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Introduction

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As early as 2000, Gregory Stock and John Campbell noted that the achievement of the capacity to make changes to human germline cells was "inevitable."[1] "The real question," they postulated, was not "whether the technology will become feasible, but when and how it will."[2] The advent of the CRISPR/Cas9 family of genome-editing tools answered the question of how. As to when, it is now: humanity has already entered a new age. How it will unfold depends on the regulatory frameworks that each state, and all of them collectively, will be able to provide in response to this pathbreaking scientific and technological development.

The primary goal of this book is to analyze how human germline genome modification is currently regulated in a selected, but fairly broad and representative, set of developed countries, and to assess their national governance practices in the light of the existing and emerging international legal obligations states have. These include the obligation to respect international human rights, and specifically the so-called right to science and the rights of science. It is obvious, but not always recalled, that the mosaic of national regulatory frameworks does not exist in a vacuum. There is a broader international framework within which the various national frameworks need to be placed. The human rights framework constraint governs and guides states in their law-making activities.

Until relatively recently, international human rights standards had not featured prominently in bioethical analyses, even though most international documents relating to bioethics issued during the past two decades are framed on a rights-based approach and attach utmost importance to the notion of

[1] G Stock and J Campbell, *Engineering the Human Germline: An Exploration of the Science and Ethics of Altering the Genes We Pass to Our Children*, Oxford University Press, 2000, p. 6.
[2] Ibid., p. 5.

human dignity, which is a concept used also in human rights law.[3] However, bioethical analyses do not necessarily take all relevant human rights into account or use the concept of dignity in the same way as human rights law does. In this book, we link human rights law to the regulation of human germline genome modifications at the national level.[4] For all its limitations and weaknesses, the existing human rights system, with its extensive body of international standards and wide range of mechanisms, cannot be ignored. As it has been said, the human rights framework provides a "more useful approach for analysing and responding to modern public health challenges than any framework thus far available within the biomedical tradition."[5] Indeed, there are few mechanisms available other than human rights to function as "a global ethical foundation, a *Weltethik*."[6] In fact, we conclude this book by showing how international human rights standards are applicable and can be used to respond to the challenge of regulating human germline genome modifications.

Because this book speaks to a very wide and diverse audience, one made of legal scholars and biomedical scientists, nongovernmental and governmental actors, we believe it is necessary to begin by introducing some basic concepts and terms related to human germline modification that might very well be familiar to some readers but not necessarily to others. In this Introduction (Chapter 1), we discuss the science of human genome modification in general, how it relates to human reproduction, and the specific advances that CRISPR/Cas9 represents and the family of tools it has generated to date. We then explain the methodology we followed preparing this book, including how countries were selected and what we chose to focus on and why.

This is necessary background for a discussion of the national and international governance frameworks for human germline genome engineering. Chapter 2 sets out the emerging international governance framework. The aim is to introduce the reader to the key actors (international organizations,

[3] On the intersection between international bioethics law and international human rights law, see, in general, R Andorno, *Principles of International Biolaw: Seeking Common Ground at the Intersection of Bioethics and Human Rights*, Bruylant, 2013; S Holm, *The Law and Ethics of Medical Research: International Bioethics and Human Rights*, Cavendish Publ., 2005.

[4] For a condensed version of the data and arguments presented in this book, see, A Boggio, B M Knoppers, J Almqvist, and C P R Romano, "The Human Right to Science and the Regulation of Human Germline Engineering," *The CRISPR Journal*, Vol. 2 (2019), pp. 134–142.

[5] J Mann, "Health and Human Rights: Protecting Human Rights is Essential for Promoting Health," *British Medical Journal*, Vol. 312 (1996), p. 924.

[6] D Thomasma, *Proposing a New Agenda: Bioethics and International Human Rights*, Cambridge Quarterly of Healthcare Ethics, 2001, Vol.10, pp. 299–310, p. 300.

international agencies, and learned societies), both at the global and regional level, as well as the most important legal standards they have articulated so far, including in the area of human rights law. This discussion is not limited to treaties, of which there are few, but also takes so-called soft law, such as international declarations and guidelines, into account. Then Part III, the bulk of this volume, presents an analysis of the national regulatory frameworks of eighteen selected countries and one region: Europe (Chapters 3 to 21). Each "national chapter" follows a template that we explain in the "methodology" section below.[7] Finally, we conclude the book by looking at the existing national and international regulatory frameworks in light of the five foundational principles that a reading of international bioethics law combined with international human rights standards suggests. They are: (i) freedom of research, (ii) benefit sharing, (iii) solidarity, (iv) respect for dignity, and (v) the obligation to respect and to protect the rights and individual freedoms of others (Chapter 22). We conclude by offering our vision of an international governance framework that promotes science and technological development while being mindful and respectful of international human rights standards, as well as the different sensitivities with which citizens from different parts of the world approach this complex problem.[8]

I THE SCIENCE

At least since the discovery of DNA, biomedical researchers have been on a quest to develop tools to make targeted changes to the genome of living organisms for scientific, therapeutic, and economic reasons. Efficient, reliable, and low-cost genome-editing tools can be used, and are already used, for different purposes. They can help advance science, for instance by understanding what functions each gene controls, and be used to prevent or cure genetic disorders, for instance by correcting errors in the genome. Or, they can be used to improve and develop certain traits in a given cell or organism. Scientists modify the DNA of animals not only to observe how changes affect the animal itself but also to predict how the same changes in the human genome might affect human health. Or, they might seek to propagate a particular suite of genes throughout a species (the so-called gene drive) to drive to extinction insects that carry pathogens (such as mosquitoes that transmit malaria), control invasive species, or eliminate herbicide or pesticide

[7] See, in this chapter, Section II.
[8] The views expressed in this and the final chapter do not necessarily reflect the views of the authors who contributed the national chapters to this book.

resistance.[9] In plant bioengineering, gene editing is used as a tool to improve resistance against fungi (corn), crop yields during drought stress (maize), and accelerate growth to boost yield (tomato and rice).[10]

A series of genome-reading (sequencing) and genome-editing tools, including ZFN, TALEN and CRISPR (Cas9 and Cas12a) gave scientists the instruments to modify the genome precisely, efficiently, and at much lower cost. This scientific breakthrough has opened unprecedented and hitherto unthinkable research and therapeutic scenarios. It is also raising major ethical and regulatory dilemmas, especially when the genome that is being edited is the one of human germline cells, as this means changing the genes passed on to future generations.

Germline cells are one of the two kinds of cells that mammals have. Gametes, that is, oocytes (eggs) in females and sperm in males, are our germline cells. All other cells that make up the human body are "somatic cells." Modifications made to somatic cells stay within the body of the person whose cells have been modified. However, modifications made to germline cells, under certain circumstances, are heritable. They might be passed on to the next generation and into the future, potentially altering the genome of entire populations.

There are valid reasons for wanting to modify the genome of entire populations, if not the whole of humanity. The modification of the genome of human germline cells allows attaining two major therapeutic goals. First, it could be used to greatly reduce the burden of hundreds of hereditary genetic diseases that condemn millions to considerable pain or death every year, much as vaccines have eliminated some viruses that have plagued humanity for millennia. Second, it allows curing genetic diseases before they manifest their often-devastating effects on the human body. That is a more efficient and ethically superior goal than allowing a patient to get sick first and then cure (if there is a cure).

To achieve these therapeutic goals, two paths are being considered: editing gamete precursor cells, or gametes before fertilization, or editing embryos after fertilization. Either can be done outside the human body (in vitro or ex vivo) or directly inside the body (in vivo). The difference is whether the mutation is corrected before fertilization (editing of gamete and precursor cells) or after

[9] N Kofler and others, "Editing Nature: Local Roots of Global Governance," *Science*, Vol. 362 (2018), p. 527.

[10] C Gao, "The Future of CRISPR Technologies in Agriculture," *Nature Reviews Molecular Cell Biology*, Vol. 19 (2018), p. 275.

(editing of embryos). In the end, the goal of either method is similar: allowing parents to procreate a genetically related offspring who does not carry an undesirable mutation.[11] Since both methods necessarily involve human reproduction, we introduce some basic concepts of human reproduction and embryology before turning to the science of gene editing.

1 *Germline Cells, Reproduction, and Embryo Development*

Sexual reproduction involves a process that begins with the fertilization of an egg (Figure 1.1). This occurs when the male gamete (sperm) and the female gamete (oocyte) fuse to form the "zygote," a cell that carries two complete sets of chromosomes: one set matching the sperm DNA and the other the oocyte DNA. Subsequently, the zygote (which scientists sometimes call a "one-cell embryo") develops into a ball of cells and undergoes a series of divisions, called "cleavages." As this ball of cells develops, it assumes different names. At the first stage of development, it is called a "morula," consisting of sixteen cells. The morula then develops into a "blastocyst" when the internal cells are pushed outward forming a cavity. The outer cells become epithelial tissue, which will eventually form the placenta that supports the embryo in its growth in the uterus.

It is important to note that the cells of a blastocyst, known as "inner cell mass," are undifferentiated and pluripotent. This means that they have not yet committed to becoming any particular type of cell and that they can still become any tissue of the human body, including gametes. It follows that, if the cells of blastocysts are edited, any modification will have effects on the germline of the human being who will develop from a modified blastocyst and their progeny.

Upon entering the uterus (or being artificially transferred into the uterus, if fertilization occurred outside, in vitro), the blastocyst implants in the endometrium, the mucous membrane lining the uterus, and develops into a "gastrula." From a single-layer structure, the cells of the ball are reorganized into a multilayered one. The transition from blastocyst to gastrula, known as "gastrulation," is characterized by the formation of the primitive streak, which is a structure that forms on the back of the balls of cells. After the appearance of the primitive streak, the internal cells are reorganized in three germ layers (endoderm, ectoderm, and mesoderm), each of which will produce a distinct cell lineage that form the various types of tissue that form the human body.

Gastrulation, which takes place about fourteen days after fertilization, is a turning point in cell development because, after the formation of germ

[11] N Kofler and K L Kraschel, *Treatment of Heritable Diseases Using CRISPR: Hopes, Fears, and Reality*, Seminars in Perinatology, Vol. 48 (2018), pp. 515–521.

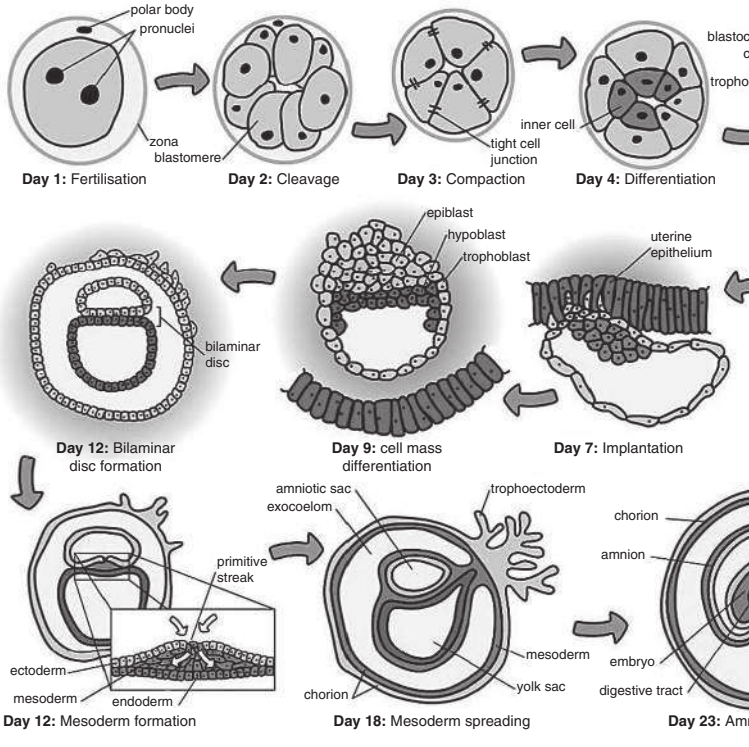

FIGURE 1.1 The first few weeks of embryogenesis in humans: beginning at the fertilized egg, en
Zephyris – SVG version, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.

layers, cells are no longer pluripotent. They are differentiated, meaning that they are able to develop only into certain types of tissue. This happens during the next stage of development, when the outermost layer of cells of the embryo (ectoderm) thickens, activating the formation of the neural plate, which will give rise to both the spinal cord and the brain. The outcome of this process, called "neurulation," is the transformation of the gastrula into a "neurula." The other two germ layers, interacting with each other and the emerging neural system, contribute to the development of all individual organs by way of cell movements and cell differentiation (organogenesis). These organs include the gonads, the reproductive glands that produce the gametes.

Theoretically, gametes or their precursor cells can be edited in vivo, cells in the fetus in utero, within the testis, or early embryos within the oviduct before implantation. This scenario is hypothetical because of technical and legal limitations. In fact, until recently, scientists have not been able to culture embryos in vitro during gastrulation, and in vitro experimentation on embryos beyond fourteen days has been prohibited for the past forty years across the globe. However, in May 2016, labs in the United States and the United Kingdom reported being able to sustain human embryos in vitro for up to thirteen days.[12] This means that what is now just a mere hypothetical scenario may become reality.

Fertilization and some of the early stages of reproduction can also take place in vitro, outside the female body. Since 1978, when the first child resulting from an in vitro fertilization (IVF) process was born, scientists have been refining techniques. During IVF, the gametes are retrieved from the parents-to-be and, after proper handling, combined using various techniques. The resulting zygote is then cultured in vitro and eventually is implanted in the uterus. If not immediately implanted, the morula or the blastocyst is cryopreserved for varying lengths of time for various medical reasons. Sometimes, frozen morulae or blastocysts end up not being used by the woman or couple. When that happens, some are donated to other infertile couples. Some are used in research (supernumerary or spare embryos). Those that are not donated for reproduction or research are destroyed or, in some countries, kept frozen in perpetuity.

2 *The Emergence of the CRISPR Tools*

We now turn to the technology that is seen as the most promising for gene editing: the CRISPR tools. CRISPR (the acronym for clustered regularly interspaced short palindromic repeats) is a family of DNA sequences found within

[12] J Rossant and P P L Tam, "Exploring Early Human Embryo Development," *Science*, Vol. 360 (2018), pp. 1075–1076.

the genomes of prokaryotic organisms such as bacteria and archaea. It is the mechanism of self-defense that cells possess to fend off viral attacks. When a virus attacks, the bacteria capture snippets of DNA from invading viruses and store it to remember the identity of the invader so that, in the event of a second attack, the bacteria can activate its defense mechanism. This mechanism entails releasing an enzyme called "Cas9" (short for "CRISPR-associated 9"), which acts as a pair of "molecular scissors" and cuts the DNA chain at a specific location. As a defense mechanism, Cas9 is used to disable the attacking virus. Researchers have figured out that Cas9 can be used to cut DNA of human cells to edit it. To do so, Cas9 is injected in a cell along with a piece of RNA, known as "guide RNA" or "gRNA," that is engineered to have an end that is complementary to the DNA sequence that Cas9 must cut. Once the gRNA is injected, Cas9 is guided to the right spot by the guide, which is looking for the target DNA, and cuts the DNA sequence where gRNA. Once the DNA chain is cut, scientists can add or remove DNA sequences (Figure 1.2).

To everyone but those who practice it, it might sound like magic, but it is not. It is the result of a long process of scientific research and discovery that started toward the end of the twentieth century. It is a rather long and intricate story, involving scientists in Asia, Europe, and North America, in universities and the private sector, producing a complex network of knowledge from bioinformatics, genetics, and molecular biology. It begins with some rather obscure research on bacteria and microbes. In 1987, Yoshizumi Ishino and colleagues at Osaka University discovered an unusual series of repeated sequences in DNA sequences outside of coding regions, later called clustered regularly interspaced short palindromic repeats (CRISPR), that contribute to the process of programmed cell death – the deliberate suicide of an unwanted cell – in *E. coli* bacteria.[13] Then, in the early 1990s, in Spain, Francisco Mojica made the first attempt to describe the function of CRISPR. The Spanish scientist published first a paper in 1993 describing a repeated sequence of thirty bases in the genome of the microbe *Haloferax mediterranei*.[14] Intrigued by the discovery, Mojica kept studying these curious sequences in his lab at the University of Alicante. This research led to the publication, in 2005, of the first paper presenting CRISPR's likely function: to encode the instructions for an adaptive immune system that

[13] Y Ishino and others, "Nucleotide Sequence of the Iap Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in Escherichia Coli, and Identification of the Gene Product," *Journal of Bacteriology*, Vol. 169 (1987), p. 5429.

[14] F Mojica, G Juez and F Rodriguez-Valera, "Transcription at Different Salinities of Haloferax Mediterranei Sequences Adjacent to Partially Modified PstI Sites," *Molecular Microbiology*, Vol. 9 (1993), p. 613.

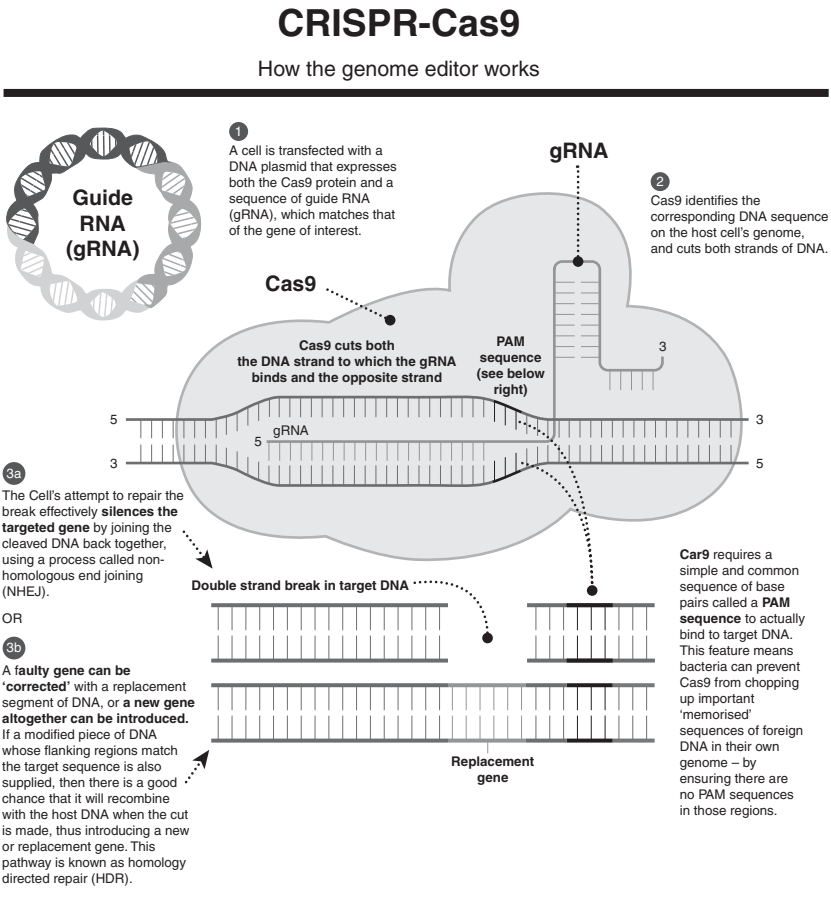

FIGURE 1.2 CRISPR-Cas 9: how the genome editor works
Adapted from J Lewin W, CRISPR-Cas 9: How the Genome Editor Works, https://commons.wikimedia.org/w/index.php?title=File:CRISPR-Cas9-biologist.jpg&oldid=269431713.

protected microbes against specific infections.[15] As Eric Lander reports, the review of the paper was painstaking, with journal editors failing to appreciate the significance of Mojica's discovery.[16]

[15] F Mojica, J García-Martínez and E Soria, "Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements," *Journal of Molecular Evolution*, Vol. 60 (2005), p. 174.
[16] E S Lander, "The Heroes of CRISPR," *Cell*, Vol. 164 (2016), p. 18.

Since then, knowledge development accelerated. Several labs started working on CRISPR and, in 2007, the first description of the biological functions of CRISPR was published, demonstrating that bacteria could acquire resistance against external attacks by integrating a genome fragment of an infectious virus into these repeated sequences. Interestingly, this study was the outcome of a collaboration between a university (Université Laval, in Quebec, Canada) and the private sector (the food company Danisco). The year after, researchers at Northwestern University, in Chicago, predicted that CRISPR could be retooled for genome-editing purposes.[17]

These developments brought the field very close to being able to edit the genome using CRISPR. The tipping point was reached in 2012, when Emmanuelle Charpentier and Jörg Vogel discovered *trans*-activating CRISPR RNA, a small RNA that, when encoded by a sequence located upstream in Cas9, recognizes and activates the DNA-cutting functions of Cas9.[18] These and other studies formed the body of knowledge that gave scientists control of CRISPR and allowed them to start experimenting with it. The road was paved by a few additional studies showing that this was a reliable method to produce a double-stranded break at any desired location. Giedrius Gasiunas and colleagues demonstrated that Cas9 could be programmed to cut the purified DNA of a target site of their choosing in vitro.[19] Jennifer Doudna and Emmanuelle Charpentier simplified the technique by consolidating the two RNAs into a single-guide RNA.[20] Another leap forward came when Feng Zhang and George Church showed that CRISPR could be used to edit genomes beyond those of bacteria and microbes. They proved that genome editing could be done respectively on mammalian cells[21] and human cells.[22]

Since then, CRISPR has been improved and adapted for a vast range of applications and is being used in countless labs worldwide, in all kinds of life sciences. Between 2012 and 2018, more than 10,000 scientific papers have

[17] L Marraffini and E Sontheimer, "CRISPR Interference Limits Horizontal Gene Transfer in Staphylococci by Targeting DNA." *Science*, Vol. 322, (2008), pp. 1843–1845.
[18] E Deltcheva and others, "CRISPR RNA Maturation by *Trans*-Encoded Small RNA and Host Factor RNase III," *Nature*, Vol. 471 (2011), p. 602.
[19] G Gasiunas and others, "Cas9–crRNA Ribonucleoprotein Complex Mediates Specific DNA Cleavage for Adaptive Immunity in Bacteria," *Proceedings of the National Academy of Sciences*, Vol. 109 (2012), E2579-E2586.
[20] M Jinek and others, "A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity," *Science*, Vol. 337 (2012), pp. 816–821.
[21] L Cong and others, "Multiplex Genome Engineering Using CRISPR/Cas Systems," *Science*, Vol. 339 (2013), p. 819.
[22] P Mali and others, "RNA-Guided Human Genome Engineering via Cas9," *Science*, Vol. 339 (2013), pp. 823–826.