Cloning and physical mapping of the *Staphylococcus aureus* *rplL*, *rpoB* and *rpoC* genes, encoding ribosomal protein L7/L12 and RNA polymerase subunits β and β'

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Using segments of the *Escherichia coli* *rpoB* and *rpoC* genes as heterologous probes, we have identified and cloned an 8.3 kb *Pst*I fragment from the *Staphylococcus aureus* genome containing the *rpoB* and *rpoC* genes, which respectively encode the β and β' subunits of DNA-directed RNA polymerase. This region is almost certainly equivalent to the *rif* locus, located near to *fus* at interval 12/13 on the *S. aureus* linkage map. Limited DNA sequencing revealed the gene order *rpoB*–*rpoC* (transcribed from left to right) and identified the *rplL* gene, encoding ribosomal protein L7/L12, upstream of *rpoB*. This and other evidence suggests that the *rpoBC* genes of *S. aureus* form part of a large gene cluster encoding components of the transcription and translation apparatus which is well-conserved in other eubacteria.

Introduction

The eubacterial DNA-directed RNA polymerase (EC 2.7.7.6) typically consists of a catalytic core ($\alpha_2\beta\beta'$) comprising two small (approx. 40 kDa) α subunits and two large (>100 kDa) β , β' subunits, encoded by the *rpoA*, *rpoB*, and *rpoC* genes, respectively. This core enzyme uses different σ factors for specific initiation at the various promoter classes (Yura & Ishihama, 1979; Helmann & Chamberlin, 1988). Much of our understanding of RNA polymerase has arisen from genetic approaches, in particular, the isolation of mutants resistant to antibiotics to which the enzyme is normally sensitive, such as rifampicin, related ansamycins, and streptolydigin (Yura & Ishihama, 1979). Thus, Morrow & Harmon (1979) isolated *Staphylococcus aureus* RNA polymerase mutants resistant to rifampicin, rifamycin SV and streptovaricin. The mutations were tightly clustered on the *S. aureus* genome and were almost certainly located in the *rpoB* gene. These authors also identified a closely-linked cluster of mutations conferring streptolydigin resistance, although it is not clear whether these are located in *rpoB* or *rpoC* (compare Halling *et al.*, 1978, with Heil & Zillig, 1970; Iwakura *et al.*, 1973; and Listitsyn *et al.*, 1988). The rifampicin-resistance locus (*rif*) has been mapped to linkage interval

12/13 on the *S. aureus* genetic map close to *fus*, a fusidic acid-resistance locus (Pattee, 1990).

Rifampicin-resistant (Rif^r) isolates of *S. aureus* have been shown to have reduced virulence (Moorman & Mandel, 1981) and, more recently, it has been demonstrated that Rif^r mutants are associated with altered expression of exoprotein virulence factors (Exp[−] phenotype; Al-Ani *et al.*, 1991). Several relatively specific phenotypic effects have also been described for rifampicin-resistant mutants of a number of Gram-positive and Gram-negative bacteria (Yura & Ishihama, 1979; Jin & Gross, 1989).

The molecular characterization of *S. aureus* Rif^r Exp[−] mutants may give insights into the structure and function of RNA polymerase and the interaction of RNA polymerase with regulatory signals involved in post-exponential phase expression of exoprotein virulence factors (Abbas-Ali & Coleman, 1977; Janzon *et al.*, 1986; Iandolo, 1989; Kornblum *et al.*, 1990; Vandenesch *et al.*, 1991). As an essential step in this analysis, the *rpoBC* region of *S. aureus* has been cloned and mapped.

Methods

Isolation of S. aureus chromosomal DNA. Chromosomal DNA was isolated from *S. aureus* RN4220, an NTCC 8325 derivative obtained from Richard P. Novick, MD (Public Health Research Institute, 455 First Avenue, New York, NY 10016, USA). A 24 h stationary-phase

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Fig. 1. Restriction digests of (a) *S. aureus* chromosomal DNA hybridized to the *rpoB*-specific probe; (b) *S. aureus* chromosomal DNA hybridized to the *rpoC*-specific probe; (c) pMA1 alone; and (d) pMA1 hybridized to the *rpoB*-specific probe. Restriction enzymes were as follows: E, *EcoRI*; H, *HindIII*; P, *PstI*. Fragment sizes were determined from comparison with *HindIII*-digested λ , and *HaeIII*-digested ϕ X174 DNA standards.

culture grown in 3% (w/v) Tryptone Soya Broth (Oxoid) at 37 °C was harvested by centrifugation, and the cell pellet resuspended and lysed as described by Silhavy *et al.* (1984), with the modification that lysozyme was replaced by lysostaphin (Sigma) added to a final concentration of 1 mg ml⁻¹. The lysate was subject to CsCl equilibrium density gradient ultracentrifugation and DNA was isolated by ethanol precipitation of the viscous chromosomal DNA fraction (Kaiser & Murray, 1985).

Hybridization conditions. Colony hybridization (Grunstein & Hogness, 1975) was carried out as described by Sambrook *et al.* (1989), and Southern hybridization analysis (Southern, 1975) followed the protocols of Boulnois (1987), using 0.8% agarose for fragment separation. In both cases nylon membranes (Hybond-N, Amersham) were used with final washes in 1 × SSC, 0.1% SDS at 65 °C (1 × SSC is 0.15 M-NaCl, 0.015 M-sodium citrate, pH 7.0).

Hybridization probes were derived from pGR2017, which carries a 10.2 kb *HindIII* fragment containing the *rplJ rplL rpoB rpoC* genes of *E. coli* inserted into the *HindIII* site of pBR322 (G. C. Rowland, unpublished data). *EcoRI* (2.9 kb) and *PstI* (2.8 kb) restriction fragments were used, respectively, as *rpoB*- and *rpoC*-specific probes (see Fig. 2). After isolation by preparative agarose gel electrophoresis, these fragments were labelled with [α -³²P]dCTP (Amersham) using the 'random primer' method (Feinberg & Vogelstein, 1983).

Alternatively, probes were labelled by photochemical biotinylation (Photobiotin, Gibco-BRL), and detected with a streptavidin-alkaline phosphatase conjugate, using the fluorescent substrate 4-methoxy-4-(3-phosphatophenyl)spiro[1,2-dioxetane-3,2'-adamantane] according to the manufacturer's instructions (Photogene Nucleic Acid Detection System, Gibco-BRL).

Molecular biological techniques. Standard cloning procedures (Sambrook *et al.* 1989) were employed, using the plasmid vector pTZ18R (Mead *et al.*, 1986; Pharmacia-LKB) and the *E. coli* host strain DH5 α , ϕ 80d Δ (*lacZ*)M15 Δ (*lacZYA-argF*)U169 *endA1 recA1 hsdR17* (*r_K⁻m_K⁺*) *deoR thi-1 supE44 gyrA96 relA1 λ ⁻* (Gibco-BRL). Plasmid DNA was isolated by the alkaline-lysis method (Birboim & Doly, 1979). DNA sequencing was by the dideoxynucleotide chain termination method (Sanger *et al.*, 1977) modified for double-stranded templates, using T7 DNA polymerase (T7 Sequencing Kit, Pharmacia-LKB) and [α -³⁵S]dCTP α S (Amersham). 'Universal' and 'Reverse' 17-mer sequencing primers (Pharmacia-LKB) were used as appropriate. Sequence data was compiled and analysed using DNASIS/PROSIS software (LKB-Hitachi).

Results and Discussion

Identification of *S. aureus rpoBC* genes by hybridization with heterologous probes

Southern hybridization analysis indicated that the *E. coli rpoB* and *rpoC*-specific probes hybridized to a single *S. aureus* genomic *PstI* fragment of approximately 8.3 kb (Fig. 1a, b). The alternative possibility of different *PstI* fragments of similar size was unlikely, as both probes also hybridized to a *HindIII* fragment of 4.9 kb but only the *rpoC* probe hybridized to a further 4.2 kb *HindIII*

- (b)
- 60-3% identity (94-5% similarity) in 73 amino acid residue overlap.
S.a. RplL AAEKTEFDVELTSAGSSKIKVVKAVKEATGLGLKDAKELVDGAPKVIKEALPKEEAELKEQLEEVGATVELK*
E.c. RplL AEEXTDFVILKAAGANKVAVIKAVRGATGLGLKEAKDLVESAPAAKEGVSDDAEALKALEAGAEVEVK*
50 60 70 80 90 100 110 120
 - (comp) 53-7% identity (85-4% similarity) in 82 amino acid residue overlap.
S.a. RpoB DIIASMSYFFNLLSGIGYTDIDHGLGNRRILRSVGLLQNFRIQLSRMERVVRERMSIQDTESITPQQLINIRPVIAIKEF
E.c. RpoB DIIIVMKLLDIRNGKEVDDIDHGLGNRRILRSVGLMAENQFRVGLVVRERAVKERLSLGLDITLMPQDMINAKPIAAVKEF
430 440 450 460 470 480 490 500
 - 52-9% identity (92-9% similarity) in 85 amino acid residue overlap.
S.a. RpoB EFGFIETPYRKVDLTHAITDQIDYLTADEEDSYVVAQANSKLDENGFRFMDDEVVCRFRGNNTVMAKEKMDYMDVSPKQVVSAAAT
E.c. RpoB EYGFLETPYRKV--TDGVVTDIEHYLSAIEEGNYVIAQANSNLDDEGHFVEDLVTCRSKGESSLFSRDQVDYMDVSTQQVVSVA
590 600 610 620 630 640 650 660
 - (comp) 60-6% identity (94-2% similarity) in 104 amino acid residue overlap.
S.a. RpoC FGPTKDWECSCGKYKVRVYKGMVCDRCGVEVTKSKVRRERMGHIELAAPVSHIWFYFKGIPSRMGLLLDMSPRALEEVIYFASYVVVDPGPTGLEKKTLLSEAF
E.c. RpoC FGPVKDYECCLCGKYKRLKRGVICEKCGVEVTQTKVRRERMGHIELASPTAHIFLWFLKSLPSRIGLLDMLPLRDIERVLYFESYVVIIEGGMTNLERQOILTTEEY
70 80 90 100 110 120 130 140 150 160
 - (comp) 50-0% identity (85-2% similarity) in 122 amino acid residue overlap.
S.a. RpoC GQPVQRCGEVLTEGSIENKYLVSAGLNATESYLLKEVQKVYRMQGEVIDDKHVEVMVRQMLRKVRIIEAGDTKLLPGSLVDIHNFDTANREAFKHKRPATAKPVLLGITKASLETESFLSA
E.c. RpoC GERVERGDVSDGPEAPHDILRLRGVHAVTRYIVNEVQDVYRLQGVKINDKHIEVIVRQMLRKATIVNAGSSDFLEGEQVEYSRVKIANRELEANGKVGATYSRDLLGITKASLATESFISA
1210 1220 1230 1240 1250 1260 1270 1280 1290 1300 1310 1320

Fig. 2. Characterization of the *Staphylococcus aureus rplL rpoB rpoC* gene cluster. (a) Hybridization mapping, cloning, and sequencing. The top bar represents the 10-2 kb *HindIII* insert of pGR2017, encoding the *rplL rpoB rpoC* genes of *E. coli*. Immediately below are the restriction fragments used as hybridization probes, and dotted lines indicating the approximate extent of hybridization to the *S. aureus* chromosomal restriction map. The lowest bar is a more detailed restriction map of the recombinant plasmid pMA1, comprising the 8-3 kb *S. aureus PstI* fragment cloned into pTZ18R. Arrows, numbered 1 to 5, denote the direction and extent of partial DNA sequence determination. The direction of transcription of all genes is from left to right. Restriction sites are labelled as follows: E, *EcoRI*; H, *HindIII*; P, *PstI*; S, *SalI*; X, *XbaI*. DNA sequence analysis confirmed the presence of the *HindIII* site within *S. aureus rplL*. (b) Amino acid sequences encoded by the partial *S. aureus (S.a.)* sequences described in (a) aligned with the equivalent regions from *E. coli (E.c.)* reported by Post *et al.* (1979), and Ovchinnikov *et al.* (1981, 1982). 'Comp' indicates, with reference to the sequence direction shown in (a), that the complementary strand was translated. Sequences were aligned by the method of Lipman & Pearson (1987) as implemented in PROSIS software (LKB-Hitachi). The alignment is denoted by a colon for an identity and a dot for a similarity (conservative replacement). Insertions required for optimal alignment are marked by a dash. An asterisk denotes the translation termination site of *rplL*.

fragment. These results indicated that the *rpoB* and *rpoC* genes were present in single copy, adjacent to each other on the *S. aureus* chromosome, and (largely) contained within the 8.3 kb *Pst*I fragment. These data, together with results of further single and multiple restriction digests (Fig. 1*a, b*) generated a provisional restriction map of the *S. aureus rpoBC* region which was confirmed and elaborated as detailed below and in Fig. 2(*a*).

Cloning of *S. aureus rpoBC* genes

S. aureus RN4220 DNA was digested with *Pst*I and size-separated by preparative gel electrophoresis; DNA in the approx. 8 kb size range was isolated and ligated into the *Pst*I site of pTZ18R. Despite the high copy-number of the pTZ18R plasmid vector, screening the recombinants by colony hybridization was problematic, due to a high background resulting from probe hybridization to the homologous host *E. coli rpoBC* genes. Therefore, as it was expected that the DNA size-fractionation already constituted an enrichment for the *rpoBC* region, individual purified recombinant plasmids were digested with *Pst*I and screened by Southern hybridization with the *rpoB*- and *rpoC*-specific probes. Of 100 clones tested in this manner one (pMA1) was isolated with the expected 8.3 kb insert. Further restriction and hybridization analysis of the pMA1 insert (Fig. 1*c, d* and data not shown) was entirely consistent with the chromosomal data (e.g. compare the hybridization profiles (*a*) and (*d*) in Fig. 1) and allowed the construction of a more detailed restriction map of the staphylococcal *rpoBC* region (Fig. 2*a*). One feature of this analysis was that the *E. coli rpoB* probe only gave a detectable hybridization signal with the 3' half of the *S. aureus rpoB* region. This is consistent with the dissimilar base composition of *E. coli* (50% G+C) and *S. aureus* (34% G+C) coupled with the presence of the most highly-conserved domains of the β subunit towards the C-terminus (Listitsyn *et al.*, 1988; Igloi *et al.*, 1990).

Identification of the *rplL rpoB rpoC* gene cluster

In view of their large size, and the homology between the *S. aureus* and *E. coli rpoB* and *rpoC* genes, limited sequence determination from convenient restriction sites was used to confirm the location and extent of the *rpoB* and *rpoC* genes on the pMA1 insert. Nucleotide sequences were determined for one strand only and so are not reported here and have not been deposited with public databases. However, as detailed below, the derived predicted amino acid sequences allowed useful correlation of the genetic and physical maps. The vector-directed 'Universal' and 'Reverse' sequencing primers

allowed direct sequencing of the pMA1 insert ends from the flanking *Pst*I sites, and also from internal *Eco*RI sites (Fig. 2*a*), after subcloning appropriate *Eco*RI fragments into pTZ18R. Translation of these sequences revealed unambiguous homology to *E. coli rpoB* and *rpoC* genes (Fig. 2*b*) and indicated that the 8.3 kb *Pst*I insert encoded the entire *rpoB* gene, and virtually all of the *rpoC* gene.

By analogy with the *E. coli* β' subunit (1407 residues) the right-flanking *Pst*I site was expected to be located approximately 240–270 bp (80–90 codons) upstream of the termination codon of *S. aureus rpoC*. Similarly, it was possible to predict the location of the 5' end of *rpoC* and the extent of *rpoB* (Fig. 2*a*). From the estimated sizes of 3.8 kb and 3.5 kb, and assuming an amino acid composition similar to the respective *E. coli* subunits, it may be predicted that the *S. aureus rpoB* and *rpoC* genes encode polypeptides of 142 kDa and 129 kDa. This is in reasonable agreement with values of 150 kDa for the β subunit and 130 kDa for the β' subunit derived from SDS-PAGE (Al-Ani, 1990).

In addition, sequencing from the left-flanking *Pst*I site (Fig. 2*a, b*) identified a sequence homologous to the 3' half of the *E. coli rplL* gene, encoding ribosomal protein L7/L10, upstream of *rpoB* and transcribed in the same direction as *rpoBC* (Fig. 2*a, b*). In *E. coli*, the RNA polymerase β and β' subunits are encoded with ribosomal proteins L11, L1, L10, and L7/L12 by the complex *rplKALrpoBC* operon (Jinks-Robertson & Nomura, 1987). The *rplJ* and/or *rplL* genes have also been shown to be located upstream of *rpoBC* in *Salmonella typhimurium* (Sanderson & Roth, 1988), *Pseudomonas putida* (Borodin *et al.*, 1989), and *Chlamydia trachomatis* (Engel *et al.*, 1990). Perhaps most significantly, the gene order *rplKALrpoB* has also been established for *Bacillus subtilis* (Dabbs, 1984) which, as a member of the low-(G+C) subdivision of the Gram-positive bacteria (Woese, 1987), is relatively closely-related to *S. aureus*. Although we have not determined whether L11, L1, and L10 are encoded by chromosomal DNA adjacent to the left-end of the cloned 8.3 kb *Pst*I fragment (Fig. 2*a*), our results indicate that at least part of the structure of this gene cluster is conserved in *S. aureus*.

Genetic evidence suggests that other ribosomal protein genes may also be located in the *S. aureus rpoBC* chromosomal region. Protoplast fusion and transformation studies indicate that *rif* is near to the fusidic acid resistance locus *fus* on the *S. aureus* linkage map (Pattee, 1990; and personal communication). Fusidic acid resistance in *S. aureus* has been shown to be a property of the translational machinery (Chopra, 1976) and it is likely that *fus* mutations are located in the gene encoding elongation factor G (EF-G). In *E. coli* the *fusA* gene, encoding EF-G, lies within a large gene cluster (the *str*,

S10, *spc*, and α operons) encoding protein components of the translational machinery and the α subunit of RNA polymerase, located some 17 min (approx. 800 kb) from the *rplKAJLrpoBC* (L11- β) region (Jinks-Robertson & Nomura, 1987; Bachmann, 1990). However, in *B. subtilis* the *str*-S10-*spc*- α cluster is located immediately adjacent to *rplKAJLrpoBC* (Piggot & Hoch, 1985; Ziegler & Dean, 1987) and recent physical mapping suggests that this organization is conserved in another low-(G+C) Gram-positive bacterium, *Clostridium perfringens* (Canard & Cole, 1989). In the context of characterizing the staphylococcal transcriptional apparatus, the likely occurrence of a L11- β -*str*-S10-*spc*- α gene cluster implies that one strategy for cloning the *S. aureus rpoA* gene (encoding the α -subunit of RNA polymerase) may be to 'chromosome walk' downstream of the *rpoBC* region.

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