium. The relevant Working Group<sup>95</sup> made a decision that seems to spell out a leeway on how to proceed with gene drive experiments without violating international standards. This decision stressed that States should apply a precautionary approach with regard to gene drives. More specifically, it states that it

[...] also calls upon Parties and other Governments to only consider introducing organisms containing engineered gene drives into the environment, including for experimental

<sup>86</sup> Ibid., para. 44–46.

<sup>87</sup> Ibid., para. 47.

<sup>88</sup> Ibid., para. 48.

<sup>89</sup> Ibid., para. 51.

<sup>90</sup> Cf. BVL, Az. 42050 (5 February 2015); for the reasons cf. New Techniques Working Group, Final Report, 2012.

<sup>91</sup> BVL, Cibus Raps-Bescheid vom BVL zurückgenommen (17 August 2018), available at [https://www.bvl.bund.de/DE/06\\_Gentechnik/04\\_Fachmeldungen/2018/2018\\_08\\_17\\_Fa\\_Cibus\\_Raps\\_Bescheid.html](https://www.bvl.bund.de/DE/06_Gentechnik/04_Fachmeldungen/2018/2018_08_17_Fa_Cibus_Raps_Bescheid.html).

<sup>92</sup> Kahrman/Bömeke/Leggewie, EurUP 2, 2017, 180, 182.

<sup>93</sup> ZKBS, Stellungnahme zur Einstufung von gentechnischen Arbeiten zur Herstellung und Verwendung von höheren Organismen mit rekombinanten Gene-Drive-Systemen, February 2016.

<sup>94</sup> Cf. Scientific American, *Researchers to Release Genetically Engineered Mosquitoes in Africa for First Time* (2018), available at <https://www.scientificamerican.com/article/researchers-to-release-genetically-engineered-mosquitoes-in-africa-for-first-time/>.

<sup>95</sup> Conference of the Parties to the Convention on Biological Diversity, Fourteenth meeting Sharm El-Sheikh, Egypt, 17–29 November 2018, Agenda item 27, *SYNTHETIC BIOLOGY, Draft decision submitted by the Chair of Working Group II*, UN Doc. CBD/COP/14/L.31, 28 November 2018, available at <https://www.cbd.int/doc/c/2c62/5569/004e9c7a6b2a00641c3af0eb/cop-14-1-31-en.pdf>.



releases and research and development purposes, when:

- (a) Scientifically sound case-by-case risk assessments have been carried out;
- (b) Risk management measures are in place to avoid or minimize potential adverse effects, as appropriate;
- (c) Where appropriate, the “prior and informed consent”, the “free, prior and informed consent” or “approval and involvement”<sup>96</sup> of potentially affected indigenous peoples and local communities is sought or obtained, where applicable in accordance with national circumstances and legislation [...].<sup>97</sup>

The last paragraph spells out and proposes some criteria for a valid consent under the umbrella of the CBD, as it was interpreted by the Working Group. From a human rights point of view Art. 7 ICCPR might be relevant as well, if individuals are included in the experiments; according to this: ‘[...] (n) one shall be subjected without his free consent to medical or scientific experimentation’. This norm is fundamental and part of customary international law that binds every state. Besides, in the case of transboundary harm, States can be subject to liability for risks on the bases of rules of customary international law if a State violates its due diligence obligations.

### Legal framework for genome editing in humans

Concerning the legal implications of genome editing in humans there is the need to differentiate between the following ways and applications:

1. in Germany, genome editing that is part of a gene therapy and used and aimed to enhance the chance of survival of a human embryo is not prohibited by law; the same is true for somatic gene therapy;
2. human germline editing (i.e. human germline therapy) is prohibited in Germany – as in other European States – but there does not exist a universal prohibition of human

germline editing up to now (see below);

3. furthermore, the German Embryo Protection Act as part of criminal law prohibits the use of human embryos for scientific research, including generating embryonic stem cells; this is disputed, however, as far as non-viable human embryos (e.g. tri-pronuclear embryos) are used for research.<sup>98</sup> Other European States, such as the UK, Sweden and France, to the contrary, do not prohibit research with human embryos during a maximum period of the first 14 days following fertilization.

Usages of the new techniques that change the DNA of unborn human beings, as is the case with human germline editing, are highly controversial. Although it is prohibited *inter alia* in Germany<sup>99</sup> by national laws, and according to Article 13 Convention on Human Rights and Biomedicine of the Council of Europe (binding only 35 States parties), that is a regional international treaty norm, there does not exist a universal international law-based prohibition of human germline editing; even soft law UNESCO Declarations in the area of Bioethics and Biomedicine do not prohibit this type of gene editing. The German Ethics Council issued an opinion in September 2017 on this topic and argued that there is a need for global political debate and international regulation.<sup>100</sup> However, until now there was no consensus at the UNESCO to do so: in 2015 the UNESCO IBC called on member States to agree on a joint moratorium, but there was no consensus by member States. Besides, there was no consensus at the 2015 International Summit on Human Gene Editing that was organized by national science academies of three States (USA, UK and China).<sup>101</sup> The lacunae of current standards and regulations became apparent when in November 2018, a Chinese researcher informed the world of the birth of twins whose embryonic genomes had been edited. The researcher claimed that he edited two human embryos by using the CRISPR–Cas9 genome-editing technique and implanting them in a woman.<sup>102</sup> There were clear statements by the scientific community about the irresponsibility of the

<sup>96</sup> Decision XIII/18., CBD/COP/DEC/XIII/18, 17 December 2016, available at: <https://www.cbd.int/doc/decisions/cop-13/cop-13-dec-18-en.pdf>.

<sup>97</sup> Para. 9; see as well paras. 10 and 11 that state: ‘10. Recognizes that, as there could be potential adverse effects arising from organisms containing engineered gene drives, before these organisms are considered for release into the environment, research and analysis are needed, and specific guidance may be useful, to support case-by-case risk assessment; 11. Notes the conclusions of the Ad Hoc Technical Expert Group on Synthetic Biology that, given the current uncertainties regarding engineered gene drives, the free, prior and informed consent of indigenous peoples and local communities might be warranted when considering the possible release of organisms containing engineered gene drives that may impact their traditional knowledge, innovation, practices, livelihood and use of land and water; [...]’, Decision XIII/18., UN Doc. CBD/COP/DEC/XIII/18, 17 December 2016.

<sup>98</sup> Cf. Leopoldina (ed.), Discussion Paper 10: Ethical and legal assessment of genome editing in research on human cells, March 2017, 23.

<sup>99</sup> Act for the Protection of Embryos (*Embryonenschutzgesetz*), 13 December 1990, Bundesgesetzblatt (BGBl.) I, 2746, as amended on 21 November 2011, BGBl. I, 2228.

<sup>100</sup> German Ethics Council, *Germline intervention in the human embryo: German Ethics Council calls for global political debate and international regulation, Ad Hoc Recommendation*, 29 September 2017, available at <https://www.ethikrat.org/fileadmin/Publikationen/Ad-hoc-Empfehlungen/englisch/recommendation-germline-intervention-in-the-human-embryo.pdf>, at 2.

<sup>101</sup> Cf. National Academies of Sciences, Engineering, and Medicine, *On Human Gene Editing: International Summit Statement*, 3 December 2015, available at <http://www8.nationalacademies.org/onpinews/newsitem.aspx?RecordID=12032015a>. In 2017 in the US, a joint committee convened by the US National Academy of Sciences and the National Academy of Medicine argued that germline intervention were ethically defensible if this constituted the last reasonable option for a couple to have a healthy biological child, cf. Jocelyn Kaiser, ‘U.S. panel gives yellow light to human embryo editing’, *ScienceMag*, 14 February 2017, doi:10.1126/science.aal0750, available at <http://www.sciencemag.org/news/2017/02/us-panel-gives-yellow-light-human-embryo-editing>; see study report by the National Academies of Sciences, Engineering, and Medicine, *Human Genome Editing: Science, Ethics, and Governance* (2017), available at <https://www.nap.edu/catalog/24623/human-genome-editing-science-ethics-and-governance>.

<sup>102</sup> The researcher He Jiankui stated that the CCR5 gene in the embryos was modified; this gene encodes a protein that some common strains of HIV use to infect immune cells. See David Cyranoski, *First CRISPR babies: six questions that remain*, 30 November 2018, available at <https://www.nature.com/articles/d41586-018-07607-3>.



procedure.<sup>103</sup> In the aftermath, the need for the development of international norms and standards on setting limits for this kind of germline research and for creating effective oversight of germline editing was acknowledged by state officials.<sup>104</sup> As the experiments with the Chinese twins in 2018 showed, there is the need for more international discussion about the risks, benefits and human rights, even the dignity of humankind,<sup>105</sup> with regard to human germline intervention. Nevertheless, it is unclear whether there will be a chance to agree on a meaningful international consensus.

### Genome editing and dual use research of concern

There is the risk, that genome editing research results can be misused or constitute a serious harm, and can be defined as dual use research of concern.<sup>106</sup> Some of these research results may be covered by international and national rules concerning dual use activities; for others no such legal framework exists yet. After all neither the international nor the national legal framework may be fully adequate to protect against or fence in such risks.

The Biological Weapons Convention (BWC) and the Chemical Weapons Convention (CWC) do not provide sufficient protection internationally against the aforementioned risks and dangers of misuse of research that is conducted for peaceful purposes: The BWC does prohibit the production and use of biological weapons, however, it does not include a verification regime – laboratories in States cannot be monitored – and does not limit research for peaceful purposes. The CWC has many lacunae as well and only regulates specific, listed chemicals; it is (currently) not equipped to limit the risks and dangers of misuse of genome editing experiments and results.<sup>107</sup>

The possibilities of misuse and security risks of this kind must be carefully considered and analyzed, especially in times of

political unrest and terrorist activity. Several considerations and reactions are being discussed. They range from the encouragement of the researcher to reconsider the potential benefits and risks of such research over the prohibition of publication of the method of the genetic manipulation undertaken, over the prohibition of the results of such research to an outright prohibition of such research. Considering efficiency and the internationalization of research it is recommend that any such rule should be adopted not in isolation but on a broader basis be it regional or universal.

Such rules should carefully weigh the potential risks and the benefits to be expected on the basis of the research so far undertaken. It should be noted that in this context the Max Planck Society<sup>108</sup> and the DFG and Leopoldina have already enshrined these principles of responsible research in codes of conduct<sup>109</sup> that are adopted by Universities in Germany. Besides of this, federal oversight, or an EU or international committee, which can uniformly assess the rare cases of high-risk dual use research of concern experiments that are planned or conducted, would be desirable.

If such research is conducted, as the benefits outweigh the risks, it is important to enshrine questions on laboratory security as well as warning systems in case of accidents as effectively and universally as possible. In these areas, the regulations in Europe are still not coherent.<sup>110</sup>

In the long run, an international treaty for questions of dual use research of concern might be necessary. Such a treaty would offer legal certainty, especially since, in the case of transboundary harm, States can be subject to liability for risks on the bases of rules of customary international law if a State violates its due diligence obligations.

<sup>103</sup> See for instance Organizing Committee of the Second International Summit on Human Genome Editing, *Statement, On Human Genome Editing II*, 29 November 2018: '[...] At this summit we heard an unexpected and deeply disturbing claim that human embryos had been edited and implanted, resulting in a pregnancy and the birth of twins. We recommend an independent assessment to verify this claim and to ascertain whether the claimed DNA modifications have occurred. Even if the modifications are verified, the procedure was irresponsible and failed to conform with international norms. Its flaws include an inadequate medical indication, a poorly designed study protocol, a failure to meet ethical standards for protecting the welfare of research subjects, and a lack of transparency in the development, review, and conduct of the clinical procedures. [...]', available at <http://www8.nationalacademies.org/onpinews/newsitem.aspx?RecordID=11282018b>.

<sup>104</sup> US National Institutes of Health, Director Francis S. Collins, *Statement on Claim of First Gene-Edited Babies by Chinese Researcher*: "The need for development of binding international consensus on setting limits for this kind of research, now being debated in Hong Kong, has never been more apparent", available at <https://www.nih.gov/about-nih/who-we-are/nih-director/statements/statement-claim-first-gene-edited-babies-chinese-researcher>.

<sup>105</sup> Cf. Jürgen Habermas, *Die Zukunft der menschlichen Natur – Auf dem Weg zu einer liberalen Eugenik* (2001).

<sup>106</sup> According to a broad definition dual use research of concern is "research that based on current understanding, can be reasonably anticipated to provide knowledge, products, or technologies that could be directly misapplied by others to pose a threat to public health and safety, agricultural crops and other plants, animals, the environment, or material." See NATIONAL SCIENCE ADVISORY BOARD FOR BIOSECURITY (NSABB), PROPOSED FRAMEWORK FOR THE OVERSIGHT OF DUAL USE LIFE SCIENCE RESEARCH 17 (2007), available at <https://fas.org/biosecurity/resource/documents/NSABB%20draft%20guidelines%20on%20dual%20use%20research.pdf>; the term was coined by the so-called Fink Report, see NATIONAL RESEARCH COUNCIL, BIOTECHNOLOGY RESEARCH IN AN AGE OF TERRORISM (2004), available at <https://www.nap.edu/read/10827/chapter/1>.

<sup>107</sup> For a legal assessment of the treaties with regard to questions of dual use research of concern, see GERMAN ETHICS COUNCIL, BIOSECURITY – FREEDOM AND RESPONSIBILITY OF RESEARCH 214 (2014), P. 88 et seq., available at <http://www.ethikrat.org/files/opinion-biosecurity.pdf>.

<sup>108</sup> For framing risks of dual use, see esp. GUIDELINES AND RULES OF THE MAX PLANCK SOCIETY ON A RESPONSIBLE APPROACH TO FREEDOM OF RESEARCH AND RESEARCH RISKS (2010, updated 2017), available at [https://www.mpg.de/229644/Research\\_freedom](https://www.mpg.de/229644/Research_freedom) (last accessed 08-04-2018).

<sup>109</sup> Deutsche Forschungsgemeinschaft, Leopoldina – Nationale Akademie der Wissenschaften, Scientific Freedom and Scientific Responsibility, Recommendations for Handling Security-Relevant Research, 2014, available at: [https://www.leopoldina.org/uploads/tx\\_leopublication/2014\\_06\\_DFG-Leopoldina\\_Scientific\\_Freedom\\_Responsibility\\_EN.pdf](https://www.leopoldina.org/uploads/tx_leopublication/2014_06_DFG-Leopoldina_Scientific_Freedom_Responsibility_EN.pdf).

<sup>110</sup> Currently warning system are laid down in the EU-Regulation for laboratories that work with pathogens of Foot-and-Mouth disease.





## 8. Ethics, societal opportunities and challenges of genome editing

Klaus Tanner and C. Walch-Solimena

New laboratory procedures for altering genetic information are currently being used primarily in basic research. These tools have quickly gained acceptance, and their use is already widespread. The new methods of genome editing are now seen as “revolutionary” in nature. The dynamic of change has triggered intensive discussions in the fields of ethical and legal judgement. Reports and statements have been prepared in many Western democracies in which the new methods are described, ethically and legally evaluated and suggestions made for regulating the way in which they may be handled.<sup>111</sup>

In spite of this “revolutionary” character, the underlying research strategy is marked by a high degree of continuity. There are lines of continuity in the fundamental research paradigm that “genes” play a key role in the development of organisms and that consequently it can be hoped that opportunities to change genetic information through targeted human intervention will provide insights into basic developmental processes in biology. The potential for human intervention in genetically controlled development processes is linked to the hope of being able to “optimize” these processes, for example in the field of plant cultivation and animal breeding, or to better diagnose genetic diseases and perhaps to cure them. Even the latest breakthroughs in the development of CRISPR-Cas9 techniques build on a research history that began in Spain 25 years ago with research on *archaeobacteria*.<sup>112</sup>

The “genomic era” following the success of the Human Genome Project (HGP) published in 2001 simultaneously by a private initiative and a publicly funded large consortium, was at the time greeted as the beginning of a new era in medicine, mostly based on a deterministic view on the genetic basis of diseases. Much progress was indeed achieved in understanding monogenic (Mendelian) diseases. However, the vision of ‘personalized medicine’ based on patient’s genome has been slow to materialize.

The unexpectedly small number of coding genes identified in the human genome represents mere 1-2% of the human DNA

sequence, while the remaining 99% are playing regulatory roles and seem to be a key to the emergent complexity of human biology in health and disease. Insights into this so-called ‘dark matter’ of the genome were gained by projects such as ENCODE (Encyclopaedia of DNA Elements)<sup>113</sup> and the Epigenome Road Map<sup>114</sup> that revealed new layers of an extremely complex regulation of the genome by genetic elements and epigenetic mechanisms. New types of assays, including gene-editing based screens, are speeding up the confirmation of functions of regulatory elements discovered by ENCODE.<sup>115</sup> Still much remains to be discovered.

Another area of active research concerns the understanding of complex, multifactorial diseases. Whole genome sequencing and sequence-based genomic assays enable the identification and functional characterization of DNA sequence variants, and their association with disease. A better understanding of how risk-alleles exert their phenotypic impact will be needed to understand the pathways and the biology underlying diseases.<sup>116</sup>

The continuity in fundamental research is relevant in three respects:

**1) In terms of ethical reflection, there is a wealth of experience which we can draw on, ethical principles and established codes of practice that have emerged from the debate ensuing from our ability to intervene in the genome.**

When it became possible in the 1970s to effect targeted changes to DNA, this immediately led to intensive, ethical discussions, initially among scientists themselves, but then also among a wider public.

The first controversies were triggered by visions formulated by leading gene researchers at the Ciba Foundation Symposium

<sup>111</sup> By way of an example: Documents from the International Summit in Washington 2015, Nuffield Council on Bioethics. Genome editing: an ethical review 2016; for Germany: Berlin-Brandenburg Academy of Sciences (BBAW): Human Genome Surgery — towards a responsible evaluation of a new technology. An analysis by the interdisciplinary working group Gene Technology Report, Berlin 2015; German National Academy of Sciences Leopoldina, incl.: Opportunities and Limits of Genome Editing, Halle (Saale) 2015; Annual Conference of the German Ethics Council June 2016, European Academies Science Advisory Council Genome editing: scientific opportunities, public interests and policy options in the European Union; ESAC policy report 31, 2017; Discussion Paper by the Leopoldina Working Group: Ethical and legal assessment of genome editing in research on human cells, Halle (Saale) 2017.

<sup>112</sup> Lander ES. The Heroes of CRISPR, Cell Volume 164, Issues 1–2, p18–28, 14 January 2016. For the history of research into CrispCas techniques, see also John Parrington, Redesigning life. How Genome Editing will transform the world 2016 Jennifer Doudna and Samuel Sternberg, A Crack in Creation: The New Power to Control Evolution, 2017.

<sup>113</sup> The ENCODE Project Consortium. An integrated Encyclopaedia of DNA Elements in the Human Genome. *Nature* 489: 57–74 (2012).

<sup>114</sup> <http://www.roadmapepigenomics.org/overview> (last accessed September 13, 2017)

<sup>115</sup> Chi KR. The dark side of the human genome. *Nature* 538: 275–277 (2016)

<sup>116</sup> McCarthy MI, MacArthur DG. Human disease genomics: from variants to biology. *Genome Biol* 18:20 (2017). doi: 10.1186/s13059-017-1160-z (last accessed September 14, 2017).



in 1962.<sup>117</sup> The conference held in Asilomar in 1975 constitutes a milestone in the debate on how to deal responsibly with technologies to change genetic information.<sup>118</sup> Asilomar represents an example of self-regulation of scientific research, which then also led to the setting of a national standard in the USA. The decisions taken at this conference foreshadowed the suggestion of human research moratoria, which are once again under discussion today. One document that became a cornerstone for further discussions was the report of the USA President's Commission for the Study of Ethical Problems in Medicine and Biomedical and Behavioural Research entitled "Splicing life: The Social and Ethical Issues of Genetic Engineering with Human Beings" (Washington D.C., 1982). The report was an important catalyst in the creation of the Recombinant Advisory Committee of the National Institutes of Health in 1985.

In Germany, an inter-ministerial working party was set up in 1984 under the chair of the constitutional judge Ernst Benda, and in 1984 published a report "In Vitro Fertilization, Genome Analysis and Gene Therapy". Germline gene therapy was already the subject of intense discussion and the Commission regarded it as "currently" unjustifiable, but did not rule out germline interventions for serious monogenic disorders in the future. In 1987, the Enquete Commission "Prospects and Risks of Gene Technology" produced a report in which genetic interventions were discussed from a regulatory perspective for the first time.

A consensus crystallized on basic bioethical principles, which offers an initial guide to ethical questions. The "Georgetown Mantra", as set out and refined by Beauchamp and Childress, states four key principles in reaching judgements in the fields of application for bio- and medical ethics<sup>119</sup>: (1) respect for autonomy, (2) the principle of non-maleficence (3) beneficence and (4) justice. The Mantra represents an important reference point in many reports on CRISPR-Cas9 technologies. However, as the orientational strength of any regulatory approach based solely on principles is limited, judgements and rules are required based on the specific context of applications.

As CRISPR-Cas9 techniques represent a form of human action, all judgements and rules are shaped through the situation, the position, and the time they were made in. Due to

changes over time in the field of human actions and research, norms and rules have to be reviewed if new challenges emerge. Rules for human actions and research can only be effective if they are appropriate and therefore also reflect changes in the scientific landscape. Although interest in improving conditions for life, and in maintaining safety and protection is a constant, these goals can only be effectively attained if they are also appropriate to the new courses of action available. All laws must therefore be amenable to amendment. To what extent "adaptation" is required from an ethical viewpoint in order to reflect changed circumstances, or must be rejected for the same reason, will remain a matter of fierce political controversy in the future. As it is a question here of setting standards that will always be influenced by cultural factors, scientific knowledge can only provide limited answers to questions of regulation.<sup>120</sup> This is demonstrated by the consistently articulated demands for ethical and legal judgements or for "public debates" and consideration for the opinions of stakeholders in society.

## 2) The ethical discussions on recombinant DNA culminated in political debates that led to the development of legally codified regulations that still govern this field of research to this day.

In Germany's case, for example, mention can be made in this context of the Genetic Engineering Act, the Embryo Protection Act and Animal Welfare Acts. Internationally, legal frameworks often saw the creation of new institutions, which provided new platforms for structuring ethical deliberation. Numerous ethics commissions are now working, also in Germany, in an advisory capacity or are involved in approval procedures for research work.

## 3) Debates spawned pro and contra positions that also continue to shape the deliberations about "genetic engineering" to this day.

Institutions and forums representing a "critical" counter-culture have established themselves and influence discussions

<sup>117</sup> Prominent biologists and molecular geneticists such as F. Crick, J. B. S. Haldane, J. Lederberg and H. J. Muller were among the participants. The conference report "Man and his Future" 1963 (English) was published in German in 1966 under the title "Das umstrittene Experiment: der Mensch. Elemente einer biologischen Revolution". In a German-speaking context, books such as Richard Kaufmann's "Die Menschenmacher. Die Zukunft des Menschen in einer biologisch gesteuerten Welt" (1964), Thomas Regau's "Menschen nach Maß. Werkstoff Mensch im Griff einer seelenlosen Wissenschaft" (1965) or Friedrich Wagner's "Die Wissenschaft und die gefährdete Welt" (1964) arose from the reaction to the Ciba Symposium. The first ethicist who addressed the new challenges in detail was Paul Ramsey, "Fabricated Man: The Ethics of Genetic Control", New Haven 1970.

<sup>118</sup> Paul Berg, David Baltimore, Sydney Brenner, Richard O. Roblin III, and Maxine F. Singer. "Summary Statement of the Asilomar Conference on Recombinant DNA Molecules". *Proc Natl Acad Sci* Vol. 72, No. 6, pp. 1981-1984, (June 1975): 1981;

Paul Berg and Maxine F. Singer. "The recombinant DNA controversy: Twenty years later". *Proc Natl Acad Sci* 9011-9013, (1995).

Susan Wright. "Molecular Biology or Molecular Politics? The Production of Scientific Consensus on the Hazards of Recombinant DNA Technology" *Social Studies of Science*, 16, 595-96 (1986).

Donald S. Fredrickson, Asilomar and recombinant DNA in: Kathi E. Hanna (ed.) *Biomedical Politics*, Washington D.C. 1991, 258 - 29.

<sup>119</sup> Tom L. Beauchamp, James F. Childress, *Principles of Biomedical Ethics*, 7 ed. New York /Oxford 2013.

<sup>120</sup> cf. Nuffield Council on Bioethics. *Genome Editing. An Ethical Review* (2016) <http://nuffieldbioethics.org/project/genome-editing/ethical-review-published-september-2016>. p. 114 "the focus on the technology tends to obscure rather than reveal the social and ethical issues. It also masks questions that arise at different spatio-temporal scales".



on legal regulations at a national and international level.<sup>121</sup> The extension of the opportunities for non-governmental organizations (NGOs) to participate in the EU and UN has increased the political weight of new players acting under the label of “civil society”. Equally, there are considerable cultural differences, rooted in historical experience, in the approach to the risks and opportunities associated with new biotechnologies.<sup>122</sup> The unscrupulous behavior of scientists in Germany at the time of National Socialism<sup>123</sup> and the laborious process of coming to terms with this since 1945 has bred skepticism and reservations towards “science” in Germany that still resonate in present discussions on gene therapies.

While scientific research focuses on progress as a matter of principle, attitudes heavily critical of progress have taken root since the 1970s. In his book “Das Prinzip Verantwortung” [The Imperative of Responsibility], Hans Jonas coined the phrase “heuristics of fear”. The “principle ... that greater attention must be paid to the prophets of doom than to the prophets of salvation”<sup>124</sup> became the recurring theme of many positions critical of research. This reservation is frequently formulated in the so-called “slippery slope” arguments in which consequentialist objections are used to point out the dangers of “undesirable consequences”.<sup>125</sup> As undesirable consequences can only be anticipated to a limited extent, recourse to scientific facts often provides little to counter these arguments.

There is a need for forms of communication, which reinforce the trust and show that scientists themselves can deal with the new possibilities responsibly.<sup>126</sup> One important factor in creating and strengthening trust is transparency.<sup>127</sup> Rules offering maximum clarity, labelling obligations and publicly accessible registers represent concrete steps to promote this transparency. Individual researchers themselves cannot directly generate trust. Instead, the work of individual scientists remains dependent on the trust placed in the institutions in which they work. Trust is fragile and can be quickly destroyed. The reckless actions of one individual, carelessness, raising unrealistic expectations which are then disappointed will always damage an entire field of research.

In media societies in which scientists themselves make use of the media to announce their successes, there is a danger of promising too much too early in order to prepare the ground for further funding for the research. This interplay between science and the media contains, on the one hand, opportunities to create transparency but at the same time dangers that can be labeled as “hype”.<sup>128</sup>

## Six key questions relating to research programs employing CRISPR-Cas9 currently being conducted in MPG laboratories.

When it comes to the ethical and legal assessment of research tools, there are basic questions that help to reflect on the problems arising from new methods such as genome editing. Six key questions:

- 1) How is the method by which genetic modifications are carried out described?
- 2) How safe is it to use the method?
- 3) In what areas is it to be deployed?
- 4) What are the objectives pursued?
- 5) How can the research field be regulated?
- 6) What role do economic interests play in shaping the research field?

### 1) How is the method described? Does the description and imagery chosen suitably reflect its actual effect, scope and the depth of the intervention?<sup>129</sup>

Looking at the mechanism by which the nucleases deployed modify genetic information, the question is whether the mechanistic images of a “pair of gene scissors” adequately cover biological processes. If the term “genetic surgery” is used, this shifts the range of meanings. Every surgeon knows that any operation on complex organic structures carries a higher risk than editing or modifying a text.

<sup>121</sup> e.g. Greenpeace and the Testbiotech Institute, Center for Genetics and Society, in Germany the Gen-ethische Netzwerk

<sup>122</sup> For the different patterns of behaviour in the USA and Germany, cf. Sheila Jasanoff, *Designs on Nature. Science and Democracy in Europe and the United States*, Princeton 2005.

<sup>123</sup> cf. the book series “Geschichte der Kaiser-Wilhelm-Gesellschaft im Nationalsozialismus” [History of the Kaiser Wilhelm Society in National Socialism], published by Reinhard Rürup and Wolfgang Schieder on behalf of the Presidential Committee of the Max Planck Society.

<sup>124</sup> Hans Jonas, *Das Prinzip Verantwortung* [The Imperative of Responsibility] Versuch einer Ethik für die technologische Zivilisation [In Search of an Ethics for the Technological Age], Frankfurt a. M. 1979, p. 64 ff, p. 70. Jürgen Habermas also warned of a “liberal eugenics” with reference to Hans Jonas. Jürgen Habermas, *Die Zukunft der menschlichen Natur* [The Future of Human Nature]. Auf dem Weg zu einer liberalen Eugenik? [On the Way to Liberal Eugenics?], Frankfurt a. M. 2001, p. 40 and 84 ff.

<sup>125</sup> Douglas Walton, *Slippery slope arguments*, Oxford, New York 1992.

<sup>126</sup> In the “Guidelines and rules of the Max Planck Society on a responsible approach to freedom of research and research risks” 2010 (updated 2017), pointed reference is made to the fact that individual scientists “must not content themselves with observing statutory rules, but must take more far-reaching ethical principles into consideration” (p. 6 cf. also p. 8). cf. also the “Code of Conduct: Working with highly pathogenic micro-organisms and toxins” published by the German Research Foundation’s Senate Commission for Basic Questions of Genetic Research, March 2013.

<sup>127</sup> In the report of the National Academies of Sciences, Engineering, Medicine “Human Genome Editing. Science, Ethics and Governance”, “transparency” is explicitly mentioned as an important principle alongside classical bioethical principles. Summary p. 11 Box S-1.

<sup>128</sup> Zubin Master and David B. Resnik *Hype and Public Trust in Science*, in: *Sci Eng Ethics*. 19, 321–335; David B. Resnik, *Scientific Research and the Public Trust*, in: *Sci Eng Ethics*. 17, 399–409 (2011).

<sup>129</sup> cf. perhaps Evelyn Fox Keller, *Making Sense of Life. Explaining Biological Development with Models, Metaphors and Machines*, Harvard 2002.



The language used to describe genome editing has the overall tendency of reinforcing ideas of genetic determination,<sup>130</sup> giving the impression that it should therefore be possible to draw direct conclusions for the phenotype from our knowledge of genotypes. Instead, the complex regulation of the genome has to be taken into consideration when discussing this technology and its consequences.

## 2) How safe is it to use the tool? What undesirable side effects and risks can reasonably be expected?

Unexpected mutations have been repeatedly found when using various methods of genome editing. Scientists have not yet reached a definitive judgement on their frequency or the risks associated with such "off-target mutations".<sup>131</sup> Judgements on the risk potential in biology and medicine correlate with assumptions of how the development processes in cells and organisms are regulated.

Knowledge of the complexity of these regulation relationships has expanded enormously as a result of genome research<sup>132</sup> CRISPR-Cas9 methods are one example. Sequences in the DNA, which were long regarded as meaningless, proved to be the repository of important information, and the significance of epigenetic regulation now plays a pivotal role.

Judgements on risk potential are closely related to the "locations" where the intervention is made, and to the varying complexity of the organisms operated on. It makes a difference whether mutations are generated "in vitro" in a cell culture in a security laboratory or "in vivo" in a research animal, whether genetically modified plants are released, a human embryo is operated on or ecological systems are to be modified via the methods of gene drive.

With the aid of the so-called "precautionary principle" that has been increasingly included in international documents and European Community law since the 1990s, an attempt is made to legitimize preventive state action to minimize risks even if a "lack of complete scientific certainty" is given.<sup>133</sup> The power of this principle, as an ethical principle, is frequently the subject of critical debate,<sup>134</sup> also nearly all States have ratified treaties

including this principle. An attempt to assess what dangers may be incurred if new technological capabilities are not used, must also form part of the risk assessment. The responsibility for risks must be balanced against the responsibility for innovation.<sup>135</sup>

## 3) In what areas will or should the method be deployed?

The paradigm of genome research is associated with a leveling tendency. As genetic information plays an important role in all organisms, the impression can be created that it makes no difference whether the method is used in cell cultures, plant cultivation, animal breeding or in human medicine. However, species-specific differences play a central role in everyday research life, both with regard to the efficacy of the methods and with respect to the legal framework conditions of the respective research field (most notably in the protection of the human germline). Depending on the area of application, there are different regulations that have to be taken into account when employing the methods. The legally codified standards represent the crystallization of ethical convictions and the interests of protection.

## 4) What are the objectives that the new methods are to achieve?

The aim of improving the living conditions of humans is uncontroversial. There is an ethical obligation to fight disease and alleviate suffering. The controversies are sparked by the question of what means may be used to achieve these objectives and what risks have to be accepted. The rejection of use of the new technologies to develop bioweapons is likewise ethically undisputed. As any technology can be used for the attainment of positive aims or for destructive purposes, there are difficult problems of demarcation, which are discussed under the heading of dual-use problems.<sup>136</sup>

Objectives, such as improving methods in basic research or increasing nutritional options through genetically modified plants or animals, must be evaluated differently from an ethical standpoint than weapons development. There is high sensitivity to research in the human domain, in attempts to modify

<sup>130</sup> cf. Nuffield on Bioethics. Genome Editing. An Ethical Review (2016) <http://nuffieldbioethics.org/project/genome-editing/ethical-review-published-september-2016>. Council, p. 114.

<sup>131</sup> Schaefer, K. A., Wen-Hsuan W., Colgan, D. F., Tsang, S. H., Bussuk, A. G. & Mahajan, V. B. Unexpected mutations after CrispCas) editing in vivo. *Nat Methods*, 14, 547 – 548 (2017).

Editorial: CRISPR off-targets: a reassessment. *Nat Methods*, 15, 229–230 (2018).

Ravindran, S. New Methods to Detect CRISPR Off-Target Mutations. *The Scientist*, March 1, 2018.

<sup>132</sup> The complexity of the regulation mechanisms was revealed by the ENCODE project of the National Human Genome Research Institute (NHGRI)2 <https://www.encodeproject.org/>

<sup>133</sup> For application in the EU: European Commission Notification from the Commission dated 2 February 2000 on the applicability of the precautionary principle. Brussels, 2000 For Cass R. Sunstein's critical appraisal, *Laws of Fear: Beyond the Precautionary Principle*. New York 2005 (German: *Gesetz der Angst: Jenseits des Vorsorgeprinzips*. Frankfurt a. M. 2007); Göran Hermeren: The principle of proportionality revisited: interpretations and applications, in: *Med Health Care Philos*. 15: 373–82, (2012).

<sup>134</sup> However others defend an interpretation of the precautionary principle from the perspective of philosophy of science, Daniel Steel, *Philosophy and the Precautionary Principle – Science, Evidence, and Environmental Policy*, 2015.

<sup>135</sup> Alfons Bora A. Zukunftsfähigkeit und Innovationsverantwortung – Zum gesellschaftlichen Umgang mit komplexer Temporalität [Future Viability and Responsibility for Innovation – On the social treatment of complex temporality], in: Eifert M, Hoffmann-Riem W, eds. *Innovationsverantwortung [Responsibility for Innovation]*. Innovation und Recht [Innovation and Law]. Innovation und Recht. Vol 3. Berlin 2009: 45–67.

<sup>136</sup> cf. Statement by the German Ethics Council in May 2014 "Biosecurity – Freedom and Responsibility in Science"; DFG and Leopoldina "Scientific Freedom and Scientific Responsibility – Recommendations for Handling Safety-relevant Research" Bonn and Halle (Saale) 2014.



somatic and germline cells. Therapeutic objectives must be differentiated from experiments aimed at “enhancement” or “optimization” of human traits. The most contentious issue concerns interventions in the human germline<sup>137</sup> and embryo research driven by the promise of medical applications.

It is of note that personal judgements concerning technologies are usually steeped in convictions, presumptions, hopes and fears with regard to the “future” and deeply rooted in the biographies of the individuals concerned. These “prejudices” inform their individual judgement. As these “frameworks” differ widely in modern societies depending on group allegiance, modern technologies are becoming focal points for cultural conflicts in which it is never simply a matter of the particular technology concerned. Anyone wanting to promote “public debate” must keep an eye on this symbolic dimension that can only be “influenced” to a limited degree by providing additional technological information.

### 5) How can the research field be regulated in such a way that both scientific progress and freedom of research are treated as justified interests to be protected?

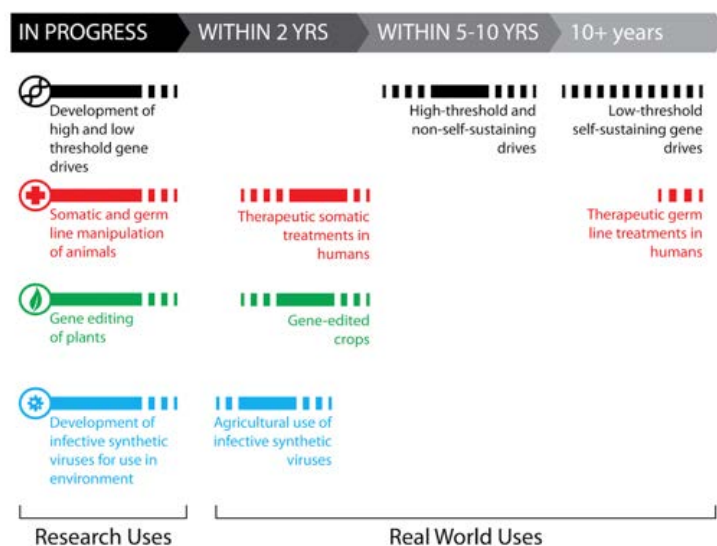
As research is international, national regulations only have limited power. Furthermore, there is need to constantly weigh anew the benefits of an innovative endeavour to find a conciliatory balance between contrasting interests, which will nec-

essarily have an international dimension. This is despite the fact that deliberations about regulation and governance are generally processes that take place within political cultures.<sup>138</sup>

### 6) The debate about CRISPR-Cas9 techniques also has an economic dimension.

For example, it is being emphasized that the new methods are less expensive than previous ones. This economic dimension becomes even more evident in the patent disputes currently being fought out between the main scientific players.<sup>139</sup> Companies involved in life sciences have licensed these technologies for commercial purposes. The Max Planck Society should make every effort to ensure that license practices do not impede the freedom of scientific research related to this technique. It has been proposed by the Human Genome Organization (HUGO) Committee of Ethics, Law, and Society (CELS) to apply to genome editing the principle of “genomic solidarity and priority on public good”. According to this, everyone is entitled to access the benefits of research such as medical advances, with the public and scientists as joint owners in discovery and opportunity.<sup>140</sup>

Since research constitutes action in time, it makes sense to differentiate between urgent problems that already require a stance to be taken in the present day, and futuristic scenarios that are still far off (Figure 3).



<sup>137</sup> Edward Lanphier, Fyodor Urnov et al., Don't edit the human germ line. *Nature* 519, 410–411 (2015).; David Baltimore, Paul Berg et al., A prudent path forward for genomic engineering and germline genome modification. *Science* 348, 36–38 (2015).

<sup>138</sup> Sheila Jasanoff (ed.), *Reframing Rights. Bioconstitutionalism in the Genetic Age*, Cambridge 2011.

<sup>139</sup> Heidi Leford, Bitter CRISPR patent war intensifies, *Nature* 26 oct 2017 (<https://www.nature.com/news/bitter-crispr-patent-war-intensifies-1.22892>),

Why the CRISPR patent verdict isn't the end of the story, *Nature* 17 feb 2017; DOI: 10.1038/nature.2017.21510

Paul B. G. Van Erp et al. The history and market impact of CRISPR RNA- guided nuclease, *Curr Opin Virol* 12, 85–90 (2015).

Knut J. Egelie et al. The emerging patent landscape of CRISPR-CAS gene editing technology, *Nature Biotechnol* vol 34 no 10, 1025–1031, 2016;

Jacob S. Sherkow, Pursuit of Profit poisons collaboration, *Nature* 532, 172–173 (2016).

<sup>140</sup> Mulvihill B, Capps B, Joly Y, Lysaght T, Zwart HAE, Chadwick R. The International Human Genome Organisation (HUGO) Committee of Ethics, Law and Society (CELS). Ethical issues of CRISPR technology and gene editing through the lens of solidarity. *Br Med Bull* 122: 17–29 (2017).

Capps B, Chadwick R, Joly Y, Mulvihill JJ, Lysaght T, Zwart H. Falling giants and the rise of gene editing: ethics, private interests and the public good. *Human Genomics* 11:20 (2017).



**Figure 2: Estimated timeline for research and development with CRISPR gene editing in different systems.**

The fear of designer babies is one of the scenarios for which there is no scientifically solid foundation of feasibility given the current status of research.

There is no consensus at the moment on some pressing problems due to already existing practices. The following examples are to be mentioned in this context:

1. There is the question of classifying genetically modified organisms within the framework of existing legal regulations. A distinction has to be drawn here between the fact that every intervention in the genome of an organism represents an action for which humans are responsible, and the question of how such an intervention is to be regulated.

The current fierce debate on this question is more than a game played with the cards face down on the table. Those who reject genetic modifications on principle are fighting for regulation through existing genetic engineering legislation. Those who advocate the technologies, desire fast certification procedures, and they are arguing that the existing rules should not be applied at least to modifications that are indistinguishable from mutations occurring in nature. In essence, this conflict is fueled by differing political and economic interests.

2. Research with and on human embryos as well as interventions in the germline represent sensitive issues, also among the wider public. In Germany, genome editing that is part of a gene therapy and used and aimed to enhance the chance of survival of a human embryo is not prohibited by law; the same is true for somatic gene therapy. Human germline editing (i.e. human germline therapy) is prohibited in Germany – as in other European States – but there does not exist a universal prohibition of human germline editing

up to now. Furthermore, the German Embryo Protection Act as part of criminal law prohibits the use of human embryos for scientific research, including generating embryonic stem cells. This is disputed, however, as far as non-viable human embryos (e.g. tri-pronuclear embryos) are used for research. Other European States, such as the UK, Sweden and France, to the contrary, do not prohibit research with human embryos during a maximum period of the first 14 days following fertilization. In England, Sweden, in privately funded research in the USA, and also in China, such experiments have already been carried out.<sup>141</sup>

Whether the changed research situation offers enough reasons for adjusting the legal regulations (Embryo Protection Act - ESchG), is the subject of heated discussion in Germany. Intervention in the germline is viewed with great reservations around the world and caution is advised.

German researchers are de facto using the results from the research on human ES cells abroad, as the data acquired there represent the “gold standard” for judgements on the ability of other cells to develop and differentiate.

3. The statement submitted by the Leopoldina pleads for a moratorium on editing of the human germline. The summit in Washington, instead, refrained from demanding a moratorium. There are differing assessments as to whether a moratorium in certain areas of research is a suitable instrument under today's conditions.

A moratorium would not necessarily entail any categorical ban on research but it might be understood as such. For the sake of properly assessing the risks and the potential of such technology the door is to be kept open for possible research in the future. Apart from that it is not clear who could authoritatively announce a moratorium and monitor its observance or draw up the criteria that must be met for the moratorium to be ended.

<sup>141</sup> cf. the first result of the experiments with orphaned embryos from in vitro fertilization approved in England in 2016: Fogarty NME et al., Genome editing reveals a role for OCT4 in human embryogenesis, *Nature*, 550: 67–73 (2017).



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