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Genome Rearrangement and Genetic Instability in *Streptomyces* spp.

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INTRODUCTION

Members of the genus *Streptomyces* are gram-positive, multicellular, filamentous soil bacteria which undergo a complex cycle of morphological differentiation. Spores germinate to form a branched, vegetative mycelial mass which colonizes the substrate and, as nutrients become limiting, produces aerial mycelia which eventually differentiate into spores. The biosynthetic versatility of the genus is of considerable industrial importance, the powerful secondary metabolism being able to direct the synthesis of a bewildering variety of enzymes and low-molecular-weight compounds. These include numerous excreted hydrolytic enzymes, enzyme inhibitors, immunomodifiers, and over 60% of naturally occurring antibiotics (5). The genome size is typically in the range of 5×10^3 to 7×10^3 kilobases (kb) (19), which is surprisingly large, being 1.5 to 2 times the size of that of *Escherichia coli*. It has a high G+C content of 73 to 75% (19) and significant levels of both repetitive sequences (4 to 11%) and "foldback" DNA (2%). There is one major genetic linkage group which takes the form of a circular chromosome that is frequently present in multiple copies per hyphal compartment but is present only as a single copy in the spores (25).

One particularly interesting aspect of *Streptomyces* spp. is the phenomenon of genetic instability, which manifests itself as extraordinarily high mutation rates affecting certain species-specific traits. Unstable phenotypes are irreversibly lost at spontaneous frequencies as high as 0.1% of plated spores, but these frequencies can be made to approach 100% by the use of both mutagenic (ethidium bromide and UV irradiation) and apparently nonmutagenic (cold storage) treatments (29). The majority of these instabilities are the result of extensive chromosomal deletions which, interestingly, are frequently accompanied by intense DNA amplifications that take place in the absence of any obvious selection pressure. The deletions can extend to in excess of 800 kb (7), and the physically linked amplified arrays can extend to 3,000 kb (20, 21), constituting 18 and 45% of the chromosome, respectively.

The last decade has seen much progress towards an understanding of these events, reflecting the increasing importance of the genus and the development of efficient genetic systems (28) for its manipulation and exploitation. In this minireview, we present an overview of the instability phenomenon and the associated chromosomal deletion and amplification events, with particular reference to one of the best-characterized systems, *S. glaucescens*.

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GENETIC INSTABILITY

Genetic instability affects all aspects of streptomycete growth, including the primary metabolism and differentiation (pleiotropic effects), but it is particularly prevalent among traits of the secondary metabolism (13, 28). The phenomenon is widespread within the genus and may indeed be ubiquitous; a lack of reports for a particular species may merely reflect a lack of investigation or the absence of easily recognizable unstable traits.

Significantly, initial observations revealed that only certain traits were affected in any one strain. High-frequency mutation was confined to specific genes, thus demonstrating that the phenomenon was localized. The enhancement of mutation by known curing agents, such as ethidium bromide and acridine orange (28), and the specificity and irreversibility of trait loss were immediately suggestive of plasmid loss. Further supportive evidence came from mapping studies, which frequently produced unbalanced crosses with high apparent fertilities of specific traits (13). These results were initially interpreted as being indicative of the extrachromosomal location of traits, and indeed, plasmid loss and rearrangement were frequently reported (13, 28, 34). In the majority of these cases, however, the loss of a trait was never satisfactorily correlated with the loss or rearrangement of plasmids, and it now seems probable that these observations merely reflect the fact that extrachromosomal sequences are also subject to instability.

Several groups were, however, able to demonstrate a chromosomal location for genes coding for certain unstable traits (6, 11, 12), including *strS* and *melC* from *S. glaucescens*. Interestingly, in this species the resulting genetic map (20) could not easily be reconciled with subsequently obtained physical linkage data, which clearly showed that the two loci are 350 kb apart (7). Although both map to the chromosome, the apparent map interval derived from recombination frequencies suggested a substantially larger distance than this, indicating that even apparently reliable mapping data must be viewed with the utmost caution when unstable traits are involved. The fact that many such crossings were unwittingly carried out with mutants which carried gross genomic rearrangements (7) is probably of major significance. Grossly distorted segregation patterns and high apparent fertility may also be in part attributable to the high frequency of progeny unable to form aerial mycelia or spores, to a lowered viability of recombinants, or to the presence of highly recombinogenic sequences (13). We believe that the vast majority of unstable traits will prove to have a chromosomal location despite the frequently encountered anomalous marker behavior.

CHROMOSOMAL DELETION

The cloning of numerous structural genes specifying unstable traits revealed that loss of a particular function was in fact attributable to loss of the relevant genetic material (13,

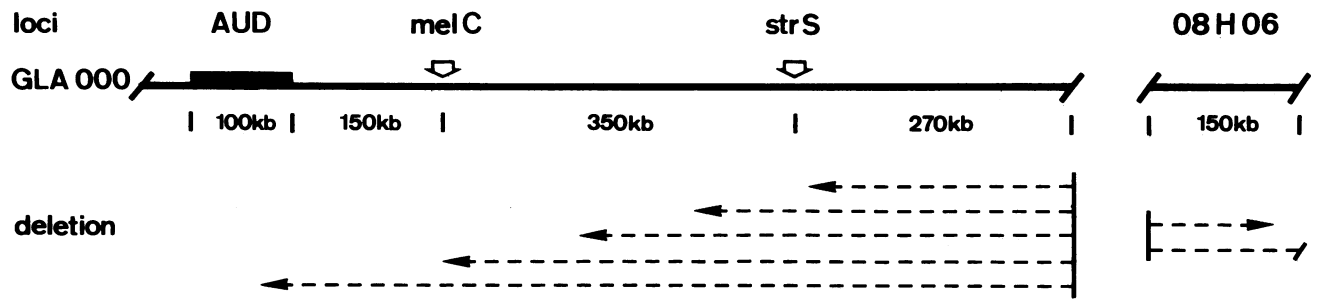

FIG. 1. Schematic representation of deletion formation in *S. glaucescens*. The solid line represents a contiguous genomic map of the area which is subject to deletion and amplification. Locus designations are as follows: melC, tyrosinase structural gene; strS, hydroxystreptomycin phosphotransferase structural gene; AUD, amplifiable unit of DNA (region of the chromosome which gives rise to amplifications in strains carrying extensive deletions and in which numerous deletions terminate); 08 H 06, region in which numerous deletion endpoints have been mapped (represented by DNA cloned in cosmid 08 H 06) (7). Symbols: ◄----, extent and direction of representative deletions; —/ /—, uncharacterized region of the chromosome.

22, 23, 28). Additionally, analyses involving DNA surrounding unstable genes demonstrated that extensive flanking sequences were also absent, implicating large chromosomal deletion events in the instabilities (6, 7, 18). In *S. glaucescens*, deletions which were responsible for the loss of the unstable genes coding for tyrosinase (*melC*) and hydroxystreptomycin phosphotransferase (*strS*) were initially characterized (22, 23). Physical mapping of these regions clearly demonstrated that the previously discrete deletions were merely components of much larger deletions which extended from 270 to in excess of 800 kb (Fig. 1). The distinctive linked appearance of the mutations seen in this strain is explained by the directionality of the deletion events (7).

Interestingly, several instability systems in *Streptomyces* spp. display a characteristic stepwise loss of markers (2, 15, 31) in which primary high-frequency mutant types give rise to secondary mutant types at an even higher frequency. The sequential nature of these phenomena may reflect the ordering of the corresponding loci in a region of the chromosome subject to polar deletions (Fig. 1). The pattern of mutant appearance in these species is highly suggestive of a two-step mechanism whereby a primary deletion facilitates further secondary events. It can be envisaged that the primary event leads to an unstable intermediate stage which is stabilized by such secondary rearrangements.

CHROMOSOMAL DNA AMPLIFICATION

Amplified DNA sequences (ADSs) (16) have been found in numerous instances in *Streptomyces* spp. (13, 28) and are the result of reiteration of amplifiable units of DNA (AUDs, i.e., sequences in the progenitor chromosome which give rise to amplification) (16) of between 2.9 and 93 kb to copy numbers of up to 500 (14, 20, 21).

Several cases in which amplifications were selected on the basis of increased resistance or overproduction of a particular product have been reported (18, 26, 28, 32). In many systems, however, amplification apparently takes place in the absence of any obvious selective pressure and, furthermore, is often closely linked to genetic instability (2, 3, 14, 17, 20, 21, 35), being found only in association with unstable mutations. Indeed, it has recently been demonstrated for *S. glaucescens* (7) and *S. lividans* (6) that the structural genes of unstable traits are physically linked to the AUDs in the progenitor chromosome and are removed by extensive chromosomal deletions which terminate either adjacent to or in the neighborhood of the ADSs in the corresponding amplified strains (21) (Fig. 1). Furthermore, in all cases in which they have been analyzed, ADSs are accompanied by deletions which are characteristically found on only one side of the amplification (3, 21). The amplifications themselves appear to be, at least in part, present as chromosomally integrated tandem arrays (21). The existence of circularized extrachromosomal copies of ADSs, a situation seen in *Haemophilus influenzae* (36), has not been reported for *Streptomyces* spp.

The amplifications can be classified into two basic types (27). Type I (14, 21, 27, 35) amplifications are nonreproducible and, when characterized, have been shown to originate from overlapping regions of the chromosome. In *S. glaucescens*, the majority of the AUDs have been mapped to a single chromosomal locus of 100 kb. There is, however, no DNA segment common to all AUDs, indicating that a consensus core sequence is not involved. The AUDs are present only in single copy in the progenitor chromosome and are flanked by short imperfect direct repeats (21), a situation which closely resembles the numerous smaller amplification events in *E. coli* and *Bacillus subtilis* (for a review, see reference 1). The observed heterogeneity of the ADSs probably reflects the fact that only poor DNA substrates are available for the amplification process. The use of such substrates, the regionality, and the absence of any obvious phenotypic alterations strongly suggests that there is a powerful pressure for amplification to occur at one particular locus.

Type II amplifications in contrast to type I amplifications, are repeatable, and the AUDs are either flanked by extensive (1.0- to 2.2-kb) direct repeats (17) or are already present in the chromosome as a duplication (3). It seems highly likely that the repeatability of these events is a direct result of the availability of such favorable substrates. In *S. fradiae* the flanking repeat sequences are present in three copies in the chromosome, and in *S. lividans* the repeat structure consists of three copies of a 1.0-kb repeat with two copies of a 4.7-kb sequence sandwiched between them. It is tempting to see these repeats as insertion sequences; however, the restriction patterns of the different repeats in *S. lividans* clearly show divergence (3), which suggests that the direct repeat itself, and not any other properties which it may possess, is the critical factor. This conclusion is supported by work which showed that strains carrying only two copies of the 1.0-kb repeat flanking the 4.7-kb sequence amplified at much lower frequencies (15) than did the larger duplicated repeat structure.

TOLERATION OF REARRANGEMENT

In considering these enormous deletions and amplifications, a question which immediately arises is, "How does the cell tolerate such gross changes and the concomitant loss of genetic information?" The discovery of giant linear plasmids (30) in several members of the genus provided an attractive extrachromosomal location for the events. In *S. glaucescens*, however, such molecules were not found (21), though it cannot be excluded that they may be present in a chromosomally integrated state. Therefore, in accepting a chromosomal location for these events, the problem of accomodation needs to be addressed. In *S. glaucescens* the loss of 800 kb of genetic material certainly impairs the vitality of the cells but, surprisingly, they are still able to grow on minimal media, indicating that they have not lost any essential genes. The deleted region thus apparently contains relatively dispensable DNA which may be rich in secondary metabolism genes, implying that these traits are clustered in the chromosome. Interestingly, the chromosome of the genetically best-characterized member of the genus, *S. coelicolor*, possesses long yet genetically silent arcs on opposite sides of the chromosome (24). This arrangement may be typical for *Streptomyces* spp. Significantly, an unstable trait in *S. lividans* has already been mapped to one of these arcs (6), indicating that these regions may be the site of localized genomic rearrangement. Such gross rearrangements may be facilitated by the mycelial-coenocytic growth of the cells whereby there are frequently multiple copies of the chromosome per hyphal compartment.

REASONS, MECHANISMS, AND CONSEQUENCES

Excluding selectable cases, the appearance of deletions and the appearance of amplifications are closely linked. For *S. glaucescens*, evidence is available to support a model in which deletion precedes amplification (7, 21). Amplifications were found exclusively in strains possessing the most extensive deletions, and though strains carrying only deletions were frequently characterized, in no case was amplification detected in the absence of an accompanying deletion. This model is also supported by data from an amplified strain in which an extensive deletion terminates directly adjacent to the amplified array (21). Analysis of the corresponding AUD showed that there are no flanking microhomologies, in contrast to the other investigated cases, and therefore, assuming that microhomologies are an absolute requirement for amplification, these can only have been provided through juxtaposition by the deletion event (27, 28). Such a conclusion can, however, only be regarded as tentative without sequencing data from the juxtaposed deletion end. The observation that ADSs are only present in strains carrying extremely large deletions suggests that deletion size may also be an important factor in genomic sequence reiteration. It is interesting to consider that amplification may be inadvertantly selected by screening for instability and hence for large chromosomal deletions. In considering the present evidence, it seems probable that deletion is either a prerequisite or a trigger for amplification. Further considerations in favor of a deletion-first amplification model may be (i) prevention of extension of the deletion into a region containing essential genes, (ii) deletion of a recombination repressor, (iii) activation of dormant replication origins, and (iv) juxtaposition of previously separate DNA regions.

The actual mechanism of amplification remains obscure; however, the sheer size and copy number of the reiteration would exclude strand slippage models (37). For various other high-level amplification systems, a model involving saltatory replication (33) has been developed, the central feature of which is repeated replication from an origin located within the AUD. This would lead to an "onion skin" structure which could then be resolved by interstrand recombination. However, Bostock (8) has pointed out that this would lead to a nonhomogeneous arrangement of repeats, a situation which has not been seen in *Streptomyces* spp. Furthermore, the lack of common core sequences would necessitate replication origins in all AUDs, a highly unlikely situation. At present, rolling-circle models seem to provide the best explanation for amplification in *Streptomyces* spp. (38). A requirement of such a model is the presence of extensive homologies flanking the AUD in which an initial recombination would take place, which would trap a replication fork and lead to gross overreplication. The structure would then be resolved by a second recombination event. Such extensive repeat structures are, of course, already present in the progenitor chromosomes of the type II amplifications (3, 17). For type I events, a primary rate-limiting duplication mediated by illegitimate recombination would be required to provide the necessary substrates for rolling-circle replication. Many AUDs from *S. glaucescens* have a complex structure (21) consisting of rearranged DNA from several loci, suggesting that a powerful recombination system which directs several illegitimate recombination events and which could mediate an initial duplication is involved. It is also interesting to consider that the high G+C content of *Streptomyces* spp. probably substantially increases the occurrence of small repeat sequences which could serve as substrates for illegitimate recombination.

Analysis of sequences surrounding the termini and junction of a large chromosomal deletion have so far only been determined for one event in *Streptomyces* spp. (7). In this case, only a small quasipalindromic sequence which precisely delineated the deletion termini was found. Studies of much smaller extrachromosomal deletion events in *Streptomyces* spp. and in other organisms (1) have shown that deletions frequently take place in the absence of extensive sequence similarity and are therefore examples of illegitimate recombination. Various mechanisms have been proposed to account for these events (1), but their applicability to the *Streptomyces* phenomena can still only be a matter for conjecture and must await further study.

Significantly, many deletion termini in *S. glaucescens* display clustering at two loci (Fig. 1). On one side deletions frequently end within the AUD locus, and on the other side deletions end within a locus designated 08 H 06 (7, 21). The fact that deletions terminate at numerous points within these loci suggests that the genomic environment in these regions promotes deletion termination, though it may not actually specify it. It is conceivable that sequences defining the termini of a deletion are in close physical proximity and/or are accessible because of chromosome folding. The genomic and genetic organization (see below) of *Streptomyces* spp. may make them inherently susceptible to rearrangement. The precise trigger for these rearrangements is still obscure; however, the elevated frequency of such events under the stress of mutagenic treatment and the involvement of illegitimate recombination indicate that they may be the result of an SOS-like response.

Streptomyces spp. contain a multitude of elements which are capable of chromosomal excision and integration. These include temperate phages, transposable elements, and integrative plasmids (10, 28). One obvious possibility is that

these elements are in some way involved in the events described above, for example either by triggering instability or by providing recombinogenic sequences and activities. Interestingly, in the archaebacterial family *Halobacteriaceae* (9), genetic instability has been shown to be the result of transposable elements which frequently transpose within the chromosome. This situation contrasts strongly with that in *Streptomyces* spp. Although several such elements have been characterized for *Streptomyces* spp. (10), there is little evidence to support a connection between mobile DNA elements and the phenomenon of genetic instability.

CONCLUSION

Amplification and deletion events in *Streptomyces* spp. are 2 to 3 orders of magnitude larger than similar events in *E. coli* and *B. subtilis*. The fact that genetic instability involves the deletion of such enormous stretches of DNA, in many cases without apparent lethality, indicates that large segments of the genome are free of essential genes. The clustering of secondary metabolism genes in long dispensable arcs could have important implications for horizontal evolution, especially considering the suggestion that plasmid and transposon antibiotic resistances may have their origin in the *Streptomyces* gene pool (4). Further study of these intriguing phenomena should provide us with a better understanding of genome flux and organization in this important genus.

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