Genome editing

Promises and problems

It has become relatively easy to add, alter, inactivate and remove genes from genomes. Bioethicist John Bryant explains some of the potential benefits and downsides in relation to human health and agriculture

Methods for cutting DNA out of genomes were first developed in the late 1990s. They were useful because they enabled the very precise 'knock-out' of specific genes in order to study gene function. But they were relatively cumbersome and could only be used on one gene at a time. This changed with the arrival of genome-editing methods based on a type of bacterial DNA called CRISPR.

Key words

CRISPR
germ-line
transplant
cancer

AQ/Ed: key words OK?

The CRISPR genome editing system

The story starts in the late 1980s, when repetitive DNA sequences were found in bacterial genomes. The repetitive DNA elements are 30 base pairs in length and their sequences are palindromic — they read the same in both directions (see Figure 1) — and are found in clusters. The term Clustered Regular Interspersed Short Palindromic Repeats (CRISPR) was introduced, but their function was unknown. The DNA sequences between the repeats are similar to the DNA of bacteriophages — viruses that infect bacterial cells — which suggested that they may be part of a bacterial immune reaction against bacteriophages. It took another 5 years of research to confirm this.

The sequences between the repeats are synthesised in response to exposure to a foreign genome. They are copied into RNA — CRISPR RNA (crRNA) — when the bacterial DNA next encounters the foreign genome. The crRNA, helped by another type of RNA — trans-activating crRNA (tracrRNA) — guides an enzyme that cuts DNA, a nuclease, to the invading genome and inactivates it by cutting it at precise sites (see Figure 2). In 2011 it was shown that CRISPR systems can work when put into other species. This paved the way to a technique for removing almost any sequence from almost any genome.

Medical applications of CRISPR

It is theoretically possible to use the CRISPR system to remove any segment of DNA, whether a gene, a regulatory sequence or a sequence of unknown function. Following removal of the sequence, the DNA may be repaired by the cell's own repair mechanisms, especially if the gap is short. Alternatively, repair can be effected by a DNA patch. This DNA can be the fully functioning version of a faulty gene that has been cut out. Alternatively, a DNA sequence may be inserted into a cut, for example when a scientist wishes to use inserted DNA to stop a gene from working (as in Figure 2). Gene knockout has thus become quite straightforward and the system is widely used in this way.

One potential of CRISPR was shown in 2015 when a team at Harvard used it to remove 62 retroviral sequences from the pig genome as part of a programme to develop pig organs for use in human transplants (see Box 1).

Several of the proposed medical applications of CRISPR relate to genetic diseases. For example, if an embryo was heterozygous at a locus with a dominant mutant allele, genome editing could be used to remove the mutant allele, leaving the remaining wild-type allele to function normally. For embryos that are homozygous for recessive mutations, the situation would be more complex, but nevertheless solvable. Removal of both copies of the gene would be followed by insertion of the wild-type sequence. Note that because the genome editing is done with very early embryos, before implantation into the uterus (and therefore before the formation of different types of cell, including germ-line cells), the changes in the genome are inherited — they are changes to the germ-line. This contrasts with somatic cell genome editing where the target cells are not (or do not give rise to) germ-line cells and changes to the genome are therefore not inherited.

There has been some progress along these lines in experiments with mouse embryos. Scientists in the USA have successfully clipped out the DNA containing the mutation from the mutant dystrophin gene, which causes Duchenne muscular dystrophy. Re-joining the cut ends led to a slightly shorter gene, expression of which produced a dystrophin protein that functioned much better than the mutant version, although not as well as the normal protein. It will be clear from what was said earlier that this change would be inherited, i.e. it is a change to the germ-line.

Any experimentation on human embryos raises the issue of the moral status of the embryo. For those who believe that even the earliest embryos should be treated as persons, this is a concern. For people holding this view, an experiment that would not be permitted with a born human subject should not be carried out with embryos. It is doubtful whether, at present, genome editing of born human subjects would be allowed under the provisions of the Helsinki Declaration (Ethical Principles for Medical Research Involving Human Subjects) except under particular medical circumstances. However, the majority view is that the early embryo is *not*

regarded as a person and therefore appropriate experiments may be carried out with embryos.

There is also the possibility of using genome editing as a means of human enhancement rather than confining it to therapeutic applications. The distinction between therapy and enhancement, although sometimes hard to define, has been widely used as a marker in ethical discussion of human genetic modification. The same distinction is applied in discussion of genome editing. In the context of sport, editing procedures that modify constraints on muscle growth or on the synthesis of oxygen-carrying proteins would not be permitted.

In addition to ethical concerns mentioned here and earlier, there are concerns about the safety of changing the genes of an embryo, whether by genetic modification or by genome editing. For genome editing, these risks include the possibility of 'off-target' editing, where a sequence similar to the target sequence is also recognised by the CRISPR-cas9 system.

Nevertheless, research on genome editing in human embryos is proceeding in a few countries, including the UK and China. It was in China where the first attempts were made to edit the mutant human gene that causes β-thalassaemia. Removal of the mutant gene was successful in nearly all embryos but its replacement with the correctly functioning allele failed in the majority, so the experiments were discontinued (it was never intended to implant the genome-edited embryos — the experiments were designed to see whether gene replacement was possible).

Gene edited humans

This is not the end of the story, however. In November 2018, a Chinese medical scientist announced that he had successfully edited the genomes of several embryos, which had been used to start pregnancies. Twins had already been born and further babies are on their way.

The editing procedure knocked out the CCR5 gene that enables the human immuno-deficiency virus to recognise and attack the immune system, thereby making the babies resistant to HIV (the fathers of all the embryos were HIV-positive). There was an immediate outcry across the world. The Chinese government stated that the work had been carried out without permission and even people who normally take a liberal line on human genetics condemned the work.

The main points of the condemnation of the work were that we do not yet know whether the procedure has long-term risks, that the procedure brought no benefit to the recipients (either in terms of therapy or enhancement) and may even have exposed them to specific harm. The fathers were HIV-positive but that does not mean that their children will suffer from HIV and in any case there are well-established non-genetic therapies for dealing with the virus. Furthermore, the knock-out of that particular gene may make people less resistant to other viruses.

Meanwhile, in the USA, progress has been made with CRISPR-based correction of thalassaemia and other blood protein disorders in human bone marrow cells — stem cells that give rise to the various types of blood cell. Bone marrow stem cells are removed from the patient, altered by genome editing and then replaced. This is a non-heritable, somatic cell change, similar in some ways to the correction of SCID (severe combined immuno-deficiency — see Biological Sciences Review, Vol 31, No. 2, pp. 38–41) by GM techniques. Applications to undertake clinical trials are in the pipeline.

Another example was the use at Great Ormond Street Hospital in 2015 of genome-edited T cells — one of the cell types that make up the immune system. An experimental procedure saved the life of a baby girl suffering from an aggressive form of leukaemia. This technique may be usable with other cancers.

However, for all possible applications in mammals and especially humans, a loud alarm bell has just been sounded. During experiments with mice, two research groups have reported significantly increased rates of cancer. It appears that p53, one of the master regulator proteins of the cell division cycle, is somehow activated, leading to unscheduled cell division. This only happens when the genome editing procedure involves insertion of a sequence to replace a piece of DNA that has been removed but not with procedures that only involve gene knockout. This gives a good reason to pause for thought as the biomedical community seeks to develop applications.

Agricultural applications

Many problems in agriculture arise from diseases of animals and of plants. Many pathogens are able to infect their hosts because they interact with specific host proteins. Removal of the genes that encode those proteins is a possible route to disease resistance. One example is the recent creation of pigs resistant to porcine reproductive and respiratory syndrome, a disease that causes respiratory difficulties, often leading to death of young pigs. This was done by cutting out a tiny part of the gene that encodes the pig protein with which the virus interacts. The slightly shorter protein still carries out its normal function but the virus can no longer lock onto it.

In plants, the genome of the main commercial variety of banana — *Cavendish* — has been edited to make it resistant to *Fusarium* wilt. This variety accounts for 99.9% of the global trade but it is already threatened by *Fusarium* in Australia, southeast Asia, parts of Africa and the Middle East. If the disease spreads to Latin America, the source of most exported bananas, the situation will become serious. Hence there is a need to develop resistant cultivars.

But there has been a lot of opposition to genetically manipulated (GM) crops, much of which has no scientific basis. Although genome editing does not have to involve adding genes to a genome, some campaigners have already started to oppose genome editing (although the US regulatory authorities do not intend to impose the same burdensome requirements on genome-edited crops as are imposed on GM crops). The same is not true in the EU, much to the dismay of the European plant science community. And the question remains: will the public eat genome-edited crops and animals?

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Further reading

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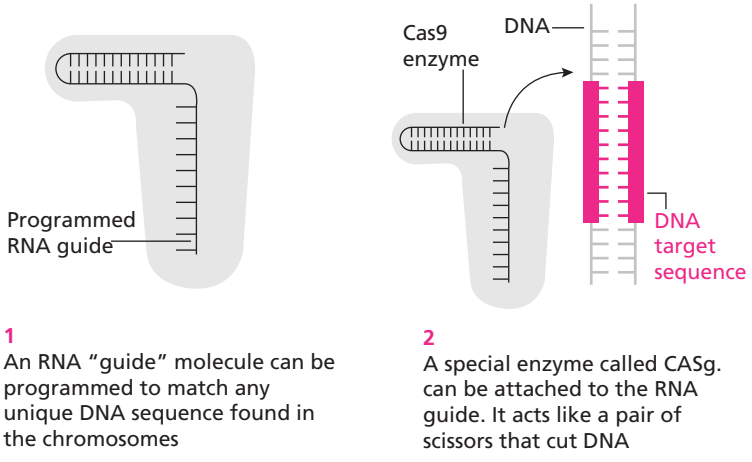

1
An RNA "guide" molecule can be programmed to match any unique DNA sequence found in the chromosomes

2
A special enzyme called CASg. can be attached to the RNA guide. It acts like a pair of scissors that cut DNA

3
The Rna aligns with the target DNA sequence and the CASg attaches and cuts both strands of the DNA double helix

4
The DNA cuts can be amended with an extra DNA insertion (above), or a deletion to eliminate the gene

Figure 2 Diagram showing how the CRISPR-cas9 system may be used to edit DNA. As shown in the last panel, when a gene is cut just once, another piece of DNA may be inserted at the cut site. Alternatively, if a second cut has been made to delete a gene or a section of a gene, the inserted DNA may be a 'patch' to guide DNA repair or may even be a normal copy of a gene (if the deletion has removed a mutant version)

CATTGCGCAATG
GTAACGCGTTAC

Figure 1 A palindromic DNA sequence (note, this is not the CRISPR sequence)

Box 1 Pig organs for human transplant

There is a shortage of donated organs available for patients who need a transplant. Medical scientists are therefore considering the possibility that pigs (with organs in the same size range as humans) might be suitable donors. Among the problems to be overcome if this xeno-transplantation (literally foreign transplantation) is to be successful is the presence in the pig's genome of retro-viruses. These are viruses which, in past infections in the evolutionary history of the pig, have inserted themselves in the pig's DNA and are now dormant. The worry is that they may be activated by transplantation into a human host and thus cause disease, hence the use of genome editing to remove them.