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Streptomyces Linear Plasmids: Their Discovery, Functions, Interactions with Other Replicons, and Evolutionary Significance

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Abstract Unusually among bacteria, streptomycetes possess linear chromosomes, and many of them also carry linear plasmids (circular plasmids are also found). The linear plasmids range in size from tens to hundreds of kilobases. The most studied is SCP1, discovered as a sex factor in the model organism *Streptomyces coelicolor* A3(2). A variety

of co-integrates and hybrids have been found between SCP1 and the host chromosome, which can greatly increase the likelihood of chromosomal transfer. Several examples of the exchange of ends between linear plasmids and linear chromosomes have been documented. These can sometimes bring about the mobilisation of chromosomal genes for antibiotic biosynthesis. Some very large linear plasmids themselves carry genes for the biosynthesis of bioactive small molecules, including antibiotics. For example, such genes occupy about two thirds of the plasmid pSLA2-L. In another case, almost identical gene sets for methylenomycin biosynthesis are present both on the linear plasmid, SCP1, and on a quite different circular plasmid, pSV1, in a related streptomycete. It appears that linear plasmids have played key roles in the architecture, accessory gene content and rapid evolution of *Streptomyces* chromosomes. They may have permitted the diversification and spread of pathways for secondary metabolism, and the evolution of some *Streptomyces*-specific families of paralogous genes. They may also have been the source of most of the genes that, because of their possession of the rare TTA codon, are dependent for expression on the cognate tRNA specified by *bldA*, a gene whose deletion has wide-ranging effects on morphological differentiation and secondary metabolism.

1 Introduction: Early Studies of Plasmids

The brilliant early studies of bacterial genetics (see Hayes, 1964, for a review) were helped by good fortune in Joshua Lederberg's choice of *Escherichia coli* K-12 as the strain for investigation, because this strain happened to have a genetic determinant, termed the F-factor, that permitted genes to be transferred between strains, albeit at a low frequency ($< 10^{-6}$). By the mid-1950s it had been deduced that all the genetic markers of *E. coli* were arranged on a single linkage group, i.e. there was a single chromosome, with the exception of F itself: its transfer was not associated with the transfer of particular chromosomal regions. F was also fairly easily lost, and such F^- variants could readily be converted to F^+ by growth in contact with a differently marked F^+ strain, at frequencies many times higher than the transfer of markers on the chromosome. Thus, F was a plasmid—a genetic element able to replicate separately from the chromosome. However, F^+ cultures contained rare variants that showed a much higher frequency of chromosomal recombination in crosses with F^- strains. In these Hfr (high frequency of recombination) variants, the F-factor was no longer easily lost, and it showed genetic linkage to chromosomal genes, a result which meant that the factor had integrated into the chromosome.

It was subsequently found that the integrated F-factor could sometimes excise from the chromosome of Hfr strains together with adjacent chromosomal DNA, to give rise to F-prime (F′) factors, which provided tools for geneticists to carry out functional tests, such as dominance and complementation. These observations provided early evidence of the kinds of molecular exchange that might be contributing to the evolution of bacterial chromosomes. Moreover, the *E. coli* chromosome was shown both genetically and physically to be cir-

cular, so the fact that the F-factor could integrate into it and excise from it suggested that F was also circular, with a single, reversible recombination event between it and the chromosome accounting for integration. Such single crossover integration events became known as "Campbell integration", after the first clear proposal by Campbell that they could account for the ability of the DNA of bacteriophage lambda to integrate into, and excise from, the *E. coli* chromosome.

Within a few years, Watanabe found that other transmissible plasmids (R-factors) were the agents of the spread of multiple antibiotic resistance among enteric bacteria, and subsequently numerous different phenotypic traits also turned out to be encoded by plasmids. It became obvious that plasmids could be found in bacteria of almost any taxonomic group. Clearly, such elements were contributing in various ways to host evolution and adaptation. This, coupled with the possibility that they might provide model systems for studies of DNA-related physiological questions, made it important to isolate and characterise them physically. The circularity of known plasmids gave rise to the first method for plasmid purification (Clewell and Helinski 1970). The method depends on the intercalating dye ethidium bromide, which is taken up to a reduced extent by covalently closed circular (CCC) plasmid DNA compared to open-circular (nicked) plasmid DNA, or linear DNA fragments such as those inevitably generated by shearing chromosomal DNA during its isolation. The CCC plasmids therefore undergo a smaller decrease in buoyant density than linear DNA during high-speed centrifugation in a CsCl–ethidium bromide solution, and form a separate band below chromosomal DNA when the density gradient reaches equilibrium. Material separated in this way, coupled with other advances such as the development by Kleinschmidt of a method for displaying DNA in the electron microscope, led quickly to a much greater understanding of the genetic and physical organisation of plasmids, and allowed their first use as vectors for gene cloning in the early 1970s. At that time, there was no reason to expect exceptions to the rule that plasmids were circular DNA molecules, and further rapid and convenient methods for their purification, all depending on their CCC nature, became available (e.g. Birnboim and Doly 1979).

2
Exploring the Genetics of Other Bacteria: The Dawn of *Streptomyces* Genetics

After the first discovery that it was possible to carry out genetic analysis in *E. coli*, genetic exchange was soon demonstrated in other phylogenetically distant bacteria, and harnessed for experimental purposes. Against this background, several scientists began in the mid-1950s to look for evidence of genetic exchange in *Streptomyces* (reviewed by Hopwood 2007). There were

three main reasons why this particular group of bacteria attracted attention. Firstly, it had become obvious that streptomycetes were a rich source of antibiotics, so there was a real possibility that success in developing genetic systems would find long-term utility. Secondly, these abundant, widespread, ecologically significant soil organisms were phylogenetically distant from *E. coli* and other genetically studied bacteria, and had an unusual mycelial growth habit like that of fungi (Fig. 1) (in fact, some people still incorrectly thought of them as intermediate between bacteria and fungi, or even as fungi). It was therefore felt that they should, on the one hand, exhibit novel features and, on the other, help to reveal what was universally true of bacteria. Thirdly, they were easy to grow at a reasonable rate on defined media, and went through a stage in their life cycle in which, as spores, they had a single copy of the genome, allowing the progeny of crosses to be classified readily; they were thus potentially experimentally convenient.

The greatest success was had with *Streptomyces coelicolor* A3(2), the organism chosen by D.A. Hopwood (Hopwood 1999). His earliest papers showed that random segments of its chromosome could be transferred between strains by conjugation, with both participants being able to act as donor or recipient (the crosses were "non-polar") and without obvious preference for particular chromosome segments (Hopwood 1967). Subsequent work revealed the involvement of a complex fertility system, involving plasmids, which is described in the next section. It was from this work that the unusual nature of some *Streptomyces* plasmids began to emerge, culminating in the 1980s with the revelation that many of them are linear.

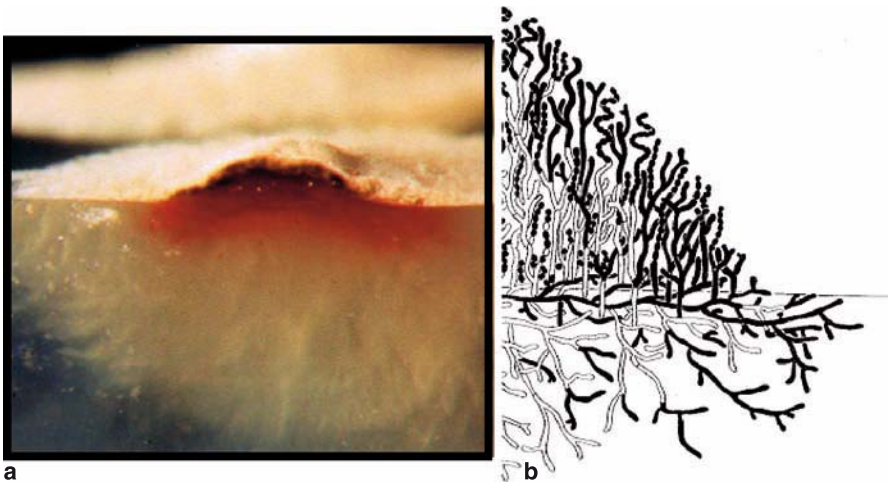

Fig. 1 The *Streptomyces* colony. **a** A mature *S. coelicolor* colony grown on agar for 5 days sliced vertically with a razor (photograph by N.J. Ryding). **b** Diagrammatic cross section showing mycelial branching and sporulation in the aerial mycelium (after Wildermuth 1970)