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Mapping of Genes Involved in Macromolecular Synthesis on the Chromosome of *Streptomyces coelicolor* A3(2)

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The genes for the β, β′, and seven σ factor subunits of RNA polymerase, for elongation factors EF-Tu1 and EF-Tu3, and for six rRNA operons were mapped on the combined genetic and physical map of the *Streptomyces coelicolor* chromosome. Like the previously mapped tRNA genes, the RNA polymerase and rRNA genes map to scattered positions. The lack of rRNA operons in the immediate vicinity of the origin of replication (*oriC*) and the absence of tRNA genes in any of the rRNA operons are novel features of the *Streptomyces* chromosome.

A combined genetic and physical map of the *Streptomyces coelicolor* A3(2) chromosome, carrying some 150 genes or gene clusters, has been constructed from a combination of the results of linkage analysis via plasmid-mediated conjugation and pulsed-field gel electrophoretic studies of chromosomal fragments generated by rare-cutting restriction enzymes (19, 21). Although the linkage analysis included a set of 28 temperature-sensitive lethal mutations (18), none of these was shown to be blocked in specific steps in DNA replication, transcription, or translation. Other such mutations were later implicated in macromolecular synthesis but were not mapped (15). In fact, until recently, the only mapped genes involved in the machinery of nucleic acid or protein synthesis were three putative ribosomal protein or RNA polymerase genes (*strA*, *spcA*, and *rifA*), which were identified by mutations that confer resistance to the antibiotics streptomycin, spectinomycin, and rifampin, respectively. This situation has changed with reports of the mapping of the chromosomal replication origin (11, 50), one putative RNA polymerase σ factor gene, *whiG* (14), and 20 tRNA genes (23, 35). Here, we report the physical mapping of eight RNA polymerase subunit genes, the two *tuf* genes for polypeptide chain elongation factors EF-Tu, and the six rRNA operons on the *S. coelicolor* chromosome. We also discuss current information on the distribution of genes for aspects of macromolecular synthesis in this member of the high-G+C gram-positive actinomycetes.

Mapping of genes encoding RNA polymerase subunits. The *S. coelicolor* genes encoding the β, β′, and seven σ subunits of RNA polymerase have recently been isolated (see Table 1 for references). Of these, *hrdB* is believed to encode the primary σ subunit and is an essential gene (7). σ^{hrdB} strongly resembles *Escherichia coli* σ^{70}; it has been shown biochemically to have a promoter specificity very similar to those of *E. coli* σ^{70} and *Bacillus subtilis* σ^{A} and to be the major polypeptide associated with the core *S. coelicolor* RNA polymerase (6). The *hrdA*, *hrdC*, and *hrdD* genes (7, 8, 39, 40) encode group 2 σ factors (26) that show a lower overall similarity to the primary σ factors of other eubacteria than does σ^{hrdB} but are very similar in their DNA-binding domains, suggesting that they recognize related promoter sequences. However, the functions of these three σ factors remain unknown: a triple *hrdACD* null mutant showed no obvious phenotypic differences from the wild type (8). The *whiG* and *sigF* genes encode σ factors involved in the control of morphological differentiation. The level of σ^{whiG} is critical in determining the developmental fate of hyphae. *whiG* mutants develop long, straight aerial hyphae that show no signs of further development into spore chains (29); conversely, overexpression of *whiG* leads to ectopic sporulation in the vegetative hyphae, which are normally fated to lyse (14). Mutations in *sigF* produce aberrant spores that fail to develop the normal thick wall and are subject to lysis (31). The *sigE* gene encodes σ^{E} (formerly σ^{28}), which has been identified biochemically as the σ subunit that directs transcription from one of four promoters (P2) of the agarase gene (*dagA*) (10). The recent cloning of *sigE* has led to the identification of a new subfamily of σ factors involved in the control of a range of different extracytoplasmic functions in a wide variety of eubacterial genera (27).

The positions of the eight new RNA polymerase genes on the *S. coelicolor* chromosome were determined by hybridizing suitable probes to Southern transfers of *Ase*I and *Dra*I digests of *S. coelicolor* M145 DNA, which were separated by pulsed-field gel electrophoresis (21). The results are summarized in Table 1, and their interpretation is given in Fig. 1B.

When the *S. coelicolor hrd* genes were originally isolated, Takahashi and coworkers (38) reported that *hrdC* was absent from the closely related strain *Streptomyces lividans* 66, whereas Buttner and coworkers (7) found it to be present and showed that the *S. lividans* 66 derivative used by Takahashi and coworkers had undergone a deletion of *hrdC*. The wild-type appearance of this strain was consistent with the lack of phenotypic consequences later associated with the construction of a *hrdC* null mutant of *S. coelicolor* (7). The *hrdC* gene of *S. coelicolor* lies on *Ase*I fragment M, in the 1 to 2 o'clock region of the chromosome, close to a chromosomal end (25) and to a region of DNA subject to deletion (20). The corresponding region of the *S. lividans* chromosome (24) is also often involved in large deletions and amplifications (33). Perhaps the spontaneous loss of *hrdC* in some isolates of *S. lividans* is related to such deletion events.

The *S. coelicolor* β and β′ subunit genes *rpoB* and *rpoC* were

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TABLE 1. Map locations of *S. coelicolor* RNA polymerase, elongation factor, and rRNA genes

Gene or operon	Fragment to which the clone hybridized		Gene product and its properties	Reference(s)
	*Ase*I	*Dra*I		
rpoBC	D	D	β, β′ subunits (*rpoB* is expected to be equivalent to *rifA*)	9
hrdA	C	G	Group 2 σ factor; nonessential and of unknown function	8, 39, 40
hrdB	B	A/B[a]	Principal σ factor; essential gene	6, 7, 36, 39
hrdC	M	—[b]	Group 2 σ factor; nonessential and of unknown function	7, 8, 39, 40
hrdD	E	A/B[a]	Group 2 σ factor; nonessential and of unknown function	7, 8, 39, 40
sigE	E	A/B[a]	σ^{E} (formerly σ^{28}); extracytoplasmic function (ECF) σ factor	10, 27
sigF	D	A/B[a]	σ^{F}; required for normal spore maturation	31
whiG[c]	B	F	σ^{whiG}; controls the onset of sporulation in aerial hyphae	14, 29
tuf-1	D	D	Major EF-Tu (EF-Tu1); *rpsL*, the first gene of the S12 operon to which *tuf-1* belongs, is probably equivalent to *strA*	43
tuf-3	G	C	Putative elongation factor EF-Tu3; not expressed during normal growth	43
rrnA	D	D	23S, 16S, and 5S rRNA	42
rrnB	I	E	23S, 16S, and 5S rRNA	4
rrnC	G	C	23S, 16S, and 5S rRNA	42
rrnD	E	A/B[a]	23S, 16S, and 5S rRNA	4
rrnE	B	A/B[a]	23S, 16S, and 5S rRNA; possibly expressed late in growth	42
rrnF	E	A/B[a]	23S, 16S, and 5S rRNA	4

[a] Hybridized to the unresolved doublet band of *Dra*I-A and -B in these experiments.
[b] —Not done.
[c] The data for *whiG* were published previously (21).

isolated using the corresponding *E. coli* genes as probes (9). Preliminary sequencing showed that the two genes are adjacent on the chromosome in the order *rpoB rpoC* (9), as in other eubacteria that have been investigated (1, 17, 28) (in the cyanobacteria, *rpoC* is split into two genes i.e., *rpoC1* and *rpoC2* [48]). Chater (13) isolated three classes of rifampin-resistant mutants of *S. coelicolor*, designated *rifA*, *rifB*, and *rifC*. Only in *rifA* mutants was RNA synthesis resistant to rifampin in extracts, as well as in whole cells. By analogy with *E. coli* and *B. subtilis*, it is expected that *rifA* and *rpoB* are the same gene. The genetic location of *rifA*, which is very close to that of *strA* (13), and the physical location of *rpoB* (Fig. 1B) are consistent with this expectation.

In the 11 gram-negative species examined, the gene encoding the principal σ factor is part of the macromolecular synthesis operon which contains three essential genes, in the order *rpsU*, *dnaG*, and *rpoD*, whose products (ribosomal protein S21, DNA primase, and principal σ factor) are necessary for the initiation of protein, DNA, and RNA syntheses, respectively (45). In gram-positive species, the macromolecular synthesis operon appears to lack *rpsU* but maintains *dnaG* next to *rpoD* (45). Interestingly, DNA sequencing of the *hrdB* region shows

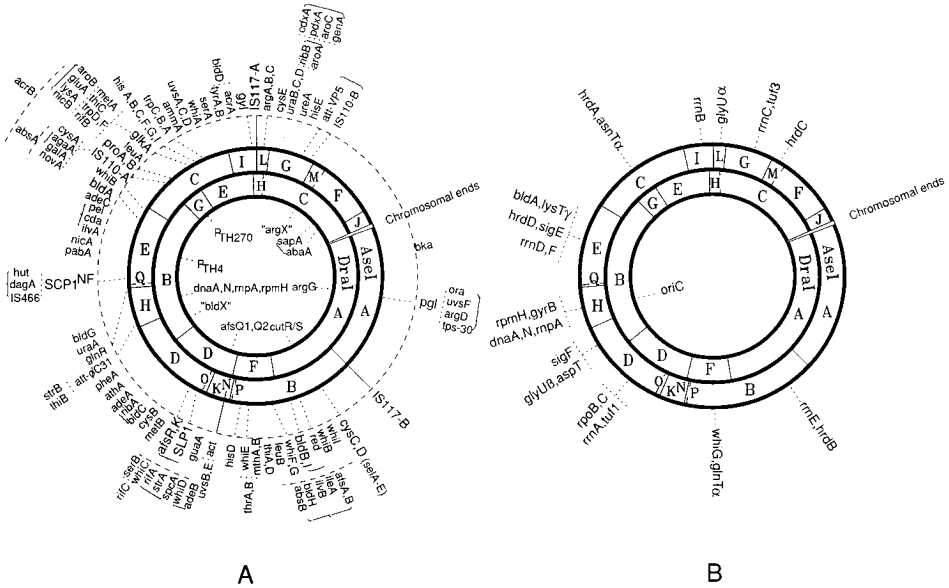

FIG. 1. Combined genetic and physical maps of the *S. coelicolor* M145 chromosome. (A) Complete map (from reference 19). The physical map for the restriction enzymes *Ase*I and *Dra*I is from previously published work (21), with correction of the position of *Ase*I fragment Q (32) and the relative positions of *Dra*I fragments A and C (20). The ends of the chromosome (25) are indicated. (B) Positions of genes for aspects of macromolecular synthesis superimposed on the physical map from panel A (genes are indicated at the midpoint of the relevant restriction fragment). Map locations of the tRNA genes are from Sedlmeier et al. (35) (only the first genes in each cluster are shown); those flanking *oriC* are from Calcutt and Schmidt (11), and *whiG* is from Kieser et al (21).

that the principal σ factor of *S. coelicolor* is not associated with *dnaG* or *rpsU*, and these genes are not found in association with *hrdA*, *hrdC*, or *hrdD* either (34, 36, 37, 40).

Mapping of the *tuf* genes. Polypeptide chain elongation factor EF-Tu, which is responsible for delivering aminoacyl-tRNA to the translating ribosome, is among the most abundant proteins in the bacterial cytoplasm and can constitute up to 10% of total protein during exponential growth in *E. coli* (46). In *E. coli*, two *tuf* genes, *tufA* and *tufB*, encode elongation factors EF-Tu that differ only in their C-terminal amino acids (2, 49). A third *E. coli* elongation factor, which is encoded by the *selB* gene, was identified; however, this protein is highly specialized, and its size is 67 kDa, much larger than the usual 43 kDa (16).

Recently, multiple *tuf* genes were found in various *Streptomyces* species. The kirromycin producers *Streptomyces ramocissimus* and *Streptomyces collinus* have three *tuf* genes that are surprisingly heterogeneous; *S. ramocissimus tuf-1* and *tuf-2* have 85% nucleotide sequence identity, whereas *tuf-3* shows only 70% identity with *tuf-1* and *tuf-2* (47). Like most other streptomycetes, *S. coelicolor* and *S. lividans* have two *tuf* genes, corresponding to *tuf-1* and *tuf-3* (43). In contrast, other actinomycetes, including *Mycobacterium* spp., *Nocardia* spp., *Corynebacterium fasciens*, *Micromonospora chalcea*, and *Saccharopolyspora erythraea*, contain only a *tuf-1* homolog (41). Therefore, both *tuf-2* and *tuf-3* may be unique for streptomycetes. The function of the *tuf-2* and *tuf-3* gene products is still unclear; however, the apparent absence of these proteins during normal growth (41) hints at a specialized role in the *Streptomyces* life cycle.

Like *E. coli tufA*, *S. coelicolor tuf-1* is probably located in the S12 operon, which also encompasses *rpsL* (encoding ribosomal protein S12), *rpsG* (encoding ribosomal protein S7), and *fus* (encoding EF-G) (43). By analogy with other microorganisms, *rpsL* and *strA* are expected to be the same locus, and the map location of *strA* is known (around 7 o'clock; Fig. 1A). Therefore, *tuf-1* was likely to be very close to *strA* and thus also to *rifA* (*rpoB*). Genetic and physical mapping of the *E. coli* chromosome revealed that *tufA* and *tufB* are well separated but that both are near the chromosomal origin of replication (22).

To locate the *tuf* genes on the *S. coelicolor* map, blots containing *Ase*I- and *Dra*I-digested genomic DNAs were hybridized with probes specific for *S. coelicolor tuf-1* and *tuf-3* (the results are summarized in Table 1 and Fig. 1B). *S. coelicolor tuf-1* maps to the overlap between *Ase*I and *Dra*I fragments D, which is consistent with genetic mapping of *strA* and *rifA*; thus, the organization around the S12 operons of *E. coli* and *S. coelicolor* may be comparable. In contrast to *S. coelicolor tuf-3*, the *E. coli tufB* gene is located in a tRNA operon and is expressed at a high level. Therefore, these genes are not comparable, and this is emphasized by their positions relative to the origins; *tufB* is close to the *E. coli* origin (22), whereas *tuf-3* is far from the *S. coelicolor* origin (Fig. 1B). A functional analysis of *tuf-3* and its gene product is currently in progress and should provide insight into the role of this unique elongation factor in *Streptomyces* protein biosynthesis.

Mapping of the rRNA operons. The organization of rRNA operons in several *Streptomyces* species has recently been investigated. Like most streptomycetes, *S. coelicolor* contains six rRNA operons, *rrnA* to *rrnF* (4, 42), and, typically, the gene order is 16S→23S→5S rDNA. In *E. coli* (and most other eubacteria), the spacer region between the 16S and the 23S rRNA genes contains a tRNA gene (5); however, in streptomycetes this is not the case (35 and references therein).

Physical and genetic mapping of the *E. coli* chromosome showed that the rRNA operons *rrnA*, *rrnB*, *rrnC*, and *rrnE* lie close to the origin of replication (3 and references therein). In contrast, none of the *S. lividans rrn* operons is adjacent to the chromosomal origin (50). For mapping the six *S. coelicolor rrn* operons, probes were obtained from λ clones by selecting fragments upstream of the 16S rRNA specifying genes, which were shown to allow specific identification of the operons.

The *S. coelicolor* rRNA operons are scattered over the well-marked segments of the chromosome (Fig. 1B). Although the operons *rrnA*, *rrnD*, and *rrnF* map in the vicinity of the chromosomal origin of replication, they are not very closely linked, which is also the case in *S. lividans* (50). The *rrnE* operon lies on *Ase*I fragment B, where many developmental genes and genes encoding pleiotropic regulators of secondary metabolism have been mapped, including *whiF*, *whiG*, *whiH*, *whiI*, *bldB*, *bldH*, *afsB*, and *absB* (21). This is interesting, since one of the *rrn* operons is probably transcribed in the transition phase in liquid cultures (44) and could therefore, in principle, be regarded as being involved in *Streptomyces* development. Preliminary experiments suggest that the operon that is transcribed late in growth may indeed be *rrnE* (41, 44); however, a transcriptional analysis of this operon is required to verify this.

The origin of replication and flanking genes. Figure 1B summarizes the arrangement of all classes of genes concerned with aspects of DNA replication, transcription, and translation that have so far been mapped. The origin of chromosomal replication has been convincingly located on *Ase*I band H. A probe of *S. lividans* DNA that hybridized to the *Pseudomonas putida dnaA* gene hybridized to this band (21), as did a cloned fragment of *S. lividans* DNA that could replicate as a minichromosome and contained characteristic DnaA boxes (50). The corresponding sequences of the *S. coelicolor* chromosome were found to be flanked by *dnaA* (encoding the replication initiator protein) and *dnaN* (for the β subunit of DNA polymerase III) (11). The *rpmH* gene (for ribosomal protein L34) and *rnpA* (for the RNase P protein) lie very close upstream of *dnaA* (11), and *gyrB* (for the DNA gyrase B subunit) is located 2 to 3 kb downstream of *dnaN* (30). Functional evidence that *oriC* is the principal replication origin was obtained by studying the overrepresentation of a sample of chromosomal genes in rapidly growing cultures (30). The location of *oriC* in the center of what is now known to be a linear chromosome in at least some derivatives of *S. coelicolor* A3(2) (25) is interesting in comparison with a similar finding for the linear chromosome of *Borrelia burgdorferi* (12). It was suggested that replication from *oriC* might be completed by priming from the protein molecules covalently bound to the free 5′ ends of the chromosome (25).

Although *S. coelicolor* resembles most other eubacteria in having a series of characteristic genes very close to *oriC*, the locations of genes involved in transcription and translation are distinctly different from those on the *E. coli* and *B. subtilis* chromosomes. As discussed in this paper, there are no rRNA operons in the vicinity of *oriC* (the closest are *rrnA*, *rrnD*, and *rrnF*, at least some few hundred kilobases away), and the set of six operons is spread around the whole chromosome. Additionally, no tRNA genes are closely linked to any of the rRNA operons, a feature that is so far unique for actinomycetes. The scattering of genes for RNA polymerase subunits, EF-Tu's, rRNAs, and tRNAs is also a striking feature of the *S. coelicolor* chromosome.

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