CONJUGATIVE SEX PLASMIDS OF STREPTOMYCES

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INTRODUCTION

Much is known about the ways in which the conjugative plasmids of gram-negative bacteria cause their hosts to mate (1). Detailed knowledge has been obtained about how the transfer of plasmid DNA occurs (2). The mechanisms by which such plasmids promote the mobilization of chromosomal genes have also been extensively studied (3-6). In contrast, very little is known about such phenomena for Streptomyces plasmids (7). However, now that many Streptomyces plasmids can be isolated with ease (8), genetically manipulated in vitro, and returned to the host cells by transformation of protoplasts (9,10) we can hope for more rapid progress in the understanding of the molecular biology of these plasmids. Here we review some facts and ideas about Streptomyces conjugation which may serve as a basis for future experimentation. As we see, conjugative Streptomyces plasmids are likely to possess properties interestingly different from those of plasmids of gram-negative bacteria and so they should repay a detailed investigation.

GENERAL FEATURES OF STREPTOMYCES PLASMIDS

The first conjugative plasmid to be identified in a gram-positive bacterium was SCP1 in the genetically studied Streptomyces coelicolor A3(2)(11). SCP1 was recognized through its influence on the recombination of host chromosomal genes ("fertility") and because it determines production of and resistance to a diffusible inhibitor of growth and sporulation, later identified as the antibiotic methylenomycin (12). Various derivatives of SCP1 have been characterized genetically, including those mediating high-frequency donation of chromosomal markers in which SCP1 seems to be stably in-
tegrated into the host chromosome, and autonomous SCPl-prime plasmids carrying segments of host DNA (13). Complete molecules of SCPl DNA have not been isolated but segments of SCPl have been cloned, including genes coding for methylenomycin resistance (14) and production (15); uncharacterized segments of the plasmid (15); and a region capable of autonomous replication when ligated with DNA carrying a selectable marker to form circular molecules (M.J. Bibb and J.M. Ward, pers. comm.). The size of SCPl has been estimated to be in the range of 150 kb to at least 200 kb (15,16).

The first Streptomyces plasmid to be isolated physically was an initially cryptic CCC molecule of 31 kb (17) which was distinct from SCPl and was later shown to be identical to a second sex plasmid, SCP2, of *S. coelicolor*. This plasmid had been identified through the occurrence of spontaneous variants (SCP2*) capable of promoting increased fertility in *S. coelicolor* A3(2)(18). The copy number of SCP2 in *S. coelicolor* is 1-4 per chromosome. This plasmid has not been shown to interact stably with the chromosome—indeed, an exhaustive search for high-frequency donors of chromosomal markers and for SCPl-prime plasmids was unsuccessful (J.A. Ewing, pers. comm.). However, the recent development of stable cloning vectors derived from SCP2* (19) has enabled segments of the host chromosome to be inserted into the plasmid, to provide artificial SCP2*-prime derivatives. Some of these, carrying segments of DNA which include genes for biosynthesis of the antibiotic actinorhodin (20), have been used in some genetic experiments described herein.

Many other plasmids have now been identified as CCC DNA (or occasionally as linear molecules; Ref. 21) in a wide variety of *Streptomyces* species (reviewed in Ref. 7). In contrast to SCP2 and (presumably) SCPl, several have a high copy number. Thus, pIJ101, a plasmid of 8.9 kb found naturally in *S. lividans* ISP5434, exists in 40-300 copies per chromosome depending on the age of the culture (22). A copy number as high as 800 has been reported for pNM100 in *S. virginiiae* (23). A further interesting series of plasmids is represented by SLPl, pIJ110, and pIJ408. These are found as autonomous plasmids in *S. lividans* 66 after mating this strain with *S. coelicolor* A3(2), *S. parvulus* ATCC 12434, or *S. glaucescens* ETH 22794 respectively (24). They arise by the "looping out" of DNA sequences which occur as part of larger replicons (in the case of SLPl in *S. coelicolor* this has been shown to be the chromosome; Ref. 25) and their conjugal transfer to *S. lividans*.

Most *Streptomyces* plasmids that have been studied give rise to "pocks" when a plasmid-carrying spore (or other plating unit) develops within a lawn of plasmid-free individuals. Pocks are circular areas, up to a few millimeters in diameter, in which the gross appearance of the culture is altered; usually the development of aerial mycelium and spores is delayed or prevented, and there may be precocious production of secondary metabolites, such as actinorhodin