

# Mobile genes

## Nicholas West & Christoph Tang

Mobile genes and transposons are found widely in nature. This article looks at prokaryotic transposons and their potential exploitation by microbiologists.

● Around 70 years ago the first observations were made regarding the existence of genes that gave rise to an increase in spontaneous mutation frequencies at other loci. These genes were originally described as 'mutation genes'. In the late 1940s, Barbara McClintock, through her now famous genetic experiments with corn, coined the phrase 'controlling elements' to describe segments of DNA that could not only alter gene activity but were also mobile in the genome. These 'controlling elements' are now known as transposable elements due to their ability to 'transpose' from one site to another. It is now recognized that transposable elements are prevalent throughout nature, being common in bacteria, plants and animals, including mammals. Transposons are particularly interesting forms of transposable elements due to their widespread distribution in nature and the extensive applications for which they have been adapted within the laboratory.

There are two general types of prokaryotic transposon, composite and non-composite. Briefly, composite transposons, such as Tn10, consist of a central element, which includes several genes that may confer resistance to antibiotics. This DNA fragment is mobilized through the activity of a transposase encoded within the insertion sequences flanking the element. Insertion sequences have short inverted repeats at either end that act as the recognition site for the transposase, leading to the excision of the complete unit. Non-composite transposons, such as Tn3, do not have insertion sequences at their extremities but simply have the inverted repeats necessary for recognition by the transposase. All the activities required for transposition, including the transposase and resolvase, are encoded by sequences in the central region.

This article covers the role of transposons in adaptive evolution and the practical applications of transposons, including some recent developments which illustrate the flexibility and versatility of this type of transposable element.

### ● Transposons in nature

Whole bacterial genome sequencing projects have emphasized the widespread distribution of transposons in microbes. Even archaea and thermophilic bacteria, such as *Thermotoga maritima*, contain multiple copies of sequences related to transposable elements. In the human pathogen *Neisseria meningitidis* over 50 potential transposable elements belonging to two major families, IS1016 and IS30, have been identified in the genome sequence. Furthermore, approximately 40% of the human genome is composed of retrotransposons, elements that utilize an RNA intermediate during mobilization. However, the contribution of transposons to adaptive evolution remains uncertain.

For intergenomic events, there are specific examples of the role of transposons in the spread of beneficial traits

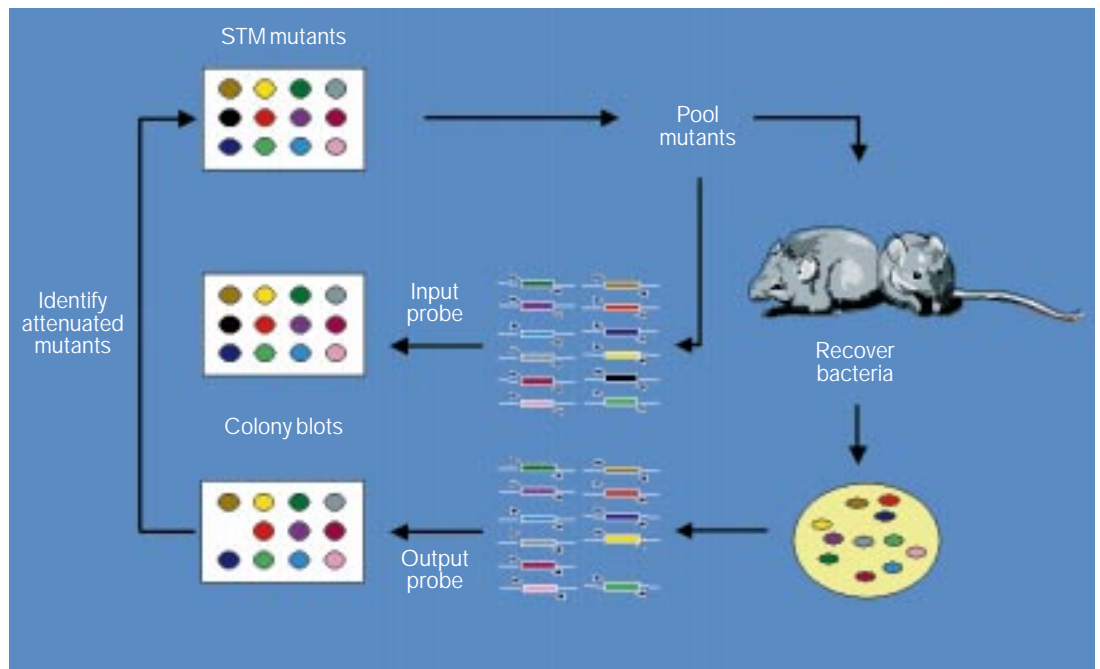
within bacterial populations. The best known is the emergence of antimicrobial resistance. The advent of multiresistant bacteria that are oblivious to most available antimicrobial interventions has become a major public health problem. Many of the genes responsible for resistance are carried on transposable elements, which have facilitated the transfer of resistance from commensals to pathogens. For example, vancomycin-resistant *Enterococcus faecium*, which is now prevalent in hospitals throughout developed countries, acquired resistance to this antibiotic on Tn1546. Transposons are in fact the predominant source of antibiotic resistance, being transferred across bacterial species and even genera.

Other examples of horizontal transfer between different species or genera further demonstrate how this type of gene 'sharing' can develop bacterial populations. For instance, the acquisition of certain genes, such as resistance to environmental metal ions, would subsequently equip the organism to exploit a new and previously unattainable environmental niche. Furthermore, due to the introduction of organic hydrocarbons into the environment, bacteria have developed mechanisms for the catabolism of many of these pollutants. The genes responsible for catabolism have been largely found on transposons. Elements have been identified with the capabilities to degrade chlorobenzoate, chlorobenzene, toluene, benzene, nylon oligomers and naphthalene, thus illustrating how naturally developed transposons may play a role in the bioremediation of environments that have become polluted.

Transposons have not only been responsible for the spread of characteristics directly related to survival, but they have also been implicated in the evolution of disease-causing bacteria. A feature of enteric pathogens, including *Escherichia coli* and *Salmonella* spp., is the presence of large genetic elements (often in excess of 10 kb) that are vital for their ability to inflict damage on their hosts. These so-called 'pathogenicity islands' are often bounded by insertion sequences, indicating that they were originally acquired by a transposition event. The implication is that transposons have caused quantum leaps in the evolution of virulence among bacterial species.

Furthermore within a population of cells derived from a single bacterium, transposition events mediate genotypic variation through intragenomic recombination, resulting in the appearance of new phenotypic traits on which selection can operate. For instance, it has been shown that expression of capsular polysaccharide, a structure required during pathogenesis by *N. meningitidis*, can be switched off by the insertion of an IS1016 element upstream of genes encoding biosynthetic steps. A further example of the importance of intragenomic events is phase variation of fimbrial

expression in *Salmonella typhimurium*. The bacterium expresses one of two flagellar types, type 1 or type 2. The genetic basis of switching between these types involves the reversible inversion of the Hin element (H antigen inversion) upstream of genes encoding type 2 flagellae and a repressor of type 1 flagellar biogenesis.



In one orientation, the promoter element within Hin activates transcription of type 2 biosynthetic genes. In the opposite orientation, the type 2 genes and the repressor are not expressed, allowing the synthesis of type 1 genes.

Although there are other specific examples of transposition events causing phenotypic variation, the overall contribution of transposon and insertion sequences to microbial evolution is uncertain. There are two opposing views. The 'selection hypothesis' holds that genetic flexibility and variability confer long-term fitness benefits to microbes, and this is the reason for the prevalence of transposons. There is some direct, albeit limited, experimental evidence supporting this theory from studies of the growth of *E. coli* in chemostat cultures. Strains containing the transposon Tn10 have a competitive advantage over isogenic strains without Tn10; this fitness gain is lost when Tn10 is deleted from the bacterium. Some argue that evolution is blind to the future and that long-term views on benefits are far more important in the minds of evolutionary biologists than in nature itself. They suggest that transposons exist purely as selfish genetic elements rather than as symbionts in the bacterial cell. The truth probably lies somewhere between these divergent views; many transposons may well act solely as passengers in bacteria while others enhance the survival of the cells in which they exist.

### ● Transposons in the laboratory

Researchers have long used the natural properties of transposons and adapted them as incredibly versatile tools for the genetic analysis of bacteria. Principally, transposons have been used as insertional mutagens, which may include the construction of libraries of strains, each carrying a single transposon at a different location. The libraries can then be analysed for mutants with the desired phenotype and the affected gene(s) can be easily identified for further study by the presence of the transposon.

Natural transposons have several limitations when used in the laboratory. First, they are usually large elements that are difficult to manipulate, and second, they tend to be unstable and move to other locations. To

prevent these problems, mini-transposons have been developed. These lack the resolvase gene, which is required for the recombinational events of transposition, improving their stability. This also allows a significant reduction in size, making them much more user-friendly. Transposons have now been developed for generating both reporter fusions, to monitor levels of expression of genes, and to identify gene products which are translocated to the cell surface. Transposons have also been used to generate large-scale genetic maps by introducing recognition sites for infrequently cutting restriction enzymes. However, this approach is rapidly becoming obsolete given the use of shotgun cloning for whole genome sequencing projects.

### ● Recent developments in transposons

A major drawback of using mutant libraries was that each mutant had to be screened individually. Therefore, researchers were limited to analysing large libraries of mutants in simple assays. Signature-tagged mutagenesis (STM) was devised to overcome this (Fig. 1). In STM, each mutant is marked with a unique 40 bp DNA sequence identifier, so that it can be readily distinguished. Mutants can therefore be analysed in pools containing many individual mutants, allowing high-throughput screening of libraries in complex assays. STM has been mainly used to study the pathogenic mechanisms of Gram-negative and Gram-positive bacteria, and fungi in animal models of disease. The results have provided unexpected insights into the genetic basis of disease and the environments that bacteria encounter during pathogenesis. For instance work on *S. typhimurium* led to the discovery of a previously unidentified genetic region containing clusters of genes required for pathogenesis. STM and similar methods do not have to be restricted to use in studies on pathogenesis; they can also be adapted to high-throughput analysis of gene function within the laboratory.

Conventional transposons are usually delivered into the organism of interest where they excise from a donor plasmid into the chromosome. For a number of microbes, this approach is not effective. Recently, *in*

ABOVE: Fig. 1. In STM, an insertional mutagen (transposon) is modified by incorporation of DNA signature tags. A collection of different insertional mutants of a bacterial pathogen, each carrying a different tag, is assembled in a microtitre dish. The mutants are pooled and used as the inoculum for an appropriate animal model of infection. Following infection, bacteria are recovered from the host and the unique DNA tags are amplified alongside those present in the initial inoculum using primers that anneal to invariant sequences flanking the tags. The product of the PCR is then utilized to probe nylon membranes carrying DNA from the mutants in the inoculum. An avirulent mutant is identified by the failure to yield a signal on the membrane hybridized with the tags recovered from the animal.

# Letter



PHOTOS: IAN ATHERTON

## Further reading

**Akerley, B.J. & others (1998).** Systematic identification of essential genes by *in vitro* mariner mutagenesis. *Proc Natl Acad Sci USA* **95**, 8927–8932.

**Chao, L. & McBroom, S.M. (1985).** Evolution of transposable elements: an IS10 insertion increases fitness in *Escherichia coli*. *Mol Biol Evol* **2**, 359–369.

**Shea, J.E. & others (1996).** Identification of a virulence locus encoding a second type III secretion system in *Salmonella typhimurium*. *Proc Natl Acad Sci USA* **93**, 2593–2597.

**Tan, H.M. (1999).** Bacterial catabolic transposons. *Appl Microbiol Biotechnol* **51**, 1–12.

**Winzeler, E.A. & others (1999).** Functional characterization of the *S. cerevisiae* genome by gene deletion and parallel analysis. *Science* **285**, 901–906.

*in vitro* mutagenesis has been successfully employed to previously intractable bacteria. In this method genomic DNA from the host bacterium is modified by combining it *in vitro* with a transposon along with its purified cognate transposase. The DNA is then returned to the host by transformation. The main constraint on this approach is that to be successful, the host bacterium must be transformable. A number of systems for *in vitro* mutagenesis are available, including Tn7, Tn10 and mariner, and these have been applied to a variety of micro-organisms, including *Haemophilus influenzae*, *N. meningitidis* and *Campylobacter jejuni*.

*In vitro* mutagenesis has been adapted to identify essential genes. Proof-in-principle for this method was demonstrated in work on *H. influenzae*. Large fragments of genomic DNA were amplified by PCR, then subjected to *in vitro* mutagenesis. The modified products were then returned to the host by transformation. 'Essential' genes were identified by comparing the profile of insertions in the PCR products with insertions in the bacterium. This allows a systematic analysis of essential genes in transformable bacterial pathogens which may be useful for drug development, and if surface-located, for vaccine development.

## ● Future directions

One of the major challenges facing microbiologists is how to exploit the wealth of information from whole genome sequencing. A striking feature is the large proportion of genes of unknown function. Some of these genes are conserved across genera, suggesting that they have important functions which have so far eluded researchers. There is now an urgent need for systematic analysis of gene function and central to this is the construction of ordered libraries of mutants which contain strains with deletions in each and every gene. So far, transposons have been used for making libraries of random mutants, but now they must be adapted to constructing ordered libraries that will allow large-scale and truly comprehensive analyses of gene function.

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## Dear Editor

I always enjoy reading *Microbiology Today* but, as someone with a special interest in the fungi, the August 2000 issue was delightful. Although, as the articles clearly show, there is no doubt about the importance of the fungi in the biosphere and the well-being of humans, there is some anxiety about the future for the discipline of mycology. I am optimistic that the exposure of mycology in *Microbiology Today* will contribute to persuading a few young microbiologists that the fungi are an important and interesting group of organisms worthy of their attention.

You ask for names of the splendid group of photographs on the front cover. It is generally considered unwise to give names on the basis of photographs alone, but one can say with certainty that they are all fruit bodies of basidiomycetes! Top left is probably *Coprinus plicatilis*, a member of the grassland flora, although there are several similar species of these delicate and very beautiful *Coprinus*. They all have black spores and many species liquefy on maturity giving them the popular name of 'Inkcaps'. Middle left is undoubtedly *Auricularia (Hirneola) auricula-judae*, commonly known as the 'Jews' Ear', which is most frequently found on dead elder wood. These fruit bodies have the remarkable ability to dry out completely as thin tough structures which, after rainfall, can absorb water to expand considerably and continue disseminating their spores. At the bottom is a splendid photograph of a species of *Russula*. It is indeed dangerous to name species of *Russula* without additional information about habitat, spore colour and microscopic examination. However, the colours look perfect for *Russula atropurpurea* (= *R. krombolzii*), which may be mycorrhizal with a number of species of trees but is especially associated with oak.

Top right clearly has white gills and looks like an old clump of the honey fungus *Armillaria mellea* after the scales which are usually present have worn off. If this is correct then this would represent a fourth habitat, for this species is capable of parasitizing shrubs and trees and then continuing to grow and eventually fruit on the dead wood. Even if the names are not completely correct these four photographs do demonstrate the diversity of habitats of the basidiomycetes; growing on organic material in the soil, growing on and decomposing dead wood, growing symbiotically in a mutualistic association with the roots of trees and growing as a pathogen of living trees.

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