

PERSPECTIVES



BIOTECHNOLOGY

A prudent path forward for genomic engineering and germline gene modification

A framework for open discourse on the use of CRISPR-Cas9 technology to manipulate the human genome is urgently needed

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Genome engineering technology offers unparalleled potential for modifying human and nonhuman genomes. In humans, it holds the promise of curing genetic disease, while in other organisms it provides methods to reshape the biosphere for the benefit of the environment and human societies. However, with such enormous opportunities come un-

known risks to human health and well-being. In January, a group of interested stakeholders met in Napa, California (1), to discuss the scientific, medical, legal, and ethical implications of these new prospects for genome biology. The goal was to initiate an informed discussion of the uses of genome engineering technology, and to identify those areas where action is essential to prepare for fu-

ture developments. The meeting identified immediate steps to take toward ensuring that the application of genome engineering technology is performed safely and ethically.

The promise of so-called “precision medicine” is propelled in part by synergies between two powerful technologies: DNA sequencing and genome engineering. Advances in DNA sequencing capabilities and genome-wide association studies have provided critical information about the genetic changes that influence the development of disease. In the past, without the means to make specific and efficient modifications to a genome, the ability to act on this information was limited. However, this limitation has been upended by the rapid development and widespread adoption of a simple, inexpensive, and remarkably effective genome engineering method known as clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9 (2). Building on predecessor platforms, a rapidly expanding family of CRISPR-Cas9–derived technologies is revolutionizing the fields of genetics and molecular biology as researchers employ these methods to change DNA sequences—by introducing or correcting genetic mutations—in a wide variety of cells and organisms.

CURRENT APPLICATIONS. The simplicity of the CRISPR-Cas9 system allows any researcher with knowledge of molecular biology to modify genomes, making feasible experiments that were previously difficult or impossible to conduct. For example, the CRISPR-Cas9 system enables introduction of DNA sequence changes that correct genetic defects in whole animals, such as replacing a mutated gene underlying liver-based metabolic disease in a mouse model (3). The technique also allows DNA sequence changes in pluripotent embryonic stem cells (4) that can then be cultured to produce specific tissues, such as cardiomyocytes or neurons (5). Such studies are laying the groundwork for refined approaches that could eventually treat human disease. CRISPR-Cas9 technology can also be used to replicate precisely the genetic basis for human diseases in model organisms, leading to unprecedented insights into previously enigmatic disorders.

In addition to facilitating changes in differentiated somatic cells of animals and plants, CRISPR-Cas9 technology, as well as other genome engineering methods, can be used to change the DNA in the nuclei of reproductive cells that transmit information from one generation to the next (an

organism's "germ line"). Thus, it is now possible to carry out genome modification in fertilized animal eggs or embryos, thereby altering the genetic makeup of every differentiated cell in an organism and so ensuring that the changes will be passed on to the organism's progeny. Humans are no exception—changes to the human germ line could be made using this simple and widely available technology.

MOVING FORWARD. Given these rapid developments, it would be wise to begin a discussion that bridges the research community, relevant industries, medical centers, regulatory bodies, and the public to explore responsible uses of this technology. To initiate this conversation, developers and users of the CRISPR-Cas9 technology, and experts in genetics, law, and bioethics, discussed the implications and rapid expansion of the genome engineering field (1). This group, all from the United States, and which included some of the leaders in the original 1970s discussions about recombinant DNA research at Asilomar and elsewhere, focused on the issue of human germline engineering, as the methods have already been demonstrated in mice (6) and monkeys (7). The Napa discussion did not address mitochondrial transfer (8, 9), a technique that does not use CRISPR-Cas9. Although characterized by some as another form of "germline" engineering, mitochondrial transfer raises different issues and has already been approved by the Human Fertilisation and Embryology Authority and by Parliament in the United Kingdom (10) and is being considered by the Institute of Medicine and the Food and Drug Administration in the United States (11). At the Napa meeting, "genome modification" and "germline engineering" referred to changes in the DNA of the nucleus of a germ cell.

The possibility of human germline engineering has long been a source of excitement and unease among the general public, especially in light of concerns about initiating a "slippery slope" from disease-curing applications toward uses with less compelling or even troubling implications. Assuming the safety and efficacy of the technology can be ensured, a key point of discussion is whether the treatment or cure of severe

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diseases in humans would be a responsible use of genome engineering, and if so, under what circumstances. For example, would it be appropriate to use the technology to change a disease-causing genetic mutation to a sequence more typical among healthy people? Even this seemingly straightforward scenario raises serious concerns, including the potential for unintended consequences of heritable germline modifications, because there are limits to our knowledge of human genetics, gene-environment interactions, and the pathways of disease (including the interplay between one disease and other conditions or diseases in the same patient). In the United States, such human research currently would require an Investigational New Drug exemption from the Food and Drug Administration, but value judgments about the balance between actions in the present and consequences in the future need deeper consideration of the ethical implications of human germline genome editing than the Investigational New Drug process provides.

RECOMMENDATIONS. To better inform future public conversations recommended by the Napa meeting, research is needed to understand and manage risks arising from the use of the CRISPR-Cas9 technology. Considerations include the possibility of off-target alterations, as well as on-target events that have unintended consequences. It is critical to implement appropriate and standardized benchmarking methods to determine the frequency of off-target effects and to assess the physiology of cells and tissues that have undergone genome editing. At present, the potential safety and efficacy issues arising from the use of this technology must be thoroughly investigated and understood be-

fore any attempts at human engineering are sanctioned, if ever, for clinical testing. As with any therapeutic strategy, higher risks can be tolerated when the reward of success is high, but such risks also demand higher confidence in their likely efficacy. And, for countries whose regulatory agencies focus on safety and efficacy but not on broader social and ethical concerns, another venue is needed to facilitate public conversation.

Given the speed with which the genome engineering field is evolving, the Napa meeting concluded that there is an urgent need for open discussion of the merits and risks of human genome modification by a broad cohort of scientists, clinicians, social scientists, the general public, and relevant public entities and interest groups.

In the near term, we recommend that steps be taken to:

- 1) Strongly discourage, even in those countries with lax jurisdictions where it might be permitted, any attempts at germline genome modification for clinical application in humans, while societal, environmental, and ethical implications of such activity are discussed among scientific and governmental organizations. (In countries with a highly developed bioscience capacity, germline genome modification in humans is currently illegal or tightly regulated.) This will enable pathways to responsible uses of this technology, if any, to be identified.

- 2) Create forums in which experts from the scientific and bioethics communities can provide information and education about this new era of human biology, the issues accompanying the risks and rewards of using such powerful technology for a wide variety of applications including the potential to treat or cure human genetic disease, and the attendant ethical, social, and legal implications of genome modification.

- 3) Encourage and support transparent research to evaluate the efficacy and specificity of CRISPR-Cas9 genome engineering technology in human and nonhuman model systems relevant to its potential applications for germline gene therapy. Such research is essential to inform deliberations about what clinical applications, if any, might in the future be deemed permissible.

- 4) Convene a globally representative group of developers and users of genome

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engineering technology and experts in genetics, law, and bioethics, as well as members of the scientific community, the public, and relevant government agencies and interest groups, to further consider these important issues, and where appropriate, recommend policies.

CONCLUSIONS. At the dawn of the recombinant DNA era, the most important lesson learned was that public trust in science ultimately begins with and requires ongoing transparency and open discussion. That lesson is amplified today with the emergence of CRISPR-Cas9 technology and the imminent prospects for genome engineering. Initiating these fascinating and challenging discussions now will optimize the decisions society will make at the advent of a new era in biology and genetics. ■

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GEOLOGY

Defining the epoch we live in

Is a formally designated “Anthropocene” a good idea?

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Human alterations of Earth's environments are pervasive. Visible changes include the built environment, conversion of forests and grasslands to agriculture, algal blooms, smog, and the siltation of dams and estuaries. Less obvious transformations include increases in ozone, carbon dioxide (CO₂), and methane (CH₄) in the atmosphere, and ocean acidification. Motivated by the pervasiveness of these alterations, Crutzen and Stoermer argued in 2000 that we live in the “Anthropocene,” a time in which humans have replaced nature as the dominant environmental force on Earth (1). Many of these wide-ranging changes first emerged during the past 200 years and accelerated rapidly in the 20th century (2). Yet, a focus on the most recent changes risks overlooking pervasive human transformations of Earth's surface for thousands of years, with profound effects on the atmosphere, climate, and biodiversity.

Crutzen and Stoermer originally favored placing the start of the Anthropocene in the late 1700s because of the industrial revolution initiated by James Watt's invention of the steam engine at that time. However, this choice lacked a key requirement for formal stratigraphic designation: a “golden spike” marker that is widely detectable in geologic records. Recently, a working group of the subcommission of Quaternary Stratigraphy of the Geological Society of London released a preliminary recommendation to mark the start of the Anthropocene on 16 July 1945, when the first atomic bomb test took place in Alamogordo, New Mexico (3). The working group chose that time because the isotopic by-products of bomb testing provide a distinctive marker horizon in ice cores, ocean and lake sediments, and soils.

This “stratigraphically optimal” choice [as it was called in (3)] faces intense scrutiny from scientists studying the long history of large and profound human effects on this planet (see the figure). For example, about 65% of the genera of large mammals became extinct between 50,000 and 12,500 years ago, with the two most abrupt extinction episodes in Australia and the Americas (4). Climate cannot be the major factor in

these episodes because most of these genera had survived some 50 previous glacial-interglacial cycles. Hunting and burning by recently arrived humans is the most plausible explanation of these dramatic and unprecedented collapses.

With the beginning of the Holocene around 11,600 years ago, an even more profound human alteration of Earth's surface had begun: the Neolithic agricultural revolution (see the figure). Subsequent millennia

“Does it really make sense to define the start of a human-dominated era millennia after most forests in arable regions had been cut for agriculture...?”

saw global-scale changes that include domestication of the world's crops after 11,000 years ago and livestock after 9000 years ago, followed by the spread of agriculture across all of Earth's arable lands (5), clearance of forested regions with resulting carbon dioxide emissions after 7000 years ago (6), and the spread of methane-emitting rice agriculture and livestock after 5000 years ago (7). Reversals of a natural downward trend in atmospheric carbon dioxide after 7000 years ago and methane after 5000 years ago have both been attributed to gas emissions from farming (8). Other early changes include the transformation of Earth's natural biome vegetation to “anthromes” modified by human activities, with increasing habitat fragmentation (9); disturbance and erosion of soils by human activity (10, 11); the onset of the Bronze Age 5000 years ago and of the Iron Age 3000 years ago; and the appearance of urban areas in Mesopotamia by 5000 years ago. Although these changes began slowly and at different times in different regions,

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