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Cloning of a chromosomal locus (*exp*) which regulates the expression of several exoprotein genes in *Staphylococcus aureus*

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Summary. Insertion of the erythromycin resistance transposon Tn*551* into a single site of the *Staphylococcus aureus* chromosome resulted in decreased production of alpha-toxin, serine and metallo-proteinases and several other extracellular proteins and a simultaneous increase in the production of protein A. The site of insertion, designated *exp,* was separate from the structural gene for alpha-toxin and protein A. Hybridization analysis showed that the effect of the insertional mutation on the expression of the alpha-toxin and protein A was at the level of transcription. The chromosomal DNA flanking the transposon and the corresponding DNA of the wild-type strain was cloned in *Escherichia coli.* Northern blot hybridization experiments revealed that the *exp* locus codes for a major RNA of approximately 3.5 kb. This RNA was not found in the insertional mutant nor in a spontaneous *exp* mutant. A map of the *exp* locus constructed by Northern blot and restriction enzyme analysis showed that the insertional mutation was located in the middle of the coding sequence of the 3.5 kb RNA. The insertional mutant was reverted to wild type by inserting a recombinant plasmid containing most of the coding sequence of the 3.5 kb RNA.

Key words: Gene regulation – Exoproteins – Pleiotropism – Cloning – *Staphylococcus aureus*

Introduction

Staphylococcus aureus produces a large number of extracellular proteins which altogether constitute up to 30% of the total protein synthesis (Abbas-Ali and Coleman 1977). Many of the extracellular proteins are toxic to man and animals and are important virulence factors (Smith 1979). Coordinate control of exoprotein synthesis has been suggested on the basis of the observation that the production is strictly synchronized and appears mainly during the post-exponential or stationary phase of growth (Abbas-Ali and Coleman 1977; Coleman and Abbas-Ali 1977). Moreover, mutants have been isolated in which the production of several exoproteins is affected, suggesting a common regulation of exoproteins (Coleman 1981; Yoshikawa et al. 1974; Björklind and Arvidson 1980).

One class of pleiotropic mutants (Exp$^-$) with a decreased production of alpha-toxin, serine proteinase, metallo-proteinase, staphylokinase, nuclease and acid phosphatase, and a simultaneous increase in the production of protein A and coagulase has been isolated in our laboratory from continuous cultures of *S. aureus* V8 (Björklind and Arvidson 1980). Analysis of specific exoprotein mRNAs in one *exp* mutant (K68:12-1) revealed that the regulation of exoprotein gene expression exerted by *exp* was at the level of transcription (Janzon et al. 1986). A mutant (RN4256) with almost the same phenotype as the *exp* mutants has recently been described by Recsei et al. (1986). In this strain of *S. aureus* the erythromycin resistance transposon Tn*551* has been inserted in the chromosome at a site which maps between *purB* and *ilv*. Results have been presented which indicate that the transposon has inactivated a *trans*-active element exerting positive control on the transcription of the alpha-toxin and toxic shock syndrome toxin (TSST-1) genes. This element has been designated *agr* for accessory gene regulator.

To study further the nature of the regulatory mechanism of *exp* and to clone this element, mutants with a typical Exp$^-$ phenotype were constructed using the transposable element Tn*551* which can insert into various sites of the staphylococcal chromosome (Novick et al. 1979b); Pattee 1981). Using an internal Tn*551* fragment as a probe in Southern blot hybridization experiments, the chromosomal DNA fragments flanking an insertion of Tn*551* in *exp* were cloned and the extent of the *exp* locus determined.

Materials and methods

Bacterial strains and plasmids. S. aureus strains and plasmids, their characteristics and origin, are listed in Table 1. *Escherichia coli* HB101 (Boyer and Roulland-Dussoix 1969) was used as a host in cloning experiments. pBR322 (Bolivar et al. 1977) and pSP64 (Melton et al. 1984) were used as *E. coli* cloning vectors.

Cultivation and exoprotein assays. Cultivation of *S. aureus* for determination of exoprotein production was done in Brain Heart Infusion (BHI, Difco) as described by Janzon et al. (1986).

Cell-bound protein A was determined by rocket immunoelectrophoresis of bacterial lysates (Janzon et al. 1986). Assays of staphylococcal serine proteinase and metallo-proteinase were also made by rocket immunoelectrophoresis as described earlier (Björklind and Arvidson 1980). Production of alpha-toxin and proteinase was screened on agar

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Table 1. Bacterial strains, their characteristics and origin

Strain of *Staphylococcus aureus*	Relevant characteristics	Origin
V8	Wild type; Exp$^+$, Em^s	Björklind and Arvidson (1980)
K68:12-1	Spontaneous *exp* mutant from strain V8	Björklind and Arvidson (1980)
8325-4	NCTC 8325 cured from phages, Exp$^+$ phenotype	Novick (1967)
SA113	Restriction-deficient mutant of NCTC 8325	Iordanescu (1975)
RN2906	8325 harbouring pRN3208 which is a temperature-sensitive derivative of plasmid pI258 (*repA36*) which contains Tn*551*	Novick (1974)
RN4256	RN4282 with Tn*551* inserted into *agr*. Exp$^-$ phenotype	Recsei et al. (1986)
RN4102	Contains pRN4102, a derivative of pT181 with an *Eco*RI site	Supplied by R.P. Novick
WA205	V8 with Tn*551* inserted into *exp*. Exp$^-$, Em^r	This study
WA250	8325-4 with Tn*551* inserted into *exp*. Exp$^-$Em^r	This study
WA251	WA250 which has regained the Exp$^+$ phenotype by recombination with pEX42	This study

plates as previously described (Björklind and Arvidson 1980).

Preparation of DNA and RNA. Chromosomal *S. aureus* DNA was prepared by the method described by Löfdahl et al. (1983). Plasmid DNA from *S. aureus* was extracted by a modified alkaline method (Hattori and Sakai 1986). The boiling lysate method was used for *E. coli* plasmids (Barnes 1977). Total RNA from *S. aureus* was prepared as previously described (Janzon et al. 1986).

Hybridization. Colony hybridization was performed as described by Grunstein and Hogness (1975) and Southern blot hybridization as modified by Botchan et al. (1976). After hybridizaton, filters were washed twice in 0.2 × SSC or 2 × SSC at 65° C for 1 h. A 609 bp *Sau*3AI fragment of plasmid pSPA1 (Löfdahl et al. 1983) within the structural gene for protein A and a 722 bp *Cla*I fragment from the alpha-toxin gene (Kehoe et al. 1983) were used as probes. DNA probes were labelled with [α-^{32}P]dATP by nick translation (Meinkoth and Wahl 1984). DNA/RNA hybridization was done as described previously (Janzon et al. 1986).

Transformation and transduction. S. aureus protoplasts were transformed in the presence of polyethylene glycol (PEG) as described by Lindberg (1981). Transformants were selected on DM3 agar containing 5 μg/ml tetracycline. Transduction to *S. aureus* strains was carried out using phage 80α. Selection was on nutrient agar plates containing 5 μg/ml erythromycin.

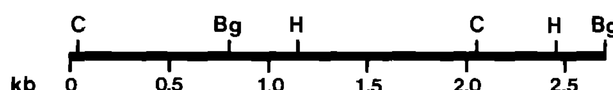


Fig. 1. Revised restriction map of one half of Tn*551*. Parts of Tn*551* from pI258 were subcloned in *Escherichia coli* and the sites for various restriction enzymes were determined. The positions of the *Bgl*II (Bg) sites were revised from earlier publications (Novick et al. 1979a; Khan and Novick 1980). The *Cla*I (C) site 29 bp from the end is the *Taq*I site found by Khan and Novick (1980). H, *Hin*dIII site. The zero coordinate indicates the junction between Tn*551* and the sequence of pI258 in which it was originally found. This coordinate was referred to as the right end of the transposon by Khan and Novick (1980)

Results

Insertional inactivation of the exp *gene by Tn551*

The temperature-sensitive plasmid pRN3208 containing the transposon Tn*551* was transduced from RN2906 to strain V8, using phage 80α. Erythromycin-resistant colonies were selected at 43° C as described by Berger-Bächi (1983). About 2000 colonies were transferred to penicillin agar plates by replica plating. Approximately two-thirds of the colonies were penicillin sensitive, indicating that they did not contain the plasmid pRN3208, but were erythromycin resistant due to the integration of Tn*551* into the chromosome. Three of these colonies did not show a zone of proteolysis on casein agar. Analysis by rocket immunoelectrophoresis revealed that they had lost both the serine proteinase and the metallo-proteinase. All three strains were also negative for alpha-toxin and had an increased production of protein A as compared with the wild-type strain. This pleiotropic change in exoprotein production is typical of *exp* mutants (Björklind and Arvidson 1980), and indicated that Tn*551* had integrated in, or next to, the *exp* locus. From one of these mutants, designated WA205, the insertional mutation was transferred to strain 8325-4 by transduction with phage 80α. More than 90% of the erythromycin-resistant transductants had the Exp$^-$ phenotype, indicating that Tn*551* was closely coupled to the *exp* locus. One transductant, designated WA 250, was chosen for further experiments.

In order to ensure that Tn*551* had integrated into a single site of the WA250 chromosome, this strain was analysed by Southern blot hybridization. Genomic DNA was digested with *Eco*RI, and hybridized with a subcloned 1.9 kb *Bgl*II fragment from Tn*551* (Fig. 1). Since Tn*551* contains no *Eco*RI site, only one band hybridizing with this probe would be expected. A single *Eco*RI fragment of approximately 20 kb was demonstrated (Fig. 2) confirming that the Exp$^-$ phenotype of WA250 was due to the integration of Tn*551* at a single site in the chromosome. Southern blot analysis of WA250 DNA was also carried out using internal fragments of the alpha-toxin (*hla*) and protein A (*spa*) genes as probes. Since these probes hybridized with *Eco*RI fragments of approximately 5.5 and 5.1 kb, respectively, it was concluded that the *exp* locus is separate from these genes (data not shown).

Northern blot analysis of WA250 demonstrated that the transcription of protein A and alpha-toxin was in-

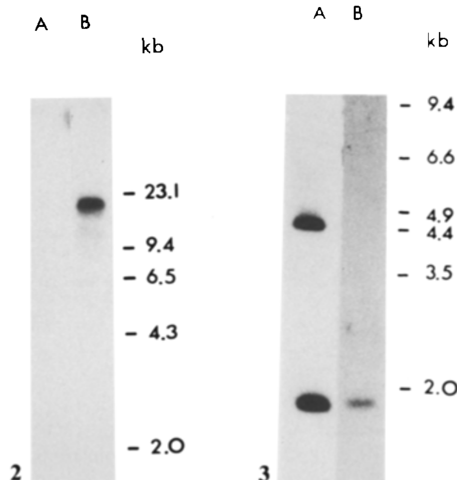


Fig. 2. Southern blot analysis of genomic DNA from A, 8325-4 and B, WA250 digested with *Eco*RI. A subcloned 1.9 kb *Bgl*II fragment inside Tn*551* was used as a probe. After hybridization, filters were washed in 2 × SSC at 65° C

Fig. 3. Southern blot analysis of *Bgl*II-digested WA250 DNA using a subcloned 2.0 kb *Cla*I internal Tn*551* fragment (A), and a 1.9 kb *Bgl*II internal Tn*551* fragment (B), as probes

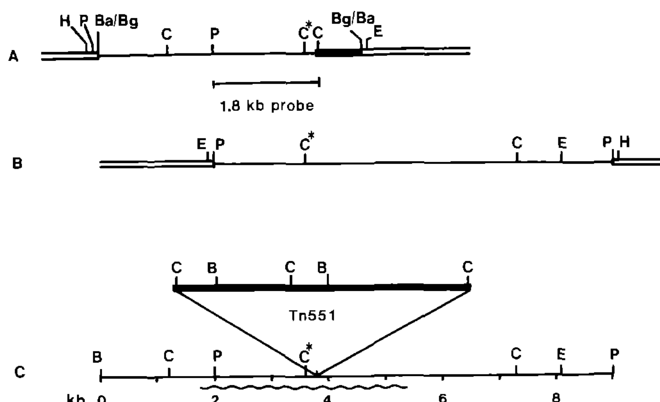


Fig. 4A–C. Restriction map of the DNA cloned in (**A**) pEXO (**B**) pEX4 and (**C**) the combined sequences including Tn*551* as it would appear in WA250. *Single lines* represent chromosomal DNA, *open bars* represent pSP64 and the *filled bars* represent Tn*551* DNA. The *wavy line* indicates the tentative extent of the *exp* gene. Restriction site designations are: Ba, *Bam*HI; Bg, *Bgl*II; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; P *Pst*I; C*, a *Cla*I site which is methylated in *Escherichia coli*

fluenced by the insertional mutation. An increase in protein A mRNA and a decrease in alpha-toxin mRNA was observed (data not shown). This is in accordance with earlier results with a spontaneous *exp* mutant (Janzon et al. 1986).

Molecular cloning of the exp *region*

Attempts to clone the *Eco*RI fragment of the WA250 chromosome containing Tn*551* with both plasmid and phage vectors were unsuccessful. In order to find a smaller DNA fragment containing Tn*551*, or part of it, chromosomal DNA from WA250 was digested with several different restriction enzymes and analysed by Southern blot hybridization using the specific Tn*551* probe. Most useful enzymes lacking sites within Tn*551* turned out to give very large restriction fragments which we failed to clone. For this reason attempts were made to clone a DNA fragment containing only one end of Tn*551* plus the adjacent DNA from the WA250 chromosome.

Digestion of WA250 DNA with *Bgl*II, which has a site at position 770 in Tn*551*, gave two DNA fragments of 4.6 and 1.9 kb, respectively, which hybridized with a subcloned *Cla*I fragment (position 29 to 2050) of Tn*551* (Fig. 3). The 1.9 kb fragment is the internal *Bgl*II fragment of Tn*551* (Fig. 1) while the 4.6 kb fragment consists of 770 bp from Tn*551* plus approximately 3.8 kb of the adjacent chromosomal DNA.

A *Bgl*II digest of WA250 DNA was ligated to *Bam*HI-digested pSP64 and transformed into *E. coli* HB101. Transformants were screened by colony hybridization using a *Cla*I–*Bgl*II fragment from map position 29–770 of Tn*551* as a probe. This probe will not pick up the internal 1.9 kb *Bgl*II fragment of Tn*551*. Several transformants which hybridized with this probe were obtained. Restriction mapping of plasmid DNA from one of the transformants revealed the presence of a 4.5 kb insert, consisting of 770 bases from *Tn551* plus 3,800 bases of chromosomal DNA (Fig. 4A). This plasmid was designated pEXO.

Since pEXO only contains sequences from one side of the insert, additional cloning was probably needed in order to obtain the entire gene. Southern blot analysis of WA250 DNA had revealed a *Pst*I fragment of approximately 12 kb containing Tn*551*. The corresponding *Pst*I fragment in the wild-type strain 8325-4, lacking Tn*551*, should contain the chromosomal sequences from both sides of the insert. The combined sequences of pEXO and the *Pst*I fragment would probably cover the whole *exp* region.

A *Pst*I digest of 8325-4 DNA was ligated to *Pst*I-digested pSP64 and transformed into *E. coli* HB101. Transformants were screened by colony hybridization using a 1.8 kb *Pst*I–*Cla*I fragment of pEXO as a probe (Fig. 4A). A plasmid, pEX4, containing a 7 kb insert which hybridized to the probe was isolated (Fig. 4B). From the combined restriction analysis of pEXO and pEX4, a restriction map of the DNA flanking Tn*551* in WA250 could be constructed (Fig. 4C).

Identification of the RNAs transcribed from the exp *region*

In order to determine the approximate size of the *exp* gene, the RNAs encoded by the cloned DNA were analysed. A Northern blot of the total RNA from the wild-type strains 8325-4 and V8, and the corresponding *exp* mutants, WA250 and K68:12-1, was hybridized with the 1.8 kb *Pst*I–*Cla*I fragment from pEXO. As shown in Fig. 5A, this DNA probe hybridized with one major RNA of approximately 3.5 kb in both wild-type strains. Minor hybridization signals were also demonstrated with RNAs of approximately 2.5 and 1.4 kb. No hybridization was observed with RNA from the *exp* mutants indicating that the Exp^- phenotype is correlated with the reduced levels of these RNAs. The fact that the same RNAs were missing in both the spontaneous *exp* mutant and the insertional mutant also confirmed that Tn*551* had inactivated the *exp* locus and that the cloned DNA was indeed a part of this locus. Whether the smaller RNAs are degradation products of the larger RNA or if they are separate RNAs is not yet known.

To determine the region of DNA spanned by the 3.5 kb RNA, DNA fragments to the left of the 1.8 kb *Pst*I–*Cla*I

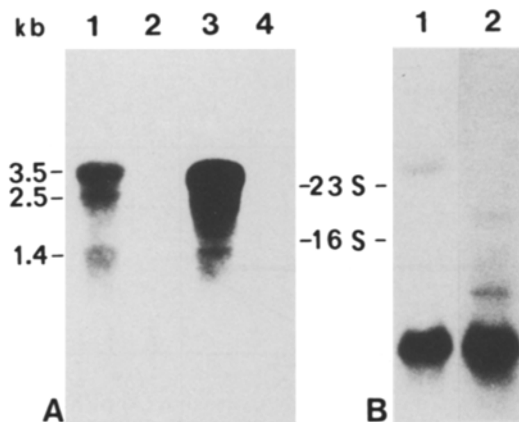


Fig. 5. A Northern blot analysis of whole cell RNA from *Staphylococcus aureus* 8325-4 (lane 1), WA250 (lane 2), V8 (lane 3) and K68:12-1 (lane 4). The 1.8 kb *Pst*I–*Cla*I fragment from pEXO was used as probe. **B** RNA from *S. aureus* 8325-4 was hybridized with the 0.9 kb *Cla*I–*Pst*I fragment (lane 1) and the 1.2 kb *Pst*I–*Cla*I fragment (lane 2) from pEXO. The positions of the ribosomal RNAs, as indicated by *arrows*, were determined by ethidium bromide staining of a parallel strip of the gel

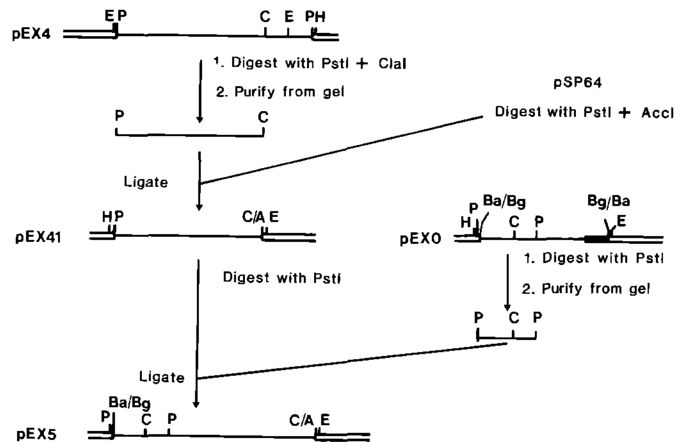


Fig. 6. Schematic presentation of the construction of plasmids pEX41 and pEX5. *Single lines* represent *Staphylococcus aureus* chromosomal DNA, *open bars* pSP64 and *filled bars* Tn*551* DNA. Restriction sites: A, *Acc*I; Ba, BamHI; Bg, BglII; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; P, *Pst*I

fragment of pEXO were used as probes in Northern blot hybridization experiments (Fig. 5B). The 0.9 kb *Cla*I–*Pst*I fragment but not the 1.2 kb *Pst*I–*Cla*I fragment generated a weak hybridization signal with the 3.5 kb RNA indicating that this RNA spans from a site somewhere within the 0.9 kb *Cla*I–*Pst*I fragment of pEXO. This means that the Tn*551* insertion is located almost in the middle of the *exp* gene.

Since the *agr* mutation in strain RN4256 described by Recsei et al. (1986) showed a typical Exp$^-$ phenotype, Northern blot analysis was carried out as described above. The same transcripts as in strains 8325-4 and V8 appeared in the wild-type strain of RN4256 and were missing in the mutant (data not shown). This means that *agr* and *exp* are probably identical.

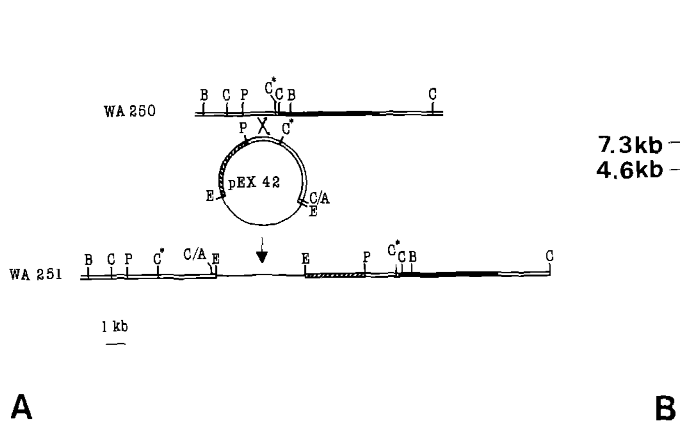
Reversion of the exp *mutation by recombination with the cloned DNA*

On the basis of the Northern blot analysis described above, plasmid pEX5 which should contain the entire *exp* gene was constructed as described in Fig. 6. To enable pEX5 to be transferred to *S. aureus* strains it was inserted in the *Eco*RI site of pRN4102 to yield pEX52, which was isolated in *E. coli* HB101. The restriction-deficient mutant, *S. aureus* SA113, was used as a primary recipient when plasmids from *E. coli* were used to transform *S. aureus*. The transformation frequency was generally very low and varied between 10 and 200 transformants per μg of DNA. pEX52 was transformed into *S. aureus* SA113 yielding about 50 tetracycline-resistant transformants. However, only deleted derivatives of pEX52 were recovered, indicating that a plasmid carrying the entire *exp* gene cannot be maintained in *S. aureus*. This was supported by the fact that plasmid pEX42 which lacks 100–800 bp at the left end of the *exp* gene was stable in *S. aureus* SA113. pEX42 was constructed by inserting pEX41 (Fig. 6) into the *Eco*RI site of pRN4102. pEX42 was isolated from strain SA113 and used to transform the insertional mutant WA250. About 5×10^3 tetracycline-resistant transformants were obtained per μg of DNA. One hundred colonies which were streaked on casein agar plates had a small zone of proteolysis around the streaks, while the recipient strain WA250 was completely negative. Isolation of single colonies from streaks with the largest zones revealed that only about 0.05% of the colonies were proteinase positive giving zones of approximately the same size as the wild-type strain 8325-4. Proteinase-positive colonies had also regained alpha-toxin production and had lost the ability to produce protein A. Furthermore, one isolate, which was tested by Northern blot hybridization with the 1.8 kb *Pst*I–*Cla*I fragment from pEXO as a probe, had regained the ability to produce the 3.5 kb RNA which was absent in WA250 (data not shown), indicating a complete recovery of the Exp$^+$ phenotype. All revertants were still erythromycin resistant indicating the presence of Tn*551*.

These results suggested that the *exp* gene had been restored by recombination between pEX42 and the WA250 chromosome, most likely by "Campbell-like" integration of the plasmid on either side of Tn*551*. To analyse this, chromosomal DNA from WA250 and one of the revertants, designated WA251, was digested by *Bgl*II and *Eco*RI and hybridized with the 0.9 kb *Cla*I–*Pst*I fragment from pEXO. Since this fragment is not present in plasmid pEX42, it will only hybridize with chromosomal fragments. It can be predicted that digestion of WA250 chromosomal DNA with *Bgl*II and *Eco*RI would give a 4.6 kb *Bgl*II fragment hybridizing with the 0.9 kb probe. The same fragment would also appear if pEX42 had integrated to the right of Tn*551*. However, integration to the left of Tn*551* would create a *Bgl*II–*Eco*RI fragment of 7.3 kb as indicated in Fig. 7A. As shown in Fig. 7B, the predicted 7.3 kb *Bgl*II–*Eco*RI fragment was present in WA251 indicating that pEX42 had integrated at the left end of Tn*551*, thereby restoring a complete *exp* gene. These results also confirmed that the *exp* gene must extend into the 0.9 kb *Cla*I–*Pst*I fragment, approximately 1.8 kb to the left of the insertion of Tn*551*.

Discussion

Insertion of the erythromycin-resistant transposon Tn*551* into a single site of the *S. aureus* chromosome resulted in



1 2 3

7.3kb –
4.6kb –

B

Fig. 7. A The integration of pEX42 into the chromosome of *Staphylococcus aureus* strain WA250. Single lines represents pT181, open bars chromosomal DNA, hatched bars pSP64 and filled bars Tn*551* DNA. Restriction sites: A, *Acc*I; B, *Bgl*II; C, *Cla*I; E, *Eco*RI; P, *Pst*I; C*, a *Cla*I site which is methylated in *Escherichia coli*. **B** Southern blot analysis. Chromosomal DNA from *S. aureus* WA250 (lane 1) and WA251 (lane 2) and DNA of plasmid pEX42 (lane 3) was double digested with *Bgl*II and *Eco*RI. The 0.9 kb *Cla*I–*Pst*I fragment from pEXO was used as a probe

a depressed production of several extracellular proteins including alpha-toxin, proteinases and nuclease and a simultaneous increase in the production of protein A and coagulase. The fact that the site of insertion was separate from the protein A gene (*spa*) and the alpha-haemolysin gene (*hla*) suggests that Tn*551* had inactivated a *trans*-active control element, acting both as a repressor and an activator.

Using this insertional mutant, we cloned the chromosomal DNA flanking the transposon and the corresponding DNA from the wild type. This DNA was shown to code for one major RNA of approximately 3.5 kb which was not detected in the mutant strain, indicating that this RNA, or its product(s), is the regulator of exoprotein gene expression. Since Tn*551* was found to be located somewhere between 1800 and 2600 bp from one end of the gene, it seems unlikely that this would interfere with transcription. We therefore suggest that the presence of the transposon in the middle of the gene results in the production of very unstable transcripts.

Spontaneous mutants of *S. aureus* with the same phenotype as that of the insertional mutant have previously been isolated in our laboratory and were designated Exp$^-$ (Björklind and Arvidson 1980; Janzon et al. 1986). Since both kinds of mutants had a reduced amount of the 3.5 kb RNA, it was concluded that the insertional mutation was located in the *exp* gene.

The *agr* mutant, RN4256, described by Recsei et al. (1986), was shown to have a reduced level of the 3.5 kb RNA as compared with the wild-type strain, indicating that *agr* is identical to *exp*. Taking together the present knowledge of *exp* (*agr*) (Björklind and Arvidson 1980; Janzon et al. 1986; Recsei et al. 1986), it can thus be concluded that *exp* probably codes for a *trans*-active element which regulates the expression of at least 12 different exoproteins including alpha-, beta- and delta-toxin, TSST-1, staphylokinase, nuclease, serine proteinase, metallo-proteinase, acid phosphatase, leucocidin (our laboratory, unpublished), protein A and coagulase. In the case of alpha-toxin, TSST-1 and protein A, it has been shown that the regulation is probably at the level of transcription (Janzon et al. 1986, Recsei et al. 1986).

From the results of restriction enzyme mapping and the DNA/RNA hybridization experiments represented in Fig. 5, a preliminary map of the *exp* region was constructed (Fig. 4C). Based on this map, a plasmid (pEX52) was constructed which was considered to contain the entire *exp* gene and which should therefore be able to restore the wild-type properties of different *exp* mutants. Unfortunately, we were not able to establish this plasmid in *S. aureus*. The reason for this could be that overproduction of the gene product, due to the use of a multi-copy plasmid, is toxic to the bacteria. In this respect, it is interesting to note that *agr* (= *exp*) also seems to be involved in the regulation of several intracellular proteins (Recsei et al. 1986).

Another plasmid, pEX42, containing most of the coding sequence of the 3.5 kb RNA, but lacking 100–800 bases from the left of the *exp* gene was however stable in *S. aureus*. This plasmid could reconstitute a functional *exp* gene by integration into the chromosome of the insertional mutant WA250. This result confirms that we had cloned the exoprotein regulating gene *exp*, and that the map of the gene as shown in Fig. 4C can be used as a basis for further studies aiming at the identification of the final gene product and its role in exoprotein regulation.

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References

Abbas-Ali B, Coleman G (1977) the characteristics of extracellular protein secretion by *Staphylococcus aureus* (Wood 46) and their relationships to the regulation of alpha-toxin. J Gen Microbiol 92:277–282

Barnes WM (1977) Plasmid detection and sizing in single colony lysates. Science 195:393–394

Berger-Bäche B (1983) Insertional inactivation of staphylococcal methicillin resistance by Tn*551*. J Bacteriol 154:479–487

Björklind A, Arvidson S (1980) Mutants of *Staphylococcus aureus* affected in the regulation of exoprotein synthesis. FEMS Microbiol Lett 7:203–206

Bolivar F, Rodriges RL, Grene PY, Betlach MC, Heynecker HL, Boyer HW, Crosa YH, Falkow S (1977) Construction and characterization of new cloning vehicles, II. A multipurpose cloning system. Gene 2:95–113

Botchan M, Topo W, Sambrook J (1976) The arrangement of Simian virus 40 sequences in the DNA of transformed cells. Cell 9:269–287

Boyer HW, Roulland-Dussoix D (1969) A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J Mol Biol 41:459–472

Coleman G (1981) Pleiotropic compensation in the regulation of extracellular protein formation by a low alpha-toxin-producing variant of *Staphylococcus aureus* (Wood 46). J Gen Microbiol 122:11–15

Coleman G, Abbas-Ali B (1977) Comparison of the patterns of increase in alpha-toxin and total extracellular protein by *Staphylococcus aureus* (Wood 46) grown in media supporting widely differing growth characteristics. Infect Immun 17:278–281

Grunstein M, Hogness DS (1975) Colony hybridization: A method for the isolation of cloned DNAs that contain a specific gene. Proc Natl Acad Sci USA 72:3961–3965

Hattori M, Sakaki Y (1986) Dideoxy sequencing method using denatured plasmid templates. Anal Biochem 152:232–238

Iordanescu S (1975) Recombinant plasmid obtained from two different compatible staphylococcal plasmids. J Bacteriol 124:597–601

Janzon L, Löfdahl S, Arvidson S (1986) Evidence for a coordinate transcriptional control of alpha-toxin and protein A in *Staphylococcus aureus*. FEMS Microbiol Lett 33:193–198

Khan SA, Novick RP (1980) Terminal nucleotide sequences of Tn*551*, a transposon specifying erythromycin resistance in *Staphylococcus aureus*: Homology with Tn*3*. Plasmid 4:148–154

Kehoe M, Duncan J, Foster T, Fairweather N, Dougan G (1983) Cloning, expression and mapping of the *Staphylococcus aureus* α-hemolysin determinant in *Escherichia coli* K-12. Infect Immun 41:1105–1111

Lindberg M (1981) Genetic studies in *Staphylococcus aureus* using protoplasts: Cell fusion and transformation. In: Jeljaszewicz J (ed) Staphylococci and staphylococcal infections. Gustav Fischer, Stuttgart, pp 534–541

Löfdahl S, Guss B, Uhlén M, Philipson L, Lindberg M (1983) Gene for staphylococcal protein A. Proc Natl Acad Sci USA 80:697–701

Meinkoth J, Wahl G (1984) Hybridization of nucleic acids immobilized on solid supports. Anal Biochem 138:267–284

Melton DA, Krieg J, Rebagliati MR, Maniatis T, Zinn K, Green M (1984) Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res 12:7035–7056

Novick RP (1967) Properties of a cryptic high frequency transducing phage in *Staphylococcus aureus*. Virology 33:155–166

Novick RP (1974) Studies on plasmid replication, III. Isolation and characterisation of replication defective mutants. Mol Gen Genet 135:131–147

Novick RP, Murphy E, Gryczan TJ, Baron E, Edelman I (1979a) Penicillinase plasmids of *Staphylococcus aureus*: Restriction deletion maps. Plasmid 2:109–129

Novick RP, Edelman I, Schweisinger MD, Gruss AD, Swanson EC, Pattee PA (1979b) Genetic translocation in *Staphylococcus aureus*. Proc Natl Acad Sci USA 76:400–404

Pattee PA (1981) Distribution of Tn*551* insertion sites responsible for auxotrophy on the *Staphylococcus aureus* chromosome. J Bacteriol 145:479–488

Recsei P, Kreiswirth B, O'Reilly M, Schlivert R, Gruss A, Novick RP (1986) Regulation of exoprotein gene expression in *Staphylococcus aureus* by *agr*. Mol Gen Genet 202:58–61

Smith JM (1979) *Staphylococcus aureus*. In: Mandell GL, Douglas R, Bennet J (eds) Principles and practice of infectious diseases. Wiley and Son, New York, pp 1530–1552

Yoshikawa M, Matsudo F, Naka M, Murofuski E, Tsunematsu Y (1974) Pleiotropic alteration of activities of several toxins and enzymes in mutants of *Staphylococcus aureus*. J Bacteriol 119:143–148

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