GENETIC ENGINEERING

A CRISPR gun for hire

Parasitic genetic elements called transposons carry CRISPR machinery that is normally used against them by bacterial cells. This paradox has now been explained, with implications for gene-therapy research. SEE ARTICLE P.219

FYODOR D. URNOV

When Hamlet is mortally wounded by Laertes' poisoned blade in a fencing match, he switches weapons and strikes back, so that Laertes is killed by his own sword. Klompe *et al.*[1] now describe an equally dramatic weapon switch in biological warfare. They report on page 219 that a molecular machine called Cascade, which bacteria use to defend themselves against genetic invaders, can also be used against them by some of those invaders. To add to the drama, this tiny instrument of war might eventually find itself serving a peaceful purpose: the genetic engineering of human cells to treat disease.

The genomes of bacteria are under constant assault from 'selfish' DNA segments (such as genes from bacterium-infecting viruses and mobile genetic elements), which enhance their own propagation and transmission, rather than their host's. One type of mobile element is called a transposon. Some transposons carry just five genes, the sole function of which is to spread the transposon among bacteria[2]. The protein products of these genes work together to insert the transposon DNA into a specific spot in a bacterium's genome at which insertion does not harm the host. The transposon thus becomes a permanent 'passenger' in that bacterium. When the opportunity arises, it transfers itself into one of the small, circular pieces of DNA that bacteria pass between each other to transfer genetic material, and can thereby move to a new host[2].

Bacteria are armed with several defence systems against such parasites. One is known as CRISPR (ref. 3), and works in a similar way to a 'wanted' poster of a criminal. When foreign DNA enters a bacterial cell, CRISPR chops it up and places a few fragments into the bacterial genome. These fragments are not dust-gathering war trophies, but 'memories' of past invasions: the bacterium copies them into short snippets of RNA, and hands them over to dedicated CRISPR-associated nuclease enzymes, of which Cas9 is the best studied[4,5]. These nucleases carry the RNA snippets and compare them with incoming DNA; if there is a match, the invading DNA is destroyed.

In 2017, a strange fact was reported by Peters *et al.*[6]: some transposons also carry genes for Cascade, a type of CRISPR defence system. This made no sense. Why would a parasitic genetic element need defence machinery that targets itself? Not all features of living things are Darwinian adaptations, but the puzzling prevalence of Cascade in transposons from many bacteria implied that it had to be there for a reason.

However, Peters *et al.* noted two peculiarities of the Cascade–transposon systems. First, although the Cascade machinery still recognized a target DNA by comparing it with an RNA snippet carried on a Cas-type protein, this machinery could not cut the DNA, and so was like a gun loaded with blanks. Second, the transposon carried all the usual genes required to integrate its DNA into a bacterial genome, but lacked the gene that directs that integration to the usual 'safe for the host' destination — thus preventing the Cascade gun from aiming at a specific target. Peters *et al.* hypothesized that these two minuses make a plus: perhaps the transposon uses Cascade to recognize a new DNA target in a bacterium, and then routes the integration of transposon DNA to that site?

Klompe and co-workers now provide a wealth of experimental data that prove and expand this idea. They show that the transposon can use the RNA-guided component of its Cascade passenger to direct Cascade to a particular position in a genome. They also report that, after recognizing the target DNA, Cascade directly binds to a protein (TniQ) that guides the insertion of the transposon to the new location in the genome (Fig. 1). This insertion is impressively specific — in all 25 cases studied by the authors, the transposon was delivered precisely and exclusively to the targeted address in the bacterial genome. Klompe and colleagues' findings illuminate how evolution in microbes can morph, shuffle and combine components to come up with radical new solutions to problems — in this case, resulting in an RNA-guided transposition of DNA.

The work will inspire researchers working on an entirely different scientific front: the genetic engineering of humans to treat disease. Therapeutic genes are conventionally installed in humans using viruses that either persist outside the cell's genome (which means that they are rapidly diluted when the cell divides) or land semi-randomly within the genomic DNA (which raises potential safety concerns)[7]. One solution to this problem is the technique called genome editing[8,9] — in which an engineered nuclease, such as Cas9, is targeted to cut DNA at a position of interest to produce a double-strand break (DSB), which is then repaired using a template that facilitates the insertion of a gene at that position[10] (Fig. 1a).

Although DSB-driven gene addition is useful, it has limitations. First, it works relatively inefficiently in non-dividing cells, many of which are potential targets for gene therapy. Second, the gene to be inserted must be flanked by DNA segments that match the sequence in the region of the genome into

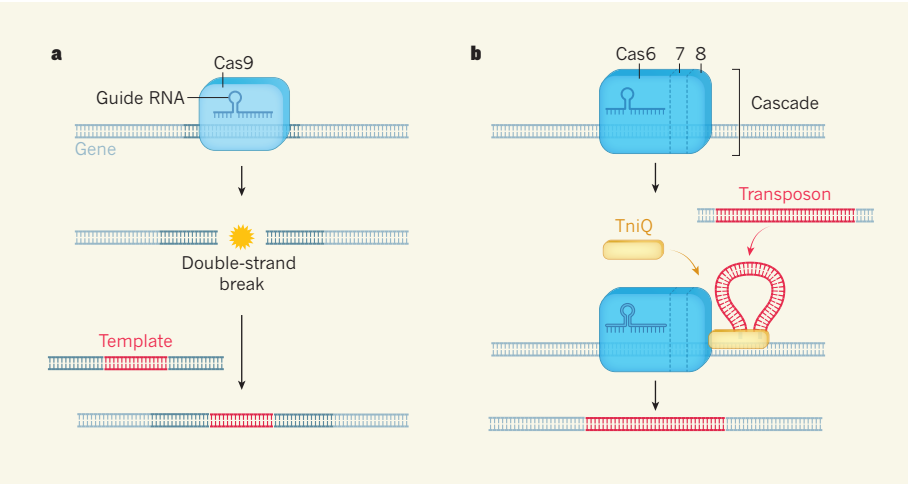

Figure 1 | Two ways in which genes can be inserted into chromosomes. **a**, In conventional gene editing, a nuclease enzyme (such as Cas9, part of the CRISPR defence mechanism in bacteria) is directed to a position on a chromosome by a guide RNA. The nuclease produces a double-strand break, which is repaired using the host cell's machinery. The repair process is guided by a DNA template in which a therapeutic gene is flanked by stretches of DNA that are identical to the chromosome, and incorporates the gene into the chromosome[10]. **b**, Klompe *et al.*[1] report that DNA elements called transposons use CRISPR machinery called Cascade (formed from Cas6, Cas7 and Cas8 proteins) to insert themselves into genomes. Cascade is directed to a chromosome by a guide RNA, but then binds a transposase-associated protein, TniQ, which in turn recruits the transposon and integrates it into the chromosome. This RNA-directed mechanism for DNA transposition avoids the need for double-strand breaks or long flanking sequences, and thus might help to address some of the shortcomings of conventional gene editing.

which it is being inserted, which takes up valuable space in the therapeutic agent. And third, the generation of a DSB has an associated risk[11], albeit a manageable one. Both Peters *et al.*[6] and Klompe *et al.* suggest that the reported transposons provide, in principle, a solution to all those issues: the transposon integration process does not require a DSB at the target (Fig. 1b), or flanking DNA in the therapeutic agent, and should work in non-dividing cells. Hence, it could be an attractive approach for human gene editing in the clinic.

However, a long checklist must be completed before clinical applications can be considered seriously. This list includes: showing that the process works efficiently at target genome positions in disease-relevant human cells (rather than in bacteria); demonstrating that it can integrate DNA fragments large enough to be clinically useful; proving its specificity in the human genome, which is about 1,000 times larger than a bacterial one; and developing ways to deliver the full complement of proteins associated with the integration process to cells without triggering the human immune response. This is a formidable workload, but a key lesson of the past 30 years of research into gene therapy is that most challenges of this type are eventually solved[7,11,12]. Therefore, a CRISPR system used by transposons to propagate themselves might well find itself repurposed for genetic medicine. ■

Fyodor D. Urnov *is at the Innovative Genomics Institute, Berkeley, California 94704, USA.*
e-mail: urnov@berkeley.edu

1. Klompe, S. E., Vo, P. L. H., Halpin-Healy, T. S. & Sternberg, S. H. *Nature* **571**, 219–225 (2019).
2. Peters, J. E., Fricker, A. D., Kapili, B. J. & Petassi, M. T. *Mol. Microbiol.* **93**, 1084–1092 (2014).
3. Barrangou, R. & Horvath, P. *Nature Microbiol.* **2**, 17092 (2017).
4. Jinek, M. *et al. Science* **337**, 816–821 (2012).
5. Jiang, F. & Doudna, J. A. *Annu. Rev. Biophys.* **46**, 505–529 (2017).
6. Peters, J. E., Makarova, K. S., Shmakov, S. & Koonin, E. V. *Proc. Natl Acad. Sci. USA* **114**, E7358–E7366 (2017).
7. Anguela, X. M. & High, K. A. *Annu. Rev. Med.* **70**, 273–288 (2019).
8. Carroll, D. *Annu. Rev. Biochem.* **83**, 409–439 (2014).
9. Urnov, F. D. *CRISPR J.* **1**, 34–46 (2018).
10. Moehle, E. A. *et al. Proc. Natl Acad. Sci. USA* **104**, 3055–3060 (2007).
11. Porteus, M. H. *N. Engl. J. Med.* **380**, 947–959 (2019).
12. Dunbar, C. E. *et al. Science* **359**, eaan4672 (2018).

This article was published online on 12 June 2019.

EVOLUTION

Fishing out a feeding paradox

If an animal's body shape is specialized in a way that aids feeding on specific organisms, does this restrict what the animal can prey on? An observation of fishes feeding in the wild might now help to settle this question.

SEBASTIAN KRUPPERT & ADAM P. SUMMERS

A chance observation of fish behaviour, made during an underwater survey along the eastern shore of Lake Tanganyika in Tanzania, has now been reported in *American Naturalist* by Golcher-Benavides and Wagner[1]. Their observation neatly ties together 40-year-old laboratory data[2] and a model of evolution based on an idea known as optimal-foraging theory[3].

The serendipitous event occurred when Golcher-Benavides was on a dive with a Tanzanian colleague, George Kazumbe, studying the species present in a region perpendicular to the lake's shoreline. They saw ahead, sparkling between the lake's surface and its rocky bottom, a massive school of juvenile sardines, estimated to comprise at least 50,000 individuals. Video footage of this event captured what happened when the sardines encountered fishes belonging to a group called the cichlids.

There are about 250 species of cichlid fish in Lake Tanganyika[4]. These species represent fishes that have a wide variety of feeding specializations, including those that have evolved in a way that allows them to target a single type of prey[5–7], as well as fishes that are capable of eating diverse sources of food. The shapes and features of the heads of some cichlid species bear witness to the adaptation that is suited to their particular food source (Fig. 1).

One example of a cichlid species that has evolved a feeding specialization is *Perissodus microlepis*. This fish has a curved head, and when it swims alongside a larger fish, it can suddenly attack and snatch a mouthful of scales[8]. The population of this species is split between fish whose head is curved to the left for attacking the right side of its fish prey, and fish whose head is bent rightward to enable an assault on the prey's left side. Other cichlid feeding specializations include those for scraping algae from rocks[9], biting out the eyes of other fish[10], and gobbling eggs knocked out

50 Years Ago

Assisted by off-stage noises which included a belching elephant seal, a giant toad in mating cry … and the song of a wren played at slow speed, the British Library of Wildlife Sounds (BLOWS) was opened recently by Mr David Attenborough … The library … aims to be the national reference collection of wildlife sounds of all descriptions … Used in conjunction with other biological reference collections, BLOWS should have an important part to play in research into animal behaviour, taxonomy and evolution … The library's target is 10,000 recordings (disk or tape) of 2,500 species of animal in five years, and Mr Attenborough appealed for copies of commercial gramophone records … and for copies of properly documented tape recordings of any animal sound made by either professional or amateur tape recordists.
From *Nature* 12 July 1969

100 Years Ago

In the April issue of the *Journal of Mental Science* … Capt. O. P. Napier Pearn describes the differences and similarities in the actual insanities (psychoses) found in military and civil practice … He has collected and tabulated the facts relating to 200 cases which made a sufficiently good recovery to warrant their being returned to duty … [W]hile at the onset of a mental disorder in civil life the friends and relatives usually co-operate with the sick person in shielding him from medical advice, such a patient in the Army … is much more likely to receive attention from his medical officer at an early stage. The effect of this early care is that these cases respond to treatment in a very gratifying way … The article, while laying claim to no new discovery, lays additional emphasis upon the urgency of the early treatment of mental disorders.
From *Nature* 10 July 1919