Base Editing and Prime Editing: Genome Editing Without Double-Strand Breaks

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Most genetic variants that contribute to disease are challenging to correct efficiently and without excess byproducts in various cell types using programmable nucleases. In this lecture I describe the development of two approaches to precision genome editing that do not require double-strand DNA breaks, donor DNA templates, or HDR. Through a combination of protein engineering and protein evolution, we developed two classes of base editors (CBE and ABE), proteins that enable all four types of transition mutations (C to T, T to C, A to G, and G to A) to be efficiently and cleanly installed or corrected at target positions in genomic DNA without making double-strand DNA breaks (Komor et al. Nature 2016; Gaudelli et al. Nature 2017). Base editing has been used by laboratories around the world in a wide range of organisms and cell types. By integrating base editors with in vivo delivery strategies, we have addressed animal models of human genetic diseases such as progeria, with phenotypic rescue and lifespan extension. I will also describe prime editing, a versatile and precise genome editing method that directly writes new genetic information into a specified DNA site using a catalytically impaired Cas9 fused to an engineered reverse transcriptase, programmed with a prime editing guide RNA (pegRNA) that both specifies the target site and encodes the desired edit (Anzalone et al. Nature 2019). We performed >175 edits in human cells including targeted insertions, deletions, and all 12 types of point mutations without requiring double-strand breaks or donor DNA templates. We applied prime editing in human cells to correct efficiently and with few byproducts the primary genetic causes of sickle cell disease (requiring a transversion in HBB) and Tay-Sachs disease (requiring a deletion in HEXA), to install a protective transversion in PRNP, and to precisely insert various tags and epitopes into target loci. Four human cell lines and primary post-mitotic mouse cortical neurons support prime editing with varying efficiencies. Prime editing offers efficiency and product purity advantages over HDR, complementary strengths and weaknesses compared to base editing, and lower off-target editing than Cas9 nuclease at known Cas9 off-target sites. Prime editing further expands the scope and capabilities of genome editing.

Cas endonuclease technology in cereals

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As an essential prerequisite for keeping pace with the rapid development of Cas endonuclease technology, a modular and versatile vector system has been developed that is based upon the use of type IIS restriction enzymes and hence allows for complex cloning procedures in single reactions. Not only multiple guide RNAs can be expressed simultaneously, but also newly emerging elements such as Cas derivatives with improved or novel functionality can be readily tested and utilized. In addition, polyethylene glycol-mediated transfection of protoplasts was shown to be a valuable means to put Cas endonuclease vectors to the test prior to their utilization for targeted genetic modification at the plant level. It was further demonstrated that the multiple genetic modifications carried by the typically chimeric primary mutants can be perfectly separated and fixed in just one step by producing doubled haploid progeny. We have been using Cas endonuclease technology in cereals to establish plant resistance to pathogenic viruses and fungi, to modify plant height, spike and grain morphology as well as malting quality.

Towards widespread somatic gene editing in the human brain

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The research area of molecular genetics is currently being revolutionized by gene editing technologies, such as those based on CRISPR (**C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats). The possibility to easily modify the genetic sequence in our cells will inevitably lead to new biological discoveries and open up for new therapeutic opportunities.

There are several reasons why the CRISPR system is revolutionary, one being the versatility of the system: it can be used on multiple organisms allowing both simple and complex gene editing strategies to be pursued in many different cell types. This has led to a rapid development of how to use the CRISPR-based systems for deletion, repair, activation and silencing of genes.

The applications of CRISPR in the brain is now rapidly moving towards use in the human brain. This will certainly allow for the development of new therapeutic possibilities but also raise a number of ethical concerns. In this talk I will discuss around the future perspective of CRISPR-based gene editing in the human brain.

Prospects and challenges for regulation of human gene editing in Europe.

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This talk will consider the regulation of clinical applications of gene editing in a European context. Gene editing using CRISPR/cas-9 and other tools is often considered a novel, disruptive technology. However, the healthcare sector is characterised by considerable existing or 'inherited' regulation that constrains and directs the way new medical technologies develop. Here we take a broad definition of 'regulation' that incorporates both 'hard' law elements and 'soft' governance through market incentives, cultural norms, professional guidelines, and standards. Two existing pathways for human clinical uses of gene editing are identified; as a medicinal product and as an assisted reproductive technology. Adult, or 'somatic' gene editing is most likely to fit within the category of 'gene therapy medicinal products' (GTMPs) under the existing EU Regulations on advanced therapy medicinal products (ATMPs). This regulatory pathway sets standards for safety and efficacy of new gene-modifying medicines. They major challenges for gene editing as a medicinal product are likely to come from health technology assessment, ensuring fair access, and infrastructural change needed to make clinical adoption institutionally viable. Reproductive, or 'germline' gene editing faces a more fragmented regulatory landscape. The major challenges will involve enforcement and monitoring or clinics and international travel by parents seeking to obtain germline gene editing services prohibited in their home jurisdiction, so called 'reproductive tourism'. Finally, the argument for granting a role for patients and the public in developing the wider regulatory framework for human uses of gene editing is made.

Patients' view on genome editing

Nick Meade
Director of Policy, Genetic Alliance UK

Genetic Alliance UK is the national charity supporting everyone living with a genetic, rare or undiagnosed condition. We represent a community with high unmet health needs, which drives an interest in technologies that might advance our understanding of these conditions and/or form a part of a new treatment paradigm. Genome editing is one such technology. In 2017 Genetic Alliance UK collaborated with the Progress Educational Trust to run a project including people living with genetic, rare and undiagnosed conditions, to understand their views on language and images in communication on genome editing, and to empower them to be able to engage in debate on genome editing. Clarity, consistency and simplicity of language was shown to be crucial to facilitating learning in this group. Realistic description of the stage of development of research projects and their future potential is important to manage this community's expectations. Clear delineation of the scope of research in genome editing is necessary for a technology with such wide potential to insulate medically important uses against controversy related to application of the technology elsewhere.

Future CRISPR-potatoes, improved properties for our health and the environment

Dr. Mariette Andersson, Swedish Agricultural University

Can our tasty and healthy potatoes be made even healthier than they already are? Will we be able to eat green potatoes in the future, the ones we sort out today because they are poisonous? Can future potato cultivation lead to products that can lead to a better environment? Plant breeding is an important tool that can contribute to solving some of the global challenges we are facing today, and there are "genetic shortcuts" we can take to get to the solutions faster.
Potato is ranked as one of the most important food crops in the world but is also one of the major crops grown for starch production. Starch produced from potatoes has many uses, both in food and technical applications, and is often chemically or physically modified to reach certain specifications. Cultivated potato is a highly heterozygous and autotetraploid crop with tetrasomic inheritance, which makes conventional cross-breeding a long term process. Therefore, introducing one or a few traits in potato with an elite genetic background and without the need of subsequent outcrossing of DNA inserts is of major interest.
Potato and CRISPR turned out to be a perfect match. A highly efficient DNA-free CRISPR method was established at the Swedish University of Agricultural Sciences (SLU) a couple of years ago. Today, the method is used in research and development of several novel traits in potato, which in the future can lead to a more sustainable agriculture and industrial production as well as food products that have clear health benefits.

On the ethics of heritable genome editing

Prof. Christine Hauskeller, University of Exeter

For several decades we saw the development, trial and practice of new forms of public discourse and decision-making on technologies that are likely to change how we live. In the past decade such bottom up approaches to policy choices and ethical discourse have vanished. This is worthy of inquiry in many aspects. One among them is that some of the technologies that have been discussed extensively when still only hypotheticals, are now doable and some advance rapidly toward application in humans. Designing human genomes, a hot topic in bioethics around the year 2000, is now possible. Yet these ethical discourses did not lead to agreed guidance, value priorities, nor sustained informed public debate. Hence now there are no viewpoints established that oblige science or politics, not locally and certainly not globally. On this basis, I will discuss issues concerning the ethics of heritable genome editing, adopting a critical lense as proposed by feminist and postcolonial critique. There is a big task ahead, trying to find answers beyond the matrix of stark contradictory interests between ethics promoting responsible choice for both present and future humanity and global science and technology development.

CRISPR-food: disrupting public opinion and politics

Dr. Sigrid Bratlie,
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Genome editing is a game-changer for crop and livestock breeding. It is also disrupting the public debate about genetically engineered food. Here, I will present results from a recent survey of Norwegian consumers' attitudes toward genome editing in agriculture and aquaculture which is part of the research project GENEinnovate – a collaboration between several Norwegian plant and animal breeding companies, academic research groups and the public sector.

In contrast to the prevailing perception that consumers (especially in Europe) are generally opposed to genetically engineered food, our findings show that attitudes to genome editing are highly nuanced. In particular, they depend on whether the application is perceived to benefit consumers or to improve sustainability in food production. We also explored aspects such as risk perception, opinions on labelling, ethical views, trust and knowledge level. Consumer attitudes and the public debate about genetically engineered food have great influence on policy and regulations, on which I will comment in light of recent developments in the genome editing field.

Transhumanism and genome editing:

Science Fiction and Science Facts: Genome editing, transhumanism and sociotechnical imaginaries

Ingrid Dunér, Lund University

In an endeavor to manage the future, humans have always made efforts to control their physical and social environment. But what of controlling our very natures? The rapid development of new gene editing techniques like CRISPR/Cas9 actualizes long-standing dreams of human enhancement. On a daily basis the lines between science fiction and science fact seem to blur.

Transhumanism as a philosophy assumes the possibility and desirability of fundamentally improving the human condition by means of science and technology. It views mankind as free to determine its own evolutionary future. A transhumanist discourse and a transhuman perspective on human beings affects mainstream thinking in our contemporary technoculture. The transhuman idea of using science and technology for the enhancement of mankind is now part of a larger cultural imagination. This sociotechnical imaginary is given life through shared understandings of forms of social life and social order attainable through, and supportive of, advances in science and technology.

While transhumanism projects visions of desirable futures, these visions — and the ideas of perfection, dreams of controlling human evolution and the longing to overcome human limitations associated with them — have an intellectual history. What can the history of transhumanism teach us about the future?