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# Characterization of an Autonomously Replicating Region from the *Streptomyces lividans* Chromosome

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**The chromosomal replication origin of the plasmidless derivative (TK21) from *Streptomyces lividans* 66 has been cloned as an autonomously replicating minichromosome (pSOR1) by using the thiostrepton resistance gene as a selectable marker. pSOR1 could be recovered as a closed circular plasmid which shows high segregational instability. pSOR1 was shown to replicate in *Streptomyces coelicolor* A3(2) and in *S. lividans* 66 and hybridized with DNA from several different *Streptomyces* strains. Physical mapping revealed that *oriC* is located on a 330-kb *Ase*I fragment of the *S. coelicolor* A3(2) chromosome. DNA sequence analyses showed that the cloned chromosomal *oriC* region contains numerous DnaA boxes which are arranged in two clusters. The preferred sequence identified in the *oriC* region of *Escherichia coli* and several other bacteria is TTATCCACA. In contrast, in *S. lividans*, which has a high GC content, the preferred sequence for DnaA boxes appears to be TTGTCCACA.**

*Streptomyces* spp. are gram-positive bacteria which form long unseptated substrate hyphae containing many molecules of chromosomal DNA (11). During vegetative growth of substrate mycelia, replication of the *Streptomyces* chromosomes is not coordinated with cell division. Septum formation occurs after depletion of nutrients in aerial mycelia in which spores are formed. However, in unicellular bacteria such as the model organisms *Escherichia coli* and *Salmonella typhimurium*, initiation of chromosomal replication is coupled with cell division (21). In order to understand the regulation of replication at different stages during the developmental cycle, it is necessary to characterize the replication origin(s) of the *Streptomyces* chromosome and its interaction with regulatory proteins required for initiation of new rounds of replication.

Bacterial chromosomal replication has been best studied in *E. coli*. DNA replication is initiated at a fixed site on the chromosome (21, 28). This origin of replication (*oriC*) has been cloned by using an ampicillin resistance gene (23). The minimal requirement which allows autonomous replication in *E. coli* and contains several highly conserved regions is 245 bp: (i) five 9-bp repeats (called DnaA boxes or R sites) with a consensus sequence 5′-TTAT(C/A)CA(C/A)A-3′, (ii) three 13-bp AT-rich direct repeats with the consensus sequence 5′-GATCTNTTNTTT-3′, and (iii) 11 GATC sites. In vitro replication studies suggest the following cascade for initiation (4, 5): 20 to 40 DnaA protein molecules interact, in the presence of ATP, with each other and with DnaA boxes located in the *oriC* region (initial complex). This causes melting of the three AT-rich 13-mers and local unwinding of the DNA strands (open complex) and thereby allows two DnaB helicases and two DnaC molecules to enter the helix. These events also require the proteins HU, SSB, and DNA gyrase. Priming by DnaG primase can then occur.

Chromosomal replication origins have been cloned from gram-negative bacteria, including several members of the family *Enterobacteriaceae* (*Enterobacter aerogenes*, *Erwinia carotovora*, *Klebsiella pneumoniae*, and *Salmonella typhimurium* [29, 30]), *Vibrio harveyi* (29) *Pseudomonas aeruginosa* (24), and *Pseudomonas putida* (24). Recently, Ogasawara et al. (13) cloned an autonomously replicating sequence from the origin region of the gram-positive bacterium *Bacillus subtilis*. Also, a putative *oriC* region has been cloned from *Micrococcus luteus* (7), which is a gram-positive bacterium with high GC content.

Here we report the cloning of the *oriC* region as autonomous replicon from a plasmid-free derivative of the gram-positive bacterium *Streptomyces lividans* 66. Sequence analysis allowed comparative studies of the *oriC* regions present in chromosomal DNAs of various G+C contents.

(A short report of this work was presented at the International Symposium on Biology of Actinomycetes, August 1991, in Madison, Wis.)

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** *Streptomyces* and *E. coli* strains, plasmids, and their origins are listed in Table 1.

**Culture conditions and transformation.** Propagation and transformation of *E. coli* and *Streptomyces* strains have been described earlier (9, 15). *Streptomyces* transformants were selected for resistance to 10 μg of thiostrepton per ml.

**DNA isolation and manipulations.** Chromosomal DNA was isolated from *Streptomyces* strains as described elsewhere (9). Plasmid DNA was isolated by an alkaline method (1) with modifications described by Zotchev et al. (27). Methods for purification of DNA fragments, Southern hybridization, and preparation of DNA probes have been described previously (15).

**PCR.** The polymerase chain reaction (PCR) was performed with a Trio-Thermoblock (Biometra) apparatus. The reaction mixture (50 μl) contained 25 μM primers, 1 μg of DNA, all four deoxynucleoside triphosphates at 0.2 mM each, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, and 2.5 U of *Taq* DNA polymerase (14). The reaction mixture was overlaid with mineral oil and subjected to 30 cycles consisting of a 1-min denaturation period at 95°C, a 1-min annealing period at 55°C, and a 3-min extension period at 72°C. Two oligonucleotides, $p_1$ (5′-CCGCATA TGAAGTATTCGCC-3′) and $p_2$ (5′-CCTCGACCTCGATT CGTCA-3′), containing homologous sequences to opposite

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TABLE 1. Bacterial strains and plasmids

| Strain or plasmid | Genotype and/or relevant characteristics[a] | Source or reference |
|---|---|---|
| Strains | | |
| *E. coli* | | |
| HB101 | *supE44 hsdS20* ($r_B^-$ $m_B^-$) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1* | 3 |
| JM109 | *recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi*Δ(*lac-proAB*) F'[*traD36 proAB*$^+$ *lacI*$^q$ *lacZ*ΔM15] | 22 |
| *S. albus* | ISP 5313 (ATCC 3004) | M. Goodfellow |
| *S. coelicolor* A3(2) | M110 *hisA1 uraA1 strA1* | D. A. Hopwood |
| *S. coelicolor* | ISP 5233 (ATCC 23899) | M. Goodfellow |
| *S. coriofaciens* Müller | ISP 5485 (ATCC 14155) | M. Goodfellow |
| *S. fradiae* | PCM 2330 (ATCC 10745) | M. Goodfellow |
| *S. griseus* | ISP 5236 (ATCC 3478) | M. Goodfellow |
| *S. lividans* TK21 | SLP2$^-$ SLP3$^-$ derivative of *S. lividans* 66 | D. A. Hopwood |
| *S. rimosus* | ISP 5260 (ATCC 10970) | S. Rowiński |
| Plasmids | | |
| pBR322 | Amp$^r$ Tet$^r$ | 2 |
| pUC18 | Amp$^r$ | 22 |
| pSOR1 | Minichromosome containing *oriC* of *S. lividans* TK21, Tsr$^r$ | This study |
| pBOR10 | pBR322 derivative containing *Eco*RI-*Sph*I-*Bcl*I fragment of pSOR1 | This study |
| pBOR11 | pBR322 derivative containing *Bcl*I-*Bgl*II-*Eco*RI fragment of pSOR1 | This study |
| pUOR1 | pUC18 derivative containing *Sph*I-*Sph*I fragment of pBOR10 (including 187-bp *Bam*HI-*Sph*I fragment of pBR322) | This study |
| pUOR2 | pUC18 derivative containing *Sph*I-*Eco*RI fragment of pBOR10 | This study |
| pUOR3 | pUC18 derivative containing *Eco*RI-*Bgl*II fragment of pBOR11 | This study |
| pUOR4 | pUC18 derivative containing *Bgl*II-*Sal*I fragment of pBOR11 | This study |

[a] ATCC, American Type Culture Collection, Rockville, Md.; ISP, International Streptomyces Project; PCM, Polish Collection of Microorganisms, Wrocław, Poland.

strands of the 1.1-kb *Bcl*I fragment carrying the thiostrepton resistance gene (*tsr*$^r$) (9, 20), were used as primers to amplify the *S. lividans oriC* region.

**DNA sequence determination and oligonucleotide synthesis.** DNA fragments to be sequenced were cloned into pUC18. DNA sequencing was performed by using the dideoxy-chain termination method (16) with Sequenase (United States Biochemical Co.) and α-$^{35}$S-ATP (Amersham Corp.). Nucleotide sequences were determined for both strands. DNA sequence data were analyzed by using the Genmon program (Gesellschaft für Biotechnologische Forschung). Oligonucleotides used for sequencing and PCR were chemically synthesized in a Pharmacia synthesizer.

**PFGE.** Agarose-embedded blocks containing *Streptomyces* mycelia were prepared according to the method of Schwartz and Cantor (19) with special modifications for *Streptomyces* spp. (8). After lysis of the mycelia within the agar blocks, DNA was digested with 10 U of *Ase*I at 37°C overnight. Pulsed-field gel electrophoresis (PFGE) was performed in 0.8% agarose at 200 V with a pulse time of 80 s in the hexagonal-array CHEF-DRII (Bio-Rad) apparatus.

**Nucleotide sequence accession number.** The nucleotide sequence presented in this paper has been deposited in GenBank under accession number M86491.

## RESULTS

**Cloning of the *S. lividans* TK21 origin region.** In order to clone any chromosomal replication origin, high-molecular-weight DNA of *S. lividans* TK21 (a plasmid-free derivative of *S. lividans* 66) was cleaved with *Bcl*I and ligated to a 1.1-kb *Bcl*I fragment containing the thiostrepton resistance (Tsr$^r$) gene from the vector pIJ702 (9, 20). The ligation mixture was used to transform *S. lividans* TK21 protoplasts, and after regeneration Tsr$^r$ colonies were selected. Three independent transformants were identified. All of them were examined for the presence of closed circular plasmid molecules. Although *S. lividans* TK21 is not a *rec* mutant strain (suitable *rec* mutant strains are not yet available for streptomycetes), plasmid DNA could be isolated from each of three transformants. Especially in the absence of selective pressure, these plasmids showed segregational instability in *S. lividans* TK21. Thus, only small quantities of the plasmid could be obtained as closed circular DNA molecules. By using hybridization studies (including the rRNA operon as an internal standard [25, 26]), the copy number could be estimated as approximately 0.6 per chromosome of the vegetative mycelium as an average. *Bcl*I cleaved all three plasmids into two fragments (2.6 kb and the 1.1-kb *tsr*$^r$ fragment). Since hybridization studies indicated that these plasmids were identical, one of them (pSOR1) was analyzed further.

Also, the wild-type *S. lividans* 66 and M110, a derivative of *Streptomyces coelicolor* A3(2), were successfully transformed by pSOR1 which could be reisolated as closed circular molecules.

Since pSOR1 was unstable and present in low copy number, PCR was used to amplify the cloned origin region by choosing two oligonucleotides complementary to the opposite strands of the 1.1-kb *Bcl*I fragment coding for the Tsr$^r$ gene. Primers were oriented so that DNA synthesis by *Taq* polymerase proceeded across the *oriC* region. Amplified DNA was used to construct a restriction map of the *S. lividans* TK21 *oriC* region (Fig. 1).

**Subcloning and sequencing of the *oriC* region.** Since mistakes could not be excluded during the amplification process, the PCR-amplified DNA was used as a probe to recover the *oriC* region directly from chromosomal DNA. On the basis of the restriction map (Fig. 1), it appeared most suitable to cleave chromosomal DNA of *S. lividans* TK21 to completion with the two enzymes *Bcl*I and *Eco*RI. Frag-

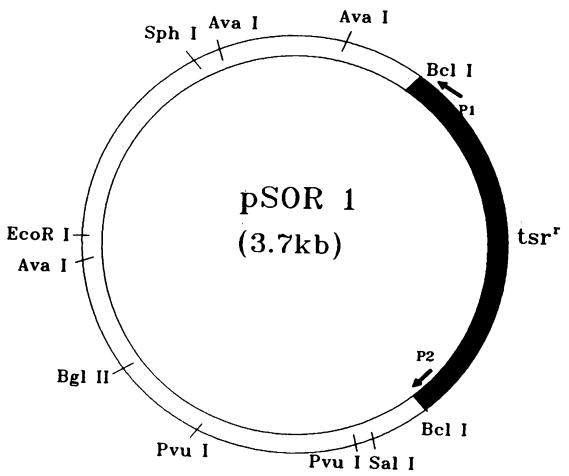


FIG. 1. Restriction map of pSOR1 containing the origin of replication (*oriC*) of *S. lividans* TK21. Arrows indicate the locations and orientations of primers ($p_1$ and $p_2$) used for amplification (PCR) of the *oriC* region.

ments of about 1.3 kb in size were recovered from the gel and cloned into *Eco*RI-*Bam*HI-cleaved pBR322. Among the plasmids isolated from 150 ampicillin-resistant transformants, pBOR10 and pBOR11 (Table 1) hybridized with the labelled PCR probe. The restriction maps of the cloned inserts of both plasmids were identical to that obtained initially.

Because of the cloning strategy employed, the possible loss of a small internal *Eco*RI fragment within the cloned plasmid could not be excluded. Therefore, the region around the *Eco*RI site (292 bp) was amplified from chromosomal DNA by using primers ($p_6$ and $p_7$; Fig. 2). Its analysis verified the presence of only one *Eco*RI site.

For further sequencing, a *Sph*I-*Sph*I (including a 187-bp *Bam*HI-*Sph*I fragment of pBR322) or *Sph*I-*Eco*RI fragment from pBOR10 and an *Eco*RI-*Bgl*II or *Bgl*II-*Sal*I fragment from pBOR11 were subcloned into pUC18 (Table 1). Figure 2 presents the nucleotide sequence of *S. lividans oriC* region. A search was made for special features within the DNA sequence which may provide signals for initiation of DNA replication. One region (250 to 850; Fig. 2) is AT rich (54%) and contains numerous repeats of 9 nucleotides. The sequence of these nucleotides is similar to DnaA boxes present within other chromosomal replication origins of many bacteria. These have been compared (Tables 2 and 3), and thus a preferred sequence, TTGTCCACA, could be identified.

```
GCAAGATCCGCAACCTGATGGCCGAGCGGCGCTCGATCTACAACCAGGTGACCGAGCTGA 60
CCAACCGCATCAAGAACGGCTGACGACCGACCCCACCGGTCACGAAGGCGCCTCCGGGAC 120
CCCGGGGGCGCCTTTCCCATGCCCCTGGGTAACCCCAGGCTGTCCCCCCGGATGCCCCTC 180
AvaI
GCATGCCCCCTCGCACGTCCCCGCGTCTCCACCGGCTCGTACGGCTGTCCTGTCCGCTGT 240
SphI                                  p3
TCGAATCCCCGCCGGGGTTACGGCCGTCCTCCACAGATTCGGCCAGTTTTTTCCGTCCACA 300
CCTTGGGGACTGGGAAGTTGTCCCGATCCTGTCCACAGGCCAGCCTGGTGAGGGATCATC 360
AGGGCAGGTCAGGTCCCTGTGGATTCGTGGACGAAGGATCTCCACAGGCTGTGGACAAAG 420
AAATGATCCACAGGCTGTGCATTCAGTTGTCCACGGACAACCCACAGGCTGAACGGGGTT 480
                  p4    p5
GTTCCCCAGCGATCACCAGCTTCTCCACATGCCTGTCCACTGTTCGGCAACGTGACGCGC 540
CCTGTCACCGCGTCGAGTGAAAGCCGTCACACCGGGCTGCCTGGTTGGGCTGTGGGAAAC 600
GTGGGTAAAGCTGGGGACGGCGCTGGGGAGAAGTCGCCTCTCCCTGTGCACGGTGTGTGC 660
AGAACTTTCTGTTCTCCACAGATACGCCCGGTTGTCCACCGGCTCCGCCCACAGGACCCG 720
                  p6
TGGACAAAATTCCTGCTCTGACCTGGGCAAACGAGGTTATCCACGGTATCCACAGGCCCT 780
ACTACTACTGACGACTAGAGAGAGAGCGGAATTCGTTTCGAAGCGGGTCCTGTGTACAAC 840
                            EcoRI
TCGGCCTCTCGGGCCCGACCGCGCCTCGGCACGACTTGACCCCGAACGGCACCTACTGTC 900
    AvaI
AGTGGGGTGCGTCAGACTGGTCCCCGGTGTTCTGCCCCTCACAGCGGCGGCGACACCGG 960
TCAGACGACGAAGGCGAAGCAGGGCGAGCAGCGCCGGCAACAGCAGGAGGCGGCAACAGT 1020
                  p7
GAAGATCCGGGTGGAACGCGACGTACTCGCGGAGGCAGTGGCCTGGGCGGCTCGCAGCCT 1080
CCCGGCCCGTCCGCCGGCGCCGGTCCTCGCGGGCCTGCTGCTGAAGGCCGAGGAGGGCAG 1140
CTGAGCCTGTCCAGCTTCGACTACGAGGTCTCGGCGCGGGTGTCGGTGGAGGCCGAGATC 1200
                  p8
GAGGAGGAGGGCACGGTCCTCGTCTCCGGCCGTCTGCTCGCCGACATCTCCCGCGCCCTG 1260
CCCAACCGGCCGGTGGAGATCT                                        1320
                  BglII
```

FIG. 2. Nucleotide sequence of a 1,283-bp fragment of pSOR1 carrying the *oriC* region of *S. lividans* TK21. DnaA boxes are indicated by boldface letters and are boxed. AT-rich regions are underlined. The oligonucleotides ($p_3$, $p_4$, $p_5$, $p_6$, $p_7$, and $p_8$) used for sequencing and PCR are indicated by arrows.

TABLE 2. List of *S. lividans* DnaA boxes

| Sequence | No. of mismatches |
|---|---|
| TCCTCCACA | 2 |
| CCGTCCACA | 2 |
| CTGTCCACA | 1 |
| TCGTCCACG | 2 |
| ATCTCCACA | 2 |
| TTGTCCACA | 0 |
| TGATCCACA | 2 |
| TTGTCCACG | 1 |
| TTCTCCACA | 1 |
| CTGTCCACT | 2 |
| TTTCCCACA | 2 |
| TTCTCCACA | 1 |
| TTGTCCACC | 1 |
| TTGTCCACG | 1 |
| TTATCCACG | 2 |
| GTATCCACA | 2 |
| TTGTCAACA | 1 |

Seventeen of the DnaA boxes diverged up to 2 nucleotides from the preferred sequence. In addition, three boxes with 3-nucleotide changes are present (205 to 213, 479 to 487, and 645 to 653; Fig. 2). These are not listed in the Table 2, since so far all presentations of *oriC* regions from other bacteria compare only boxes with differences from a consensus sequence by 2 nucleotides at a maximum. The AT-rich region is flanked by GC sequences.

**Hybridization of the *oriC* region with total chromosomal DNA derived from various *Streptomyces* strains.** High-molecular-weight DNA from M110, a derivative of *S. coelicolor* A3(2) and closely related to *S. lividans* 66 (9), was prepared in agar blocks and digested with *Ase*I, since this enzyme recognizes relatively few sites within GC-rich DNA. Fragments were separated by PFGE. Only one chromosomal fragment of 330 kb hybridized to the cloned origin sequence (Fig. 3).

In order to test whether rRNA operons are localized near *oriC*, as in *E. coli*, hybridization with the cloned *S. lividans* rRNA operon (*rrnC*, 16S-23S-5S rDNA present as a 7.2-kb *Sal*I fragment [25]) was performed (Fig. 3). The ribosomal probe hybridized to six *Ase*I fragments but not to the fragment containing *oriC*.

To test whether other *Streptomyces* strains also contain a region homologous to *oriC*, the *Eco*RI-*Sph*I fragment of pSOR1 was used as a probe. Since DNA of *S. lividans* 66 DNA and its derivatives cannot be analyzed by PFGE (18), its *Eco*RI-*Sph*I fragments were analyzed in comparison with those obtained after cleavage of DNA isolated from *Streptomyces albus*, *S. coelicolor* A3(2), *S. coelicolor* Müller, *Streptomyces coriofaciens*, *Streptomyces fradiae*, *Streptomyces griseus*, and *Streptomyces rimosus*. Southern analysis revealed the presence of only one hybridizing DNA fragment in each of the strains tested (data not shown).

TABLE 3. Frequency of nucleotide usage in *S. lividans* nonomers (DnaA boxes)

| Nucleotide | No. of times used[a] in position no.: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| A | 1 | 0 | 3 | 0 | 0 | 1 | **17** | 0 | **11** |
| C | 3 | 3 | 4 | 1 | **17** | **16** | 0 | **17** | 1 |
| G | 1 | 1 | **9** | 0 | 0 | 0 | 0 | 0 | 4 |
| T | **12** | **13** | 1 | **16** | 0 | 0 | 0 | 0 | 1 |

[a] Numbers in boldface indicate the preferred sequence.

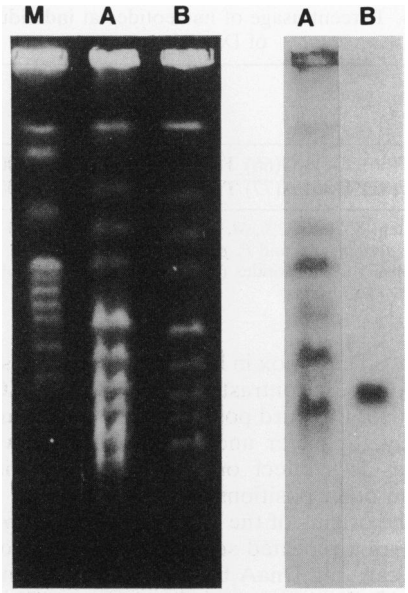


FIG. 3. Southern blot analysis of *Ase*I PFGE restriction patterns of *S. coelicolor* M110 DNA. The ethidium bromide-stained patterns are shown on the left. The blot was hybridized with the 7.2-kb *Sal*I fragment-containing rRNA operon of *S. lividans* TK21 (lanes A) or with the 0.6-kb *Eco*RI-*Sph*I fragment-containing *oriC* region of *S. lividans* TK21 (lanes B). Lane M, *Saccharomyces cerevisiae* chromosomes used as size standards (1,900, 1,640, 1,120, 1,100, 945, 915, 915, 815, 785, 745, 680, 610, 55, 450, 375, 295, and 225 kb).

## DISCUSSION

The *oriC* region of the chromosomal DNA of *S. lividans* TK21 has been cloned as an autonomously replicating minichromosome (pSOR1) by using the thiostrepton resistance gene as a selectable marker. The low copy number of pSOR1 might reflect its incompatibility with the origin in the resident chromosome and possible competition for limited amounts of DnaA protein molecules required for initiation of replication. Furthermore, it will be interesting to analyze its segregation to spores.

In contrast to the high overall G+C content of *Streptomyces* DNA (69 to 73%), the *oriC* region (~600 bp) is relatively A+T rich (54% G+C). This region contains several stop codons and does not appear to encode a protein in any possible reading frame from each strand. Interestingly, the AT-rich region contains numerous boxes (Tables 2 and 3) which share a high degree of similarity with the consensus sequence characteristic for the DnaA boxes found in the chromosomal origin regions identified within several enterobacteria: *P. aeruginosa* (24), *P. putida* (24), *B. subtilis* (12, 13), and also putative *oriC M. luteus* (7).

Comparison of the sequences of more than 90 naturally occurring DnaA boxes located in the *oriC* regions and in the promoter and/or termination regions of the *dnaA* gene shows that the fourth T, seventh A, and eighth C are highly conserved (Table 4). Interestingly, the third position in high-G+C organisms (*M. luteus* and *S. lividans*) is substituted by G (Table 4). As originally suggested by Fujita et al. (7), this change may be due to the high G+C pressure exerted during the course of evolution. Thus, the preferred

TABLE 4. Percent usage of nucleotides at individual positions of DnaA boxes

| G+C content (%)[a] | Sequence[b] (% usage) |
|---|---|
| 70–72 | T(66) T(71) G(66) **T**(97) C(93) C(100) **A**(100) **C**(97) A(78) |
| 45–65 | T(81) T(86) A(77) **T**(100) C(79) C(81) **A**(100) **C**(98) A(93) |

[a] G+C content of 70 to 72%, *M. luteus* and *S. lividans*; 45 to 65%, *E. coli*, *B. subtilis*, *P. aeruginosa*, and *P. putida*.
[b] Highly conserved nucleotides (>95%) are marked by boldface letters.

sequence of a DnaA box in high-G+C organisms seems to be TTGTCCACA, in contrast to the TTATCCACA of other bacteria. Also, the third position is the most variable (Table 4). Recently, Schaefer and Messer (17) showed that this position has less effect on the binding affinity of DnaA protein than other positions.

Within the origins of the enterobacteria *B. subtilis* and *P. putida*, AT-rich repeated sequences are located adjacent to the region carrying DnaA boxes (5, 24). In contrast, the *S. lividans oriC* does not contain obvious AT-rich repeats. Shorter AT-rich sequences are distributed among the DnaA boxes (Fig. 2).

GATC repeats which can be modified by methylation are characteristic for origins of enterobacteria (28). However, these are not highly abundant within the origin sequences of *S. lividans* and many other bacteria (13, 24). Until now, little has been known about methylation processes in *Bacillus*, *Pseudomonas*, and *Streptomyces* species.

Concomitantly, Calcutt and Schmidt (6) cloned and sequenced a 5-kb fragment of *S. coelicolor* A3(2) DNA including the *dnaA* and *dnaN* genes. The downstream (1 to 83, Fig. 2) and upstream (1019 to 1283, Fig. 2) sequences of the *S. lividans oriC* region agree completely with sequences encoding C-terminal and N-terminal ends of *S. coelicolor dnaA* and *dnaN* proteins (6), respectively. The sequence of the AT-rich segment found among both of these genes is identical to the *oriC* sequence of *S. lividans* described in this paper. In most microorganisms the *oriC* region is located close to the *dnaA* and *dnaN* genes (12).

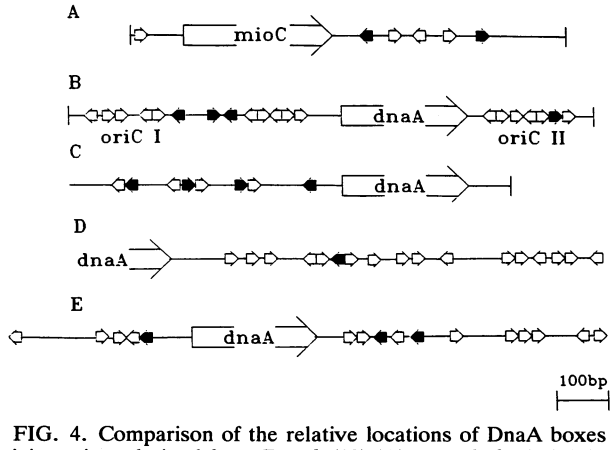


FIG. 4. Comparison of the relative locations of DnaA boxes in origin regions derived from *E. coli* (13) (A), *B. subtilis* (13) (B), *P. putida* (13) (C), *S. lividans* (D), and the putative *oriC* region of *M. luteus* (7) (E). Noncoding regions (——) are in scale with the reference size unit; sizes of the *mioC* and *dnaA* genes are reduced. Solid arrows represent the consensus TT(A/G)TCCACA sequence, and open arrows represent 1- or 2-base variations from the consensus sequence.

As shown in Fig. 4, the origin regions of *B. subtilis* (12), *P. putida* (24), and *S. lividans* are directly linked to the *dnaA* gene. In contrast, the *E. coli oriC* region is located 45 kbp away from it (21). Interestingly, the *B. subtilis oriC* region is composed of two DnaA box regions separated by the *dnaA* gene which are both required for a functional origin (13). The organization of the five DnaA boxes of *E. coli* is similar to that in *P. putida* in which two of the DnaA boxes resemble the $R_1$ and $R_4$ sites in *E. coli oriC* (24). Seventeen DnaA boxes present in the *oriC* region of *S. lividans* seem to be arranged in two clusters. This higher complexity appears to be similar to the relative location of the 11 DnaA boxes identified also downstream of the *dnaA* gene of *M. luteus* (7). However, its origin region has not yet been isolated as an autonomously replicating sequence.

Our physical mapping revealed that the replication origin is located on a 330-kb chromosomal *Ase*I fragment which is in the 8-o'clock region of the genetic map of the *S. coelicolor* A3(2) chromosome (10) and is present in one copy in all *Streptomyces* DNA tested.

The following data support the conclusion that the cloned region within pSOR1 contains the chromosomal replication origin and very likely not that one from any integrated, cryptic plasmid or phage DNA: it (i) enables autonomous replication, (ii) is segregationally unstable, (iii) contains numerous DnaA boxes and AT-rich regions, (iv) is located among sequences which agree completely with those encoding part of *dnaA* and *dnaN* for *S. coelicolor* A3(2), and (v) is present within the DNA of all *Streptomyces* strains tested and can be localized on the physical map of the *S. coelicolor* A3(2) chromosome.

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