Redesigning nature: to be or not to be?

Jayashree Das, Pritha Dey and Pradipta Banerjee*

The concept of designer babies' is indeed intriguing, wherein offspring characteristics can be modified in the embryonic stage by gene editing. Genome editing has got an immense boost with the advent of the Cas/CRISPR technology that utilizes proteins from a bacterial immune system to remove defective genes and replaces them with a rectified edition. The technique is proving to be successful in fighting a host of genetic diseases, including cancer and has even made headway with HIV. The technology has sparked a revolution in genomics with a storm brewing over its patent rights.

Keywords: Designer babies, genome editing, patent rights, redesigning nature.

THE term 'designer babies' includes children whose characteristics have been tampered with by chemical modification of their genetic material while in the womb. The resultant offspring are said to be 'designed' because genetic alteration in the zygote stage is done through technology and is a far cry from being 'natural'. Even though genetic modification seems to be the domain of science fiction, it is a regular course of action that forms the basis of evolution, a gradual process that takes thousands of years perfecting an organism to adapt to its environment. The technique holds the potential to erase genetic defects from family pedigrees plagued by inherited diseases, treat cancer in unprecedented ways and grow human organs in animals.

A designer baby can be made by isolating cells from an embryo, altering the genetic material and re-implanting it in the womb. Recently, scientists have come across genes that help shape the contours of the face[1]. Once the sequence of the genes controlling the features of the body is known, it would be a straight jump to genes controlling intelligence, and muscle and fat deposition. Imagine a readymade baby being designed in the womb, before birth, to be born with certain additional features that would make it in all possibility evolutionarily 'advanced' and better suited. Will that be a blessing, or will it spell doom for mankind? Even ten years back, such a concept would have been straight out of a medical thriller, but the gene editing technologies currently being discovered may make this into a reality.

The science behind it all – introduction to genome editing, key studies and applications

So how do we modify the genome of a baby in the womb? Genes are in fact specific regions in a long chain of polynucleotides which hold information for sequential joining of amino acid residues in order to synthesize a protein. There are 64 known codes and they are grouped as triplets, and each triplet is called a codon. Each codon specifies a particular amino acid (for example, AUG codes for methionine), and multiple such amino acid residues are linked together to form proteins, the building blocks of life. Modification of a gene involves replacing a stretch of nucleotides with a new one that codes for different amino acids to be incorporated in the protein chain. The resulting mutated protein may perform a different function leading to changes in cellular activity.

The target site in the gene to be modified is recognized by a class of nucleic acid degrading enzymes termed as nucleases which are fused with sequence-specific DNA-binding proteins. Among the many genome modifying tools invented, the most commonly used are the zinc finger nucleases (ZFNs) and TALENs.

ZFNs are fusion proteins consisting of 'zinc finger' domains obtained from transcription factors attached to the endonuclease domain from the bacterial FokI restriction enzyme (a restriction enzyme is a bacterial nuclease that cuts the DNA at a specific sequence). Each zinc finger domain recognizes a 3- to 4-base pair DNA sequence, and tandem domains can potentially bind to an extended nucleotide sequence that is unique to a genome. TALENs are similar to ZFNs, except for the substitution of a transcription activator-like effector (TALE) DNA-binding domain instead of the zinc-finger DNA-binding domain to obtain sequence specificity. The ease of engineering TALENs for a wide variety of target binding sites, their high success rate in genome editing, and lower cellular toxicity compared with ZFNs have contributed to their popularity. However, the technology does present some drawbacks. Though ZFNs have shorter nucleotide sequences thereby enabling targeted gene disruption with higher efficiency, the screening and assembly of ZFN modules is technically challenging and off-target mutations are a common side-effect. TALENs exhibit

Jayashree Das and Pritha Dey are in the Department of Biochemistry, School of Biological Sciences, Dayananda Sagar Institutions, and Pradipta Banerjee is in the School of Basic and Applied Sciences, Dayananda Sagar University, Shavige Malleshwara Hills, Kumarswamy Layout, Bengaluru 560 078, India.
*For correspondence. (e-mail: jayashreedas26@gmail.com)

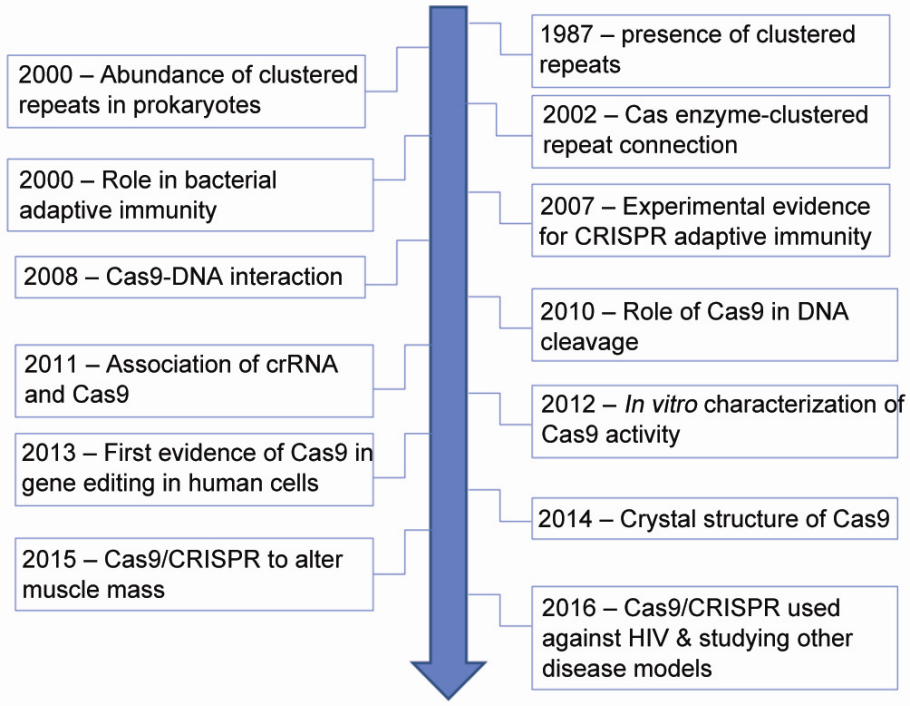

Figure 1. A timeline for the development of Cas9/CRISPR gene editing tool.

comparatively less off-target effects; however, they are less desirable owing to their surpassingly repetitive nature and substantial size, since they cannot be appropriately inserted into the limited space within a viral vector for integration of the gene of interest and subsequent transformation of the cell[2,3].

In the last five years, a new technique is rapidly gaining ground that operates with surgical precision and possesses none of the restrictions of its predecessors. This change in editing technology is due to a newly discovered set of genes known as CRISPR (clustered regularly interspaced short palindromic repeats) and CRISPR-associated proteins (Cas) in bacteria. In 2012, Jennifer Doudna (University of California, Berkeley, USA) and Emmanuelle Charpentier (Umea University, Sweden) published a paper demonstrating that the Cas9 endonuclease enzyme, a key component of a bacterial defence system can be directed to cut specific sites in isolated DNA[4]. Feng Zhang and his co-workers (Broad Institute, Massachusetts, USA) focused on using CRISPR–Cas9 to edit human embryonic cells. In January 2013, the group reported the first successful demonstration of Cas9-based genome editing in human cells in *Science*[5] in an epoch-making paper which has received 2790 citations as of June 2016. George Church and his group (Harvard University, USA) reported similar findings in the same issue of *Science*[6]. The two papers showed that Cas9 was target-specific, indicating that if utilized correctly the tool could be used to 'find' a defective gene and 'replace' it with a corrected version. Figure 1 shows the journey of understanding the Cas/CRISPR system.

At the heart of this genetic editing system, the steps of which are shown in Figure 2, lies the triad consisting of Cas9 nuclease, a guide RNA and a template DNA. The stretch of genes coding for Cas9 enzyme and a guide RNA are integrated into a vector and injected into the host cell, that is, a stem cell taken from an embryo. A second vector containing the template DNA (the corrected copy of the gene to be replaced) is also injected in the same host cell. Once inside the cell, host protein synthesis components synthesize the Cas9 protein and guide RNA from the first vector. The guide RNA molecule directs it towards the stretch of DNA to be replaced, where the latter makes a double-stranded break. This triggers a chemical alarm signal leading to the recruitment of repair proteins at the break site. During DNA repair, the cell is forced to integrate a newly introduced template at that site instead of the original sequence. This template is supplied by the corrected copy of DNA from the second vector. Thus, during the repair process, a corrected gene sequence is inserted in place of the mutated segment. The CRISPR genome is small and non-repetitive, allowing it to be packed inside a small virus vector system[7].

Redesigning nature: use of Cas/CRISPR in genome editing

Academia and research centres have wasted no time in upgrading the Cas/CRISPR system from the laboratory to the industries. Bassuk *et al.*[8] reported that this system can be used to repair genetic defects in stem cells of patients

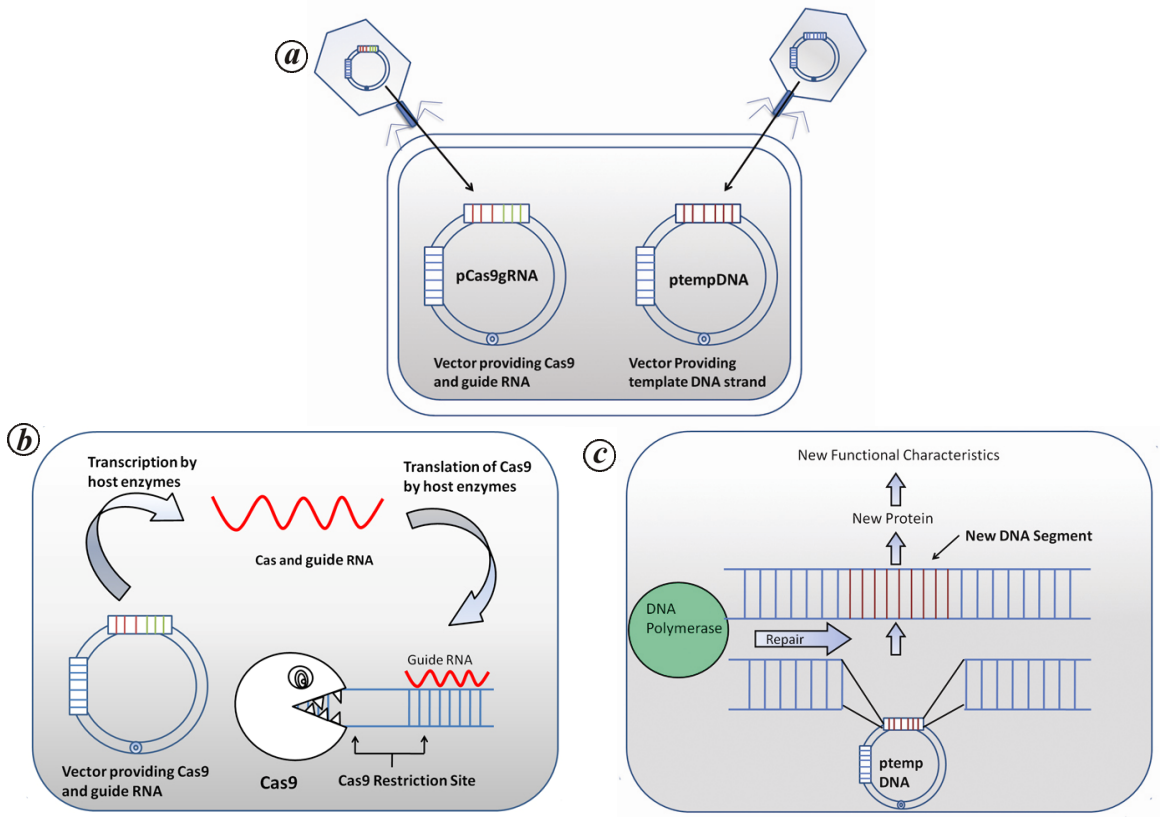

Figure 2. Utilization of Cas9-CRISPER system for genome editing in the embryo. ***a***, Insertion of two plasmids in the host embryonic cell through viral transfection. Both vectors contain an origin for replication, a marker to ensure the success of tranfection and a suitable gene of interest. The first vector, pCas9gRNA is a plasmid containing the gene for Cas9 and a guide RNA that can bind to the host defective gene to be replaced. The second vector, ptempDNA contains a corrected copy of the gene to be replaced, the template. ***b***, The host enzymes synthesize the Cas9 nuclease and the gRNA from the first vector. The gRNA binds with the gene to be replaced and Cas9 makes a double stranded cut. ***c***, Cellular repair proteins insert the template DNA from 2nd vector.

suffering from retinitis pigmentosa. They showed that 13% of the defective gene copies could be completely replaced by the corrected copy. These stem cells, with genomes bereft of the disease-causing mutation, could then be used for retinal transplantation of the patient, paving the way for rejection-free transplantation.

CRISPR has also been used to battle HIV, although with conflicting results. Kang *et al.*[9] reported that viral entry into immune cells can be blocked by the successful replacement of *CCR5* gene in human stem cells using CRISPR/Cas9. Wang *et al.*[10] reported that repairing the DNA fragmented by Cas9 by the error-prone repair machinery of the cell led to mutated sequences that disrupted the viral life cycle. Some mutations, however, led to the emergence of competent viruses that were resistant to Cas9/gRNA.

The Cas/CRISPR system has also been used for site-specific mutagenesis and allelic replacement in yeast. DiCarlo *et al.*[11] reported that double-strand breaks caused by Cas9 in yeast can increase double-strand repair mechanism rate by 130-fold compared to other nucleases, thus leading to faster production of recombinant yeast.

Another scenario where CRISPR is expected to play a major role is in combating the threat of cancer. Cellular cancer occurs due to certain specific gene mutations producing mutated altered functionality proteins that lead to unregulated growth, altered metabolism, change in the cell environment and metastasis. Cancer can be stopped at the initial stages without any radio- or chemotherapy if the mutated gene can be replaced with a target corrected copy[12].

Designer animals are not far behind. Scientists in China have successfully deleted two genes from early-stage goat embryos that suppress both hair and muscle growth, giving birth to kids exhibiting both larger muscles and longer fur, paving the way for creation of designer animals to be used for research[13]. Wang *et al.*[14] succeeded in deleting the MSTN gene in pig genome via CRISPR leading to the birth of piglets with heavy musculature. The process has already been used on cows and sheep with 50% success rate[15]. The CRISPR/Cas9 system has been used to modify three genetic loci in Bama pigs: parkin, DJ-1 and PINK1 in order to study Parkinson's disease[16].

Ramifications on the biological frontier

In January 2015, a group of interested stakeholders met in Napa, California, USA, to discuss the scientific, medical, legal and ethical implications of these new frontiers in genomics. The meeting identified immediate steps towards ensuring that the application of genome engineering technology is performed safely and ethically[17]. This meeting was the starting point for a broader conversation, the most prominent of which, the International Summit on Human Gene Editing, was held in Washington DC, USA, where nearly 500 scientists, ethicists, legal experts and advocacy groups from more than 20 countries came together to issue guidelines for the use of gene editing in humans. In reminiscent of the Asilomar Conference of 1975, which restricted DNA hybridization, the summit was organized by the US National Academies of Sciences and Medicine, the Royal Society of London and the Chinese Academy of Sciences. The meeting highlighted China's emerging prominence in genomics. The verdict was that gene modification experiments should not be condemned, but a host of ethical and safety issues should be resolved before embryos are modified for clinical applications, although gene editing after birth to correct defects in non-reproductive cells was encouraged. The meeting had mixed effects on the scientific community. Some scientists felt that it was justified to be extra cautions while dealing with new technology, as a small wrong step can lead to entire characteristics to be added to the gene pool, or worse, deleted out of the gene pool. On the other hand, many scientists felt that research that has likely benefit today and in the future should not be thwarted by sowing panic in the population about speculative harm in the distant future[18].

Patent rights and future of gene editing

Two of the most powerful universities in the US are engaged in a vicious war over the basic patent. The Broad Institute (a non-profit biomedical and genomic research centre in partnership with Massachusetts Institute of Technology and Harvard University) represents Zhang and his claim. Doudna and University of California (UC) represent the other stakeholder. Even though UC was the first to apply for the patent, the Broad, Institute paid for an accelerated review of its key patent application, and was awarded the same. Nevertheless, UC has called for an 'interference proceeding' which will resolve which organization is entitled to the key CRISPR patents.

Start-up companies devoted to CRISPR have already been launched. Doudna and Charpentier have initiated a company called Caribou Biosciences and along with Intellia Therapeutics, have obtained patents to locate precise sequences of nucleic acids that might be of interest in terms of diseases. Pharmaceutical giant Novartis has invested in both start-ups. Charpentier has co-founded another biotech company called Crispr Therapeutics, while Zhang, Church, and several others have co-founded Editas Medicine. Thus far, the four companies have raised at least US$ 158 million in venture capital[19].

However, numerous challenges lie ahead. Most importantly, successful transition from changing one or two genes to holistic clinical-level changes will depend on efficient target-specific delivery systems to diseased tissues. To simultaneously address a broad spectrum of genetic disorders, the efficiency of the template-based DNA-repair mechanism needs to be thoroughly studied and the off-targeting mutations of CRISPR need to be reduced. It will be of utmost importance to rigorously characterize the safety as well as physiological effects of gene editing in a myriad of preclinical models[20].

Designer babies: pros and cons

Editing the human germline – the genes passed on from generation to generation that have evolved naturally over millions of years to create each unique one of us – has gone from science fiction to science fact. Science now allows us to design our descendants. On the broad side, there are certain advantages in making a designer baby: it reduces the risk of genetic diseases and ensures that a defective gene gets obliterated from the family line. Along with individual achievements, gene editing will provide insight into genotype–phenotype correlation and mind–matter dichotomy. However, it is of serious concern that the 'cons' may far outweigh the 'pros'. Since the editing process is costly, families will be charged heavily for re-engineering embryonic genome restricting the technology to the financially stable class of the society. Introduction of such a protocol will lead to a gap in the society worldwide, not to mention, developing countries where there is a large difference between the rich and poor. Moreover, genes often work in tandem and there is always the chance that disrupting one gene may disrupt large signalling pathways leading to cell damage. Possibility of damage to the gene pool also becomes a real threat. Some scientists condemn gene editing on ethical grounds that the baby, whose genes are to be changed, has no say in this matter[21,22].

Conclusion

To summarize, even though gene editing technologies are accessible, caution is of utmost importance. The ease and efficiency of the CRISPR/Cas9 system enables it to be used in a variety of applications, including gene editing, gene function investigation and gene therapy in human and animals cells. Cas9-mediated genetic editing is simple and scalable, enabling researchers to decipher the

functional organization of the genome at a holistic 'systems' level and establish cause-and-effect relationships between genetic variations and their expressed phenotypes.

The saga of how a fascinating adaptive defence system of bacteria turned out to be the key behind one of the most powerful and versatile tools for genome engineering emphasizes the importance of research in fundamental biology. Just as research in bacterial restriction endonucleases, an anti-viral defence system gave rise to recombinant DNA technology, investigation into bacterial adaptive defence systems has brought forth the most recent generation of Cas–CRISPR gene editing tools. It is highly likely that nature already holds the answers to most of the problems faced today, waiting to be discovered by eager researchers. The future looks bright for genomics, no matter how far the destination lies.

Declaration: The authors declare that this article has not been submitted to any other journal and they do not have any conflict of interest. The figures have been generated by the authors.

1. Adhikari, K. *et al.*, A genome-wide association scan in admixed Latin Americans identifies loci influencing facial and scalp hair features. *Nature Commun.*, 2016, **7**, 10815.
2. Gupta, R. M. and Musunuru, K., Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. *J. Clin. Invest*, 2014, **124**, 4154–4161.
3. Tong, C. *et al.*, Generating gene knockout rats by homologous recombination in embryonic stem cells. *Nature Protoc.*, 2011, **6**, 827–844.
4. Jinek, M. *et al.*, A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*, 2012, **337**, 816–821.
5. Cong, L. *et al.*, Multiplex genome engineering using CRISPR/Cas systems. *Science*, 2013, **339**, 819–823.
6. Mali, P. *et al.*, RNA-guided human genome engineering via Cas9. *Science*, 2013, **339**, 823–826.
7. Cho, S. W. *et al.*, Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. *Nature Biotechnol.*, 2013, **31**, 230–232.
8. Bassuk, A. G. *et al.*, Precision medicine: genetic repair of retinitis pigmentosa in patient-derived stem cells. *Sci. Rep.*, **6**; doi: 10.1038/srep19969.
9. Kang, H. *et al.*, CCR5 disruption in induced pluripotent stem cells using CRISPR/Cas9 provides selective resistance of immune cells to CCR5-tropic HIV-1 Virus. *Mol. Ther. Nucleic Acids*, 2015, **4**, (accepted and published online).
10. Wang, Z. *et al.*, CRISPR/Cas9-derived mutations both inhibit HIV-1 replication and accelerate viral escape. *Cell Rep.*, 2016, **15**, 481–489.
11. DiCarlo, J. E. *et al.*, Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. *Nucleic Acids Res.*, 2013, 1–8.
12. Sánchez-Rivera, F. J. and Jacks, T., Applications of the CRISPR-Cas9 system in cancer biology. *Nature Rev. Cancer*, 2015, **15**, 387–395.
13. Ni, W. *et al.*, Efficient gene knockout in goats using CRISPR/Cas9 system. *PLoS ONE*, 2014, **9**, e106718.
14. Wang, K. *et al.*, Efficient generation of myostatin mutations in pigs using the CRISPR/Cas9 system. *Sci. Rep.*, 2015, **5**, 16623.
15. Crispo, M. *et al.*, Efficient generation of myostatin knock-out sheep using CRISPR/Cas9 technology and microinjection into zygotes. *PLoS ONE*, 2015, **10**, e0136690.
16. Wang, X. *et al.*, One-step generation of triple gene-targeted pigs using CRISPR/Cas9 system. *Sci. Rep.*, 2016, **6** (accepted, available online).
17. Baltimore, D. *et al.*, A prudent path forward for genomic engineering and germline gene modification. *Science*, 2015, **348**, 36–38.
18. Center for Genetics and Society, About human germline gene editing; http://www.geneticsandsociety.org/article.php?id=8711 (accessed on 10 June 2016).
19. Regalado, A. CRISPR patent fight now a winner–take–all match. *MIT Technol. Rev.*, 2015; https://www.technologyreview.com/s/536736/crispr-patent-fight-now-a-winner-take-all-match/ (accessed on 15 June 2016).
20. Hsu, P. D. and Lander, E. S. and Zhang, F., Development and applications of CRISPR–Cas9 for genome engineering. *Cell*, 2014, **157**, 1262–1278.
21. Sterckx, S. *et al.*, 'I prefer a child with …': designer babies, another controversial patent in the arena of direct-to-consumer genomics. *Genet. Med. Off. J. Am. Coll. Med. Genet.*, 2013, **15**, 923–924.
22. Evans, M., Designer babies – why not? *N. Z. Bioethics. J.*, 2001, **2**, 17–25.

ACKNOWLEDGEMENT. We thank Dayananda Sagar University, Bengaluru for support.

Received 27 July 2015; revised accepted 27 October 2016

doi: 10.18520/cs/v112/i07/1346-1350