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Functional Evidence that the Principal DNA Replication Origin of the *Streptomyces coelicolor* Chromosome Is Close to the *dnaA-gyrB* Region

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The degree of overrepresentation of selected chromosomal genes in rapidly growing cultures of *Streptomyces coelicolor* was assessed by quantitative DNA hybridization analysis. The results are consistent with the hypothesis that the principal origin of replication is close to the *dnaA-gyrB* region, in the center of the linear chromosome, and that replication proceeds bidirectionally.

The streptomycete bacteria are among the most complex members of the prokaryotic world and possess large (ca. 8 Mb [11]) linear genomes (12, 13). Since the *Streptomyces coelicolor* chromosome is linear, it cannot be assumed that its mode of replication will be the same as that of other bacteria, such as *Escherichia coli* or *Bacillus subtilis*. Any investigation of the mode of chromosomal replication in *Streptomyces* species is hampered by a number of methodological and physiological problems which are not encountered with unicellular bacteria. For instance, the mycelial growth habit of streptomycetes means that synchronous cultures are difficult or impossible to prepare, while thymine-requiring mutants, which enable the specific labelling of DNA (15, 17), are lacking for this genus (8). In an attempt to circumvent these difficulties, we have used quantitative DNA-DNA hybridization analysis, with specific gene probes from both homologous and heterologous sources, in order to locate the functional origin of DNA replication on the *S. coelicolor* A3(2) chromosome. This technique was used to compare the relative abundance of particular genes at different locations on the *S. coelicolor* chromosome in stationary-phase and rapidly growing cultures. This approach is a molecular biological analog of the genetic experiments performed by Masters and Broda (14) to demonstrate the bidirectional replication of the *E. coli* chromosome from a single fixed site of initiation. It is known that, in bacterial cells which are actively replicating their DNA, genes close to the replication origin will be overrepresented in comparison to those close to the terminus. This effect should be exaggerated in cells growing at, or close to, their maximum specific growth rate, when a number of rounds of chromosome replication may be proceeding at the same time (5).

Determination of gene overrepresentation in continuous cultures of *S. coelicolor*. Six gene probes of previously known map location (all listed in reference 11 except that for *gyrB* [this work]) were used to probe chromosomal DNA isolated from both stationary-phase and rapidly growing cultures: *actIII* (4), *argG* (9), *galK* (10), *gyl* operon (19), *gyrB* (20), and *leuA* (6). The *gyrB* gene was chosen as a probe because, in all bacterial genera examined to date, the replication origin has been found to lie within or close to the *dnaA-dnaN-recF-gyrB* region (16). The other five probes were chosen in order to detect genes which are widely spaced on the chromosome (11).

The experiments were performed with continuous-flow cultures of *S. coelicolor* MT1109, an $SCP1^-/SCP2^-$ derivative of the wild-type strain 1147 (7) (unpublished data); twofold-diluted Luria broth was used as the growth medium (18). Each chemostat experiment involved several shift-up steps from an initial low growth rate (dilution rate, 0.19 h^{-1}). A steady state was established at each step, until a dilution rate just below the critical dilution rate (0.32 h^{-1}; rate at which dilution exceeds growth) was attained. After establishment of the final steady state, a further increase in dilution rate was made to 0.70 h^{-1}. Since, under these conditions, the critical dilution rate was exceeded, the mycelium "washed out" of the culture vessel while growing at its maximum specific growth rate. A sample (1,000 ml) was taken 30 min after wash-out was initiated, and a second sample (500 ml) was taken after the medium flow was turned off and the culture was allowed to enter the stationary phase. In each case, the sample was immediately mixed with an equal volume of cold metabolic stop mix (95% [vol/vol] ethanol, 3% [vol/vol] toluene, 4 mM Tris-HCl [pH 7.5]) and stored for a minimum of 2 h at 4°C. The mycelium was then harvested and washed, and DNA was subsequently extracted by a standard procedure (7). The DNA samples were denatured by incubation at 100°C for 5 min and then rapidly cooled on ice, and a dilution series of each sample (0.1 to 1.0 μg) was then slot-blotted onto a Hybond N nylon membrane (Amersham) with a vacuum manifold device (Schleicher and Schuell, Dassel, Germany) and fixed by UV exposure. Each strip of membrane was loaded with DNA from both the wash-out and the stationary-phase cultures.

For each series of experiments, six replicate blotted membranes were prepared, and each was probed with one of the six gene probes. Standard radiolabelling and hybridization procedures were used (18), and each of the six blotting experiments was repeated four times. After the filters were washed, the radioactivity in each slot-blot was counted with a multiwire proportional radioactivity counter (Oxford Positron Systems). Counting was performed for a standard period of 20 min. The relative copy number of each gene locus was taken as the ratio

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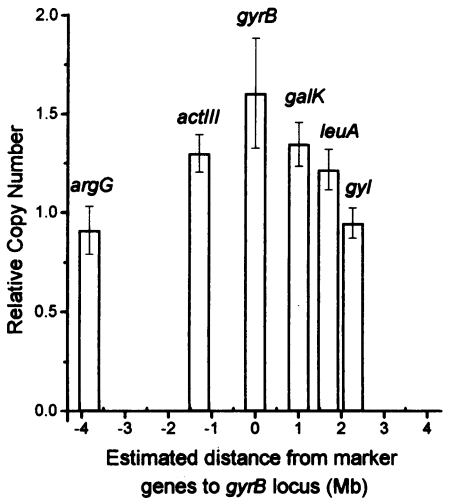

FIG. 1. Degree of overrepresentation of specific *S. coelicolor* genes in rapidly growing cultures. The relative copy number of each gene is related to its position on the linear chromosome; the *dnaA-dnaN* region maps at the center (11, 13) (designated 0 here), and hence *gyrB* does also (this work and Fig. 2). Coordinates −4 and +4 correspond to the ends of the chromosome.

of counts recorded from the wash-out DNA samples to those recorded from the stationary-phase samples.

The relative copy number value obtained for each gene was the mean of eight separate determinations (two dilutions from each replicate experiment). The ratios obtained were consistently found to fall in the following decreasing order: *gyrB*, *galK*, *actIII*, *leuA*, *gyl*, *argG*. It should be noted that these values need not represent actual gene copy numbers. These data, when related to the estimated map positions of the respective gene probes (Fig. 1), would fit with the hypothesis that chromosome replication in *S. coelicolor* is initiated in a region close to *gyrB* and proceeds bidirectionally.

Confirmation that *gyrB* is close to the *dnaA* region of the *S. coelicolor* chromosome. Having obtained evidence that the principal origin of replication of the *S. coelicolor* chromosome was close to *gyrB* (at least under the conditions employed), it was important to assess whether *gyrB* was close to the *dnaA* region, as is the case in other eubacteria studied to date (16). The *gyrB* fragment from pLST192 (20) was used as a probe to isolate flanking sequences from a λ EMBL4 library of *Streptomyces lividans* TK64 DNA (kindly provided by J. A. Cullum). One of the *gyrB*-hybridizing clones, λFF904, was shown to hybridize to the *dnaA* gene of *Pseudomonas putida* (isolated from mp18SB1.9 [3]) (Fig. 2). In order to isolate the corresponding sequences from *S. coelicolor*, the 1.57-kb *Bam*HI-*Eco*RI *dnaA*-hybridizing fragment of λFF904 (Fig. 2) was used to screen a λGEM11 library of *S. coelicolor* 1147 DNA (kindly provided by I. S. Hunter), and four clones were isolated, of which λGS3 and λGS2 extended furthest from *dnaA* (Fig. 2). The restriction maps obtained for the *S. lividans* and *S. coelicolor dnaA-gyrB* clones were identical, and the map of the *dnaA*-hybridizing region was identical to the *dnaA* regions of *S. lividans* and *S. coelicolor* reported previously (1, 21). Thus, the conservation of genetic organization in the *S. coelicolor dnaA-dnaN* region (1) also extends to *gyrB*, as in other eubacteria (16). It has been shown previously (21; our unpublished data) that sequences between *dnaA* and *dnaN* in *S. lividans* are capable of autonomous replication. The present study suggests that these sequences do indeed constitute the major origin for chromosomal replication. Our attempts to delete the noncoding putative *oriC* region between *dnaA* and *dnaN* from the chromosome (the 0.62-kb *Sph*I-*Eco*RI region; Fig. 2) have consistently failed, an expected result if these sequences fulfill a key role in replication (2a).

The observation that the principal origin of replication of the *S. coelicolor* chromosome is apparently located in (or near) the *dnaA-gyrB* region is particularly significant in the light of

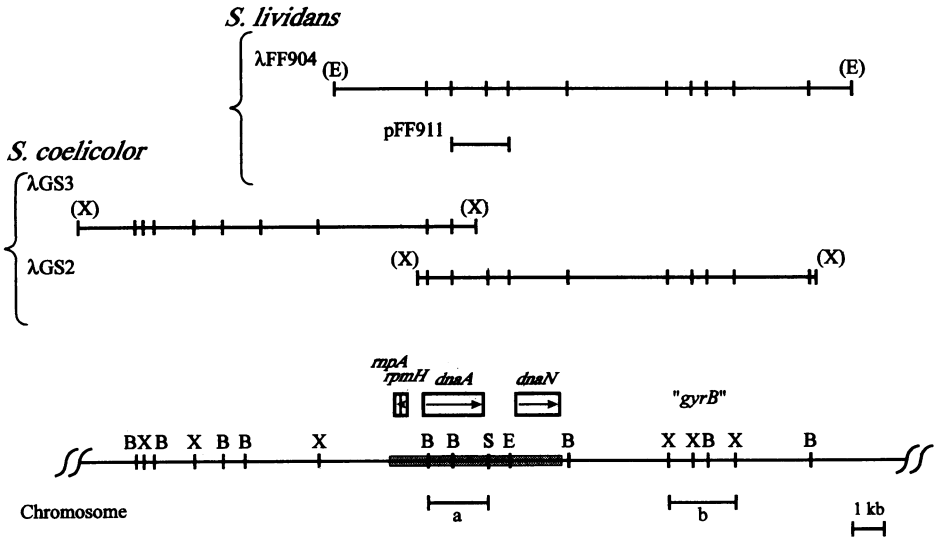

FIG. 2. Restriction map of the *dnaA-gyrB* region of *S. coelicolor* and *S. lividans*, derived from clones λFF904 (*S. lividans*) and λGS2 and λGS3 (*S. coelicolor*) and from Southern analysis of the two species. B, *Bam*HI; E, *Eco*RI; S, *Sph*I (not all sites shown); X, *Xho*I; sites in parentheses are from the respective λ vectors. Segments a and b, indicated below the restriction map, represent the sequences that hybridize to the *Streptomyces sphaeroides gyrB* gene (from pLST192) and the *P. putida dnaA* gene (from mp18SB1.9), respectively. The boxed region corresponds to the sequences characterized previously by Calcutt and Schmidt (1), and the gene organization is indicated. The nucleotide sequence of the *gyrB* region has recently been deposited in GenBank (L27063) by M. J. Calcutt.

the recent discovery that the *S. coelicolor* chromosome is linear (13). This suggests that the terminal proteins of the telomeres are unlikely to play a dominant role in priming replication of the streptomycete chromosome. To date, the spirochete *Borrelia burgdorferi* is the only nonactinomycete prokaryote known to possess a linear chromosome (2). As in *S. coelicolor*, the *dnaA-gyrB* region of the *B. burgdorferi* chromosome is centrally located, although it is not yet known whether this region functions as the origin of replication.

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