DNA NANOTECHNOLOGY

Bacteria as factories

Producing large quantities of designer DNA nanostructures at low cost has been a long-standing challenge in nanobiotechnology. It is now possible with the aid of bacteria.

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Deoxyribonucleic acid (DNA) — the molecule that encodes the genetic information in living systems — can be manipulated to build two- and three-dimensional designer nanostructures[1–3] that can then be used as templates to make other nanostructures, as well as scaffolds to organize nanoparticles and proteins. However, making these nanostructures is time-consuming and expensive. Writing in the *Proceedings of the National Academy of Sciences USA*, Hao Yan of Arizona State University, Nadrian Seeman of New York University and colleagues[4] now show that mass production of designer DNA nanostructures in bacteria with the aid of phages — viruses that have a specific affinity for (and infect) bacteria — is possible.

Using phages and bacteria such as *Escherichia coli* to make many identical copies of, or 'clone', a gene represents one of the most important biotechnological achievements in the past half-century. It involves inserting the segment of DNA to be copied into a cloning vector, such as a plasmid (a small, independently replicating, piece of DNA), to form a composite known as recombinant DNA. Introducing this composite into a bacterial host cell allows the segment to be replicated thousands or even millions of times in a collective process known as recombinant DNA technology. It has been questioned whether designer DNA nanostructures can be cloned in the same way.

In 1991, Nadrian Seeman proposed a method to clone DNA pentagonal dodecahedron structures[5]. However, the topology (that is, the connection and orientation) of this structure was too complicated to be realized experimentally because it would interfere with the replication process. It was not until 2004 that the first experimental milestone was reported by researchers at the

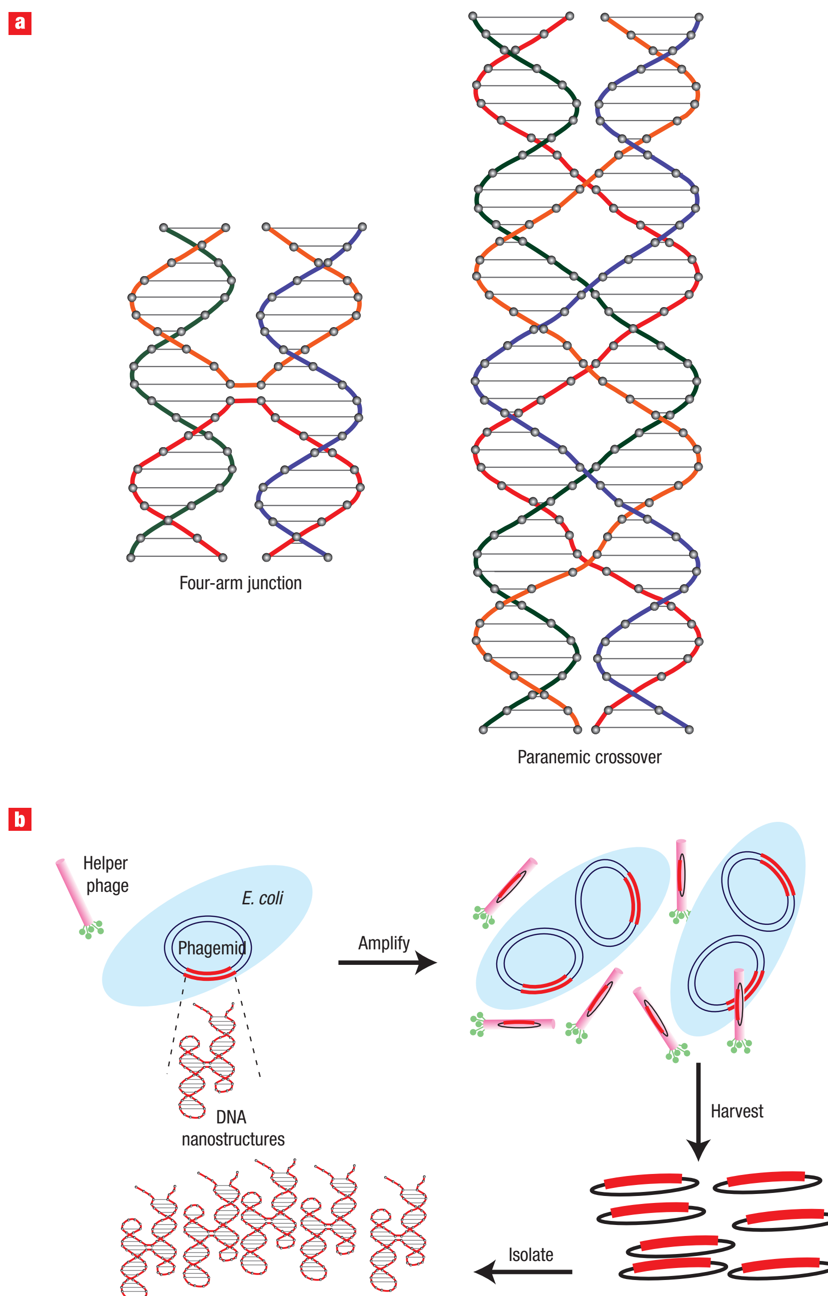

Figure 1 Cloning DNA nanostructures using bacteria and phages. **a**, A four-arm junction and a paranemic crossover. **b**, The DNA nanostructure (red segment) to be copied is inserted into a cloning vector (phagemid), which is then amplified in *E. coli* with the aid of helper phage (pink rectangles). Large numbers of phagemids are harvested and the DNA nanostructures are isolated.

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Scripps Research Institute, when they cloned a 1.7-kilobase single-stranded DNA molecule that could fold into an octahedron[6]. More recently, in his work on 'DNA origami', Paul Rothemund of Caltech developed a strategy to fold single-stranded biological genomic DNA molecules into arbitrarily designed nanostructures[7]. To create such sophisticated nanostructures, extensive intramolecular base-pairing is normally required; however, the Rothemund origami did not need such pairing, and thus could be readily cloned in bacterial cells. Although successful, both approaches required additional helper DNA strands for the cloned molecules to fold correctly. A collaboration between Ruhr-Universität Bochum and Nanogen Recognomics also explored chemical methods to replicate three-armed nanostructures[8].

In their most recent work, Yan, Seeman and colleagues managed to clone two types of DNA nanostructures inside *E. coli* using standard recombinant DNA technology. They replicated a four-arm junction and a paranemic crossover, which is a four-stranded non-coaxial DNA complex (Fig. 1a). The team first designed the DNA nanostructures and then inserted each of them into a phagemid — a small piece of modified phage DNA that functions as a cloning vector — before introducing the recombinant phagemid into the bacteria in a process known as 'transformation' (Fig. 1b). The bacteria were then infected with helper phages, and as the bacteria proliferated, the recombinant phagemids amplified exponentially. The helper phages packaged the phagemids into particles and secreted them into the culture media before the cloned nanostructures were isolated.

Production requires only a small amount of single-stranded DNA nanostructures to begin with, and can be scaled up simply by culturing bacteria in large volumes. Such *in vivo* amplification is highly efficient, and because it takes advantage of the naturally occurring cellular machinery to do the work, it is much more cost-effective than the *in vitro* amplification method developed by the same group previously[9,10]. Moreover, all the steps involved are based on well-established molecular cloning techniques, making them easy to master and reproduce.

More importantly, this work makes it possible for new and interesting DNA nanostructures to evolve and develop spontaneously *in vivo*. Currently, all the nanostructures are designed on the basis of previous experience and knowledge. However, with our limited understanding of DNA structure, we may miss many opportunities. With *in vivo* cloning, it is possible to introduce a large pool of DNA sequences into the bacteria and to allow new architectures to emerge and new functions to develop. But creating such nanostructures with evolutionary advantages is undoubtedly a considerable challenge.

The two nanostructures in the present work, which are essentially circular molecules, are topologically simple. It remains to be seen if more complicated objects, such as knots and those with multiple strands, can also be cloned using this approach. Nevertheless, the work represents another important development in the field of DNA nanotechnology.

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NANOTUBES

Giving catalysis the edge

Effective catalysts for the oxidative dehydrogenation of alkanes can be created by adding functional groups to carbon nanotubes.

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During catalysis, the distribution of products in a chemical reaction is determined by the catalyst's ability to modify the activation energy barriers of the possible reaction paths. However, the tendency of a catalyst to favour certain pathways while inhibiting others is more important than accelerating the overall rate. Needless to say, manipulating the activation energy of a specific reaction path is challenging and requires a detailed understanding of the very heart of the catalyst — its active site. Writing in *Science*, Dang Sheng Su and colleagues[1] at the Fritz Haber Institute in Berlin now report an elegant example of using the tools of nanotechnology to address this issue. By modifying the surface functionality of carbon nanotubes, they have created a highly selective oxidative dehydrogenation catalyst, clearly demonstrating that it is possible to selectively inhibit a particular reaction path by precise modification of the active site.

Su and co-workers examine the oxidative dehydrogenation of an alkane, butane (C_4H_{10}), to produce the corresponding alkenes, butene and butadiene (C_4H_8 and C_4H_6), by creating one or two new carbon–carbon double bonds in the molecule. Such alkenes are industrially important chemicals that are used to make items such as rubbers and plastics. Unfortunately, alkenes are oxidized to produce carbon dioxide and water faster than the alkanes are dehydrogenated to produce the alkenes. This reaction can therefore be written as $A \rightarrow B \rightarrow C$, where A is the starting feed (butane), B the required product (the alkenes) and C is the unwanted total oxidation product (CO_2 and water). Indeed, it is well known that high selectivities to alkenes are only obtained

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