JOURNAL OF BACTERIOLOGY, July 1993, p. 4436–4447
0021-9193/93/144436-12$02.00/0

Vol. 175, No. 14

DNA Sequence and Units of Transcription of the Conjugative Transfer Gene Complex (*trs*) of *Staphylococcus aureus* Plasmid pGO1

TERESA M. MORTON,[1] DENISE M. EATON,[1] J. LINDA JOHNSTON,[2] AND GORDON L. ARCHER[1,2]*

Departments of Microbiology and Immunology[1] and Medicine,[2] Medical College of Virginia/Virginia Commonwealth University, Richmond, Virginia 23298-0049

Received 11 March 1993/Accepted 13 May 1993

The conjugative transfer genes of 52-kb staphylococcal R plasmid pGO1 were localized to a single *Bgl*II restriction fragment and cloned in *Escherichia coli*. Sequence analysis of the 13,612-base transfer region, designated *trs*, identified 14 intact open reading frames (ORFs), 13 of which were transcribed in the same direction. Each ORF identified was preceded by a typical staphylococcal ribosomal binding sequence, and 10 of the 14 proteins predicted to be encoded by these ORFs were seen when an *E. coli* in vitro transcription-translation system was used. Functional transcription units were identified in a *Staphylococcus aureus* host by complementation of Tn*917* inserts that abolished transfer and by Northern (RNA) blot analysis of pGO1 mRNA transcripts. These studies identified three complementation groups (*trsA* through *trsC*, *trsD* through *trsK*, and *trsL-trsM*) and four mRNA transcripts (*trsA* through *trsC* [1.8 kb], *trsA-trsB* [1.3 kb], *trsL-trsM* [1.5 kb], and *trsN* [400 bases]). No definite mRNA transcript was seen for the largest complementation group, *trsD* through *trsK* (10 kb). Comparison of predicted *trs*-encoded amino acid sequences to those in the data base showed 20% identity of *trsK* to three related genes necessary for conjugative transfer of plasmids in gram-negative species and 32% identity of *trsC* to a gene required for conjugative mobilization of plasmid pC221 from staphylococci.

Conjugative plasmids were first found in staphylococci in 1979 in association with outbreaks of gentamicin-resistant staphylococcal infections in hospitals in the United States (3, 11, 17). They were found in both coagulase-positive and coagulase-negative staphylococci and were shown to mediate interspecies transfer of gentamicin resistance (3, 11, 28). The widespread dissemination in U.S. hospitals of staphylococci carrying conjugative plasmids paralleled a dramatic increase in multiresistant staphylococci that caused serious nosocomial infections (4, 38).

Staphylococcal conjugative plasmids are large, range in size from 40 to 60 kb, and transfer at a low frequency (10^4 to 10^6 transconjugants per donor) (3, 11). Transfer does not occur in broth but requires a solid substrate, such as nitrocellulose (3, 11). All of the genes necessary for transfer are plasmid encoded (43), as are a variety of antimicrobial resistance genes. Genes that encode resistance to aminoglycosides, ethidium bromide, quaternary ammonium compounds, trimethoprim, and penicillin have all been found on conjugative plasmids in staphylococci (1, 4, 13, 14). Conjugative plasmids are also capable of mobilizing smaller, nonconjugative plasmids carrying resistance to chloramphenicol and tetracycline (11). The 52-kb conjugative plasmid used in this study, pGO1, encodes resistance to aminoglycosides, trimethoprim, and quaternary ammonium compounds and is virtually identical to other conjugative staphylococcal plasmids isolated in the United States and other countries (2, 14). Therefore, this plasmid serves as a model for study of the genetic organization of the transfer region of staphylococcal conjugative plasmids.

The region of pGO1 containing the transfer genes has been previously identified by transposon insertional inactivation and subcloning (43). To determine the genetic organization and, ultimately, the function of individual genes in the transfer region of pGO1, we sequenced the 13,612-base transfer region (designated *trs*, for transfer, staphylococci), determined the number of open reading frames (ORFs) encoded by this region, and identified functional transcription units. These data constitute the first sequence and transcriptional analysis of an entire gram-positive conjugative transfer region and should provide a basis for comparison to other well-identified transfer systems.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *Staphylococcus aureus* and *Escherichia coli* strains and plasmids used in this study are listed in Table 1. Recombinant plasmids were generated in *E. coli* HB101 or XL-1 Blue (8, 9). Recombinant plasmids constructed in *E. coli* were given pGO designations. When pSK265 carrying chloramphenicol resistance was added as a staphylococcal replicon to create a shuttle plasmid, the letter C followed the pGO designation.

Materials and media. Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) was used for culture of both *E. coli* and *S. aureus* strains. Lennox L base (GIBCO-Bethesda Research Laboratories, Inc. [BRL], Gaithersburg, Md.) broth supplemented with 0.2% glucose was used exclusively for culture of *E. coli* strains, while brain heart infusion (BHI) broth (Difco, Detroit, Mich.) or Trypticase soy broth (BBL) was used exclusively for culture of *S. aureus* strains. The antibiotic concentrations used were as follows: chloramphenicol at 10 μg/ml for initial selection of *S. aureus* clones after electroporation and transduction and at 40 μg/ml for shuttle construction in *E. coli* and maintenance of *S. aureus* shuttle plasmids; gentamicin at 5 μg/ml and erythromycin at 10 μg/ml for initial selection of *S. aureus* transductants; erythromycin at 20 μg/ml for plasmid maintenance; novobiocin and rifampin each at 10 μg/ml for

* Corresponding author.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Chromosomal genotype or phenotype[a]	Remarks and source or reference
E. coli strains		
HB101	F^- *hsdS20* (r_B^- m_B^-) *recA13 ara14 proA2 lacY1 galK2 rspL20 xyl-15 mtl-1 supE44* λ^-	Recombination-defective host; 8
XL-1 Blue	*recA*$^-$ (*recA1 lac endA1 gyrA96 thi hsdR17 supE44 relA1* [F' *proAB lac-1 lac*ΔM15 Tn*10*])	Allows blue-white color selection for pBluescriptIIKS(+) plasmids; 9
S. aureus strains		
RN450	ATCC 8325-4; 8325-1 cured of Φ11, Φ12, Φ13	
RN4220	Restriction-deficient derivative of 8325-4	Host for shuttle plasmids; 21
GO57	Novr Rif^r	Recipient strain (RN4220) for conjugation; 13
RN1030	*recA*	Recombination-defective Φ11 lysogen; 30
Plasmids		
pOP203(A2$^+$)	Tc^r, 7.0 kb	Positive-selection cloning vector; 51
pBR322	Ap^r Tc^r, 4.3 kb	Cloning vector; 7
pBluescriptII	Ap^r, single-stranded DNA replication of origin, 2.9 kb	KS(+) cloning vector; 9
pUC19	Ap^r, *lacZ*, 2.7 kb	Cloning vector (GIBCO-BRL)
pSK265	Cm^r, 3.0 kb	pC194 derivative with pUC19 multiple cloning site; 35
pRN6703		Staphylococcal protein A gene probe (*spa*); 45
pGO1	Gm^r Tp^r Qam^r Trs$^+$, 52 kb	Conjugative plasmid from *S. aureus* clinical isolate from Medical College of Virginia; 1
pGO1-2P	pGO1::Tn*917-lac*(*trsI*); oriented in direction of *lacZ* transcription	This study
pGO1-43	pGO1::Tn*917-lac*(*trsK*); oriented in direction of *lacZ* transcription	This study
pGO1-230	pGO1::Tn*917-lac*(*trsC*); oriented in direction of *lacZ* transcription	This study
pGO1-83	pGO1::Tn*917*(*trsB*)	This study
pGO1-106	pGO1::Tn*917*(*trsD*)	This study
pGO1-94	pGO1::Tn*917*(*trsE*)	This study
pGO1-15	pGO1::Tn*917*(*trsF*)	This study
pGO1-86	pGO1::Tn*917*(*trsF*)	This study
pGO1-96	pGO1::Tn*917*(*trsL*)	This study
pGO53	Tc^r, 21.5 kb	14.4-kb *Bgl*II B fragment of pGO1 subcloned into *Bgl*II site of pOP203(A2$^+$); 43
pGO137	Ap^r, 6.9 kb	Gentamicin resistance gene probe; 12
pGO200	Ap^r, 18.7 kb	14.4-kb *Bgl*II B fragment of pGO1 subcloned into *Bam*HI site of pBR322; 43
pGO202	Ap^r, 10.1 kb	7.0-kb *Bgl*II-*Ava*I subclone of pGO200 produced by *Ava*I digestion of pGO200 and religation; 43
pGO203	Ap^r Tc^r, 13.6 kb	9.3-kb *Hin*dII-*Bgl*II subclone of pGO200 produced by *Hin*dIII digestion of pGO200 and religation; 43
pGO200Δ1	Ap^r, 12.4 kb	Deletion derivative of pGO200; 43
pGO210	Ap^r Tc^r, 8.7 kb	4.4-kb *Hin*dIII E fragment of pGO1 cloned onto pBR322; 43
pGO171	Ap^r, 9.1 kb	6.2-kb *Eco*RI C fragment of pGO1 subcloned into *Eco*RI site of pBluescriptIIKS(+); this study
pGO179	Ap^r, 5.3 kb	2.4-kb *Eco*RI-*Hin*cII of pGO200 subcloned into *Eco*RI-*Hin*cII site of pBluescriptIIKS(+); this study
pGO187	Ap^r, 3.7 kb	766-base *Bgl*II-*Eco*RI fragment of pGO53 subcloned into *Bam*HI-*Eco*RI site of pBluescriptIIKS(+); this study
pGO191	Ap^r, 5.1 kb	2.2-kb *Eco*RI-*Hin*dIII fragment of pGO200 subcloned into *Eco*RI-*Hin*dIII site of pBluescriptIIKS(+); this study
pGO311	Ap^r, 6.4 kb	3.7-kb *Eco*RI-*Sph*I fragment of pGO200 subcloned into *Eco*RI-*Sph*I site of pUC19; this study
pGO324	Ap^r, 8.1 kb	5.2-kb *Nco*I-*Sma*I subclone of pGO171 produced by *Nco*I-*Sma*I digestion of pGO171, treatment with Klenow fragment, and blunt-end ligation; this study
pGO325	Ap^r, 7.9 kb	5.0-kb *Acc*I-*Sma*I subclone of pGO171 produced by *Acc*I-*Sma*I digestion of pGO171, treatment with Klenow fragment, and blunt-end ligation; this study
pGO338	Ap^r, 7.4 kb	4.7-kb *Hph*I-*Eco*RI fragment of pGO171 blunt ended by treatment with mung bean nuclease and ligated into *Sma*I site of pUC19; this study
pGO349	Ap^r, 7.7 kb	3.4-kb *Hin*dIII-*Sph*I fragment of pGO210 subcloned into *Hin*dIII-*Sph*I site of pBR322; this study

[a] Abbreviations: Ap, ampicillin; Tc, tetracycline; Nov, novobiocin; Rif, rifampin; Cm, chloramphenicol; Gm, gentamicin; Tp, trimethoprim; Qam, quaternary ammonium compounds; Trs, staphylococcal conjugative transfer.

selection of plasmids transferred between *S. aureus* pairs by conjugation; ampicillin at 50 μg/ml for selection of *E. coli* HB101, XL-1 Blue, and TB1 clones; tetracycline at 12.5 μg/ml plus ampicillin for selection of all XL-1 Blue clones. The pBluescriptIIKS(+) cloning vector and the XL1-Blue host strain were from Stratagene (La Jolla, Calif.), and the pUC19 cloning vector was from New England BioLabs (Beverly, Mass.). All chemicals and antibiotics were from Sigma Chemical Co. (St. Louis, Mo.). Restriction endonucleases and other enzymes involved in DNA manipulations were obtained from BRL. Agarose and acrylamide were from International Biotechnologies Inc. (New Haven, Conn.). DNA sequencing was done with Sequenase Version 2.0 (United States Biochemical, Cleveland, Ohio) and [α-^{35}S]dATP or [α-^{33}P]dATP (DuPont, NEN Research Products, Boston, Mass.). DNA probes for Northern (RNA) analysis were generated by nick translation with [α-^{32}P] dCTP and a kit from DuPont, NEN. Vent$_R$ polymerase for polymerase chain reactions (PCRs) was from New England BioLabs.

Cloning, transformation, and DNA manipulation. All restriction endonuclease digestions, ligations, Klenow reactions, and electrophoresis were performed as recommended by the manufacturer. Transformation of recombinant plasmids into *E. coli* was done by electroporation with a Gene Pulser (Bio-Rad, Richmond, Calif.) set at 200 Ω, 25 μF, and 2.5 kV. For transformation into *S. aureus*, the Gene Pulser was set at 400 Ω, 25 μF, and 2.3 kV. *E. coli* cells were prepared in accordance with the manufacturer's specifications prior to electroporation; *S. aureus* cells were prepared in accordance with the method of Schenk and Laddaga (39). Staphylococcal plasmids were isolated for small-scale restriction digestion by the cetyltrimethylammonium bromide extraction method of Townsend et al. (44). The alkaline minilysis technique described by Sambrook et al. (37) was used to isolate *E. coli* plasmid DNA for restriction digestion and subcloning into *S. aureus*. Plasmid DNA was purified from *E. coli* for sequence analysis by the Midi-prep procedure (Qiagen, Chatsworth, Calif.).

Sequence analysis. Sequence analysis was performed by the Sanger dideoxy-chain termination method with double-stranded DNA as the template. Sequence reactions were run in accordance with the manufacturer's specifications for sequencing of double-stranded DNA with Sequenase 2.0, with the following exceptions: DNA was denatured in 0.2 M NaOH–0.2 mM EDTA for 5 min; annealing reactions were done at 37°C for 20 min; termination reactions were done at 40°C for 5 min; and samples were heated at 95°C for 2 min prior to being loaded onto gels. A 2.5-μl portion of each reaction mixture was loaded for 2-, 4-, and 6-h runs on a gel (14 by 30 cm) with an International Biotechnologies gel apparatus and a BRL model 4000 power supply at 87 V. Both strands of the 13.6-kb *trs* region were sequenced by subcloning of fragments (see Fig. 3) and by using sequentially synthesized oligomeric DNA primers to progress along cloned DNA. Sequences across restriction sites used for subcloning were confirmed on the intact *Bgl*II-B clone (pGO200). The *trs* DNA sequence was analyzed by the Genetics Computer Group (GCG; Madison, Wis.) program for potential ORFs and specific motifs.

PCR generation of *trs* fragments. PCR was used to locate sites of Tn*917* and Tn*917lac* insertions more precisely than was possible by restriction mapping, and it was also used to generate DNA probes for Northern analysis of RNA transcripts. For transposon mapping, we synthesized oligonucleotide primers that were complementary to either end of Tn*917*, on the basis of the published sequence (41), and to a nearby *trs* sequence. Oligonucleotides were chosen to generate fragments between 200 and 600 bp long. The size of the fragment generated was estimated by gel electrophoresis with comparison to a 100-bp ladder (BRL). Sequence analysis of several of the transposon-*trs* junctions confirmed that estimation of PCR fragment size located transposon insertions with an error of no greater than 50 bp.

PCR generation of DNA probes for Northern analysis used oligonucleotide primers complementary to sequences of ORFs as determined by *trs* sequence analysis. Twenty-eight cycles of PCR amplification were completed with Vent$_R$ polymerase (New England BioLabs). Annealing reactions were done at 5°C below the predicted melting temperature of the primers, and their template and extension reactions were done at 5°C above the melting temperature.

***E. coli* S30 coupled transcription-translation studies.** Clones of *trs* were analyzed for protein production by using an *E. coli* S30 coupled transcription-translation system (S30 extract minus methionine [Promega, Madison, Wis.]). Protein products were labelled with [α-^{35}S]methionine (Dupont, NEN) in accordance with the manufacturer's specifications, with the following modifications. The incubation period for all reactions was increased to 2 h. To maximize protein production, 15 μg of DNA was used in each reaction with a cloned *trs* plasmid (pGO200, pGO203C, or pGO210) and 3.5 μg of DNA was used with vector plasmid. Reaction volumes of 50 μl were precipitated with 200 μl of cold acetone and pelleted as described by the manufacturer. The protein pellet was suspended in 90 μl of sample buffer (0.0625 M Tris-HCl [pH 6.8], 2% sodium dodecyl sulfate, 10% glycerol, 0.002% bromophenol blue), 10 μl of 2-mercaptoethanol was added, samples were boiled for 2 min, and 50 μl of each sample was loaded for separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (15% resolving and 5% stacking gel) as described by Laemmli (22). The gel was dried as recommended by the manufacturer, and labelled proteins were visualized by autoradiography with overnight exposure to Kodak X-OMAT AR film at −70°C.

Northern analysis. Total cellular RNA was isolated from *S. aureus* pGO1/4220 as follows. A 150-ml volume of fresh BHI broth was added to a 250-ml overnight culture and incubated at 37°C with shaking for 1.5 h. Cells were pelleted by centrifugation at 10,000 × *g* for 10 min. The cell pellet was suspended in 5 ml of lysis buffer (20% sucrose, 20 mM Tris [pH 7.6], 10 mM EDTA, 50 mM NaCl), 1 ml of 1.5-mg/ml lysostaphin was added, and the mixture was incubated on ice for 1 h as described by Kornblum et al. (19). The lysate mixture was then centrifuged at 12,000 × *g* for 10 min at 4°C, the supernatant was discarded, and the pellet was thoroughly suspended by vortexing in 20 ml of RNAzol B (Cinna/Biotecx Laboratories, Houston, Tex.). RNAzol B is a cocktail of guanidium thiocyanate and phenol formulated specifically for isolation of RNA. The isolation procedure was done as described by the manufacturer. Briefly, this consisted of extraction of RNA from the bacterial lysate with RNAzol and 0.1 volume of chloroform, followed by precipitation in 1 volume of isopropanol and washing with 75% ethanol. The concentration of RNA was determined by measuring optical density at 260 and 280 nm. Samples were run on a 1.2% agarose minigel, stained with 1-μg/ml ethidium bromide, and examined by UV light for the presence of two distinct rRNA bands. All laboratory ware was baked at 80°C overnight, and all water and buffers were treated with diethyl pyrocarbonate to eliminate potential RNase contamination.

Northern blot procedures were done under the conditions

described by Kornblum et al. (19). Briefly, RNA was separated by electrophoresis in 1.2% agarose containing formaldehyde and transferred to nitrocellulose overnight in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Nitrocellulose was baked for 2 h in a vacuum oven and prehybridized in Denhardt's solution for 4 h at 58°C. Blots were then hybridized overnight at 58°C with Denhardt's solution containing the appropriate [α-^{32}P]dCTP-labelled probes, washed and exposed to X-ray film at −70°C for a time appropriate for optimum visualization of signals. Radiolabelled DNA probes were generated by nick translation of the PCR products of *trsA*, *trsB*, *trsC*, *trsD*, *trsH*, *trsI*, *trsJ*, *trsL*, *trsM*, and *trsN*. The clone pGO187, containing the 766-bp *Eco*RI-*Bgl*II fragment of *trs* that included *trsN*, the intervening promoter sequences between *trsN* and *trsA*, the first 244 bases of *trsA*, and the vector, was also used as a probe. Transcript sizes were determined for each *trs* probe by comparison to RNA ladders (high- and low-molecular-weight RNA markers from BRL).

Total cellular mRNA from mating mixtures of pGO1/4220 (donor) and 4220 (recipient) was also analyzed by Northern blot analysis to determine any induction of *trs* genes during the mating process. Briefly, after 4 h of filter mating, the bacteria were swabbed off the filter membrane, suspended in 1 ml of saline in a 1.5-ml Eppendorf Microfuge tube, and pelleted by centrifugation. RNA was isolated as described above by using 1/20 volumes of solutions.

Filter mating. Overnight BHI agar cultures of donor and recipient strains were resuspended in saline to a no. 3 McFarland standard, mixed in a 1:1 ratio (2 ml of donor to 2 ml of recipient), and forced through a syringe onto a 25-mm-diameter, 0.45-μm-pore-size nitrocellulose filter. The filter was placed on BHI agar, bacterium side up, and incubated at 37°C overnight. Cells were vortexed off filters in 1 ml of saline and plated on Mueller-Hinton agar containing appropriate antibiotics with selection for donors, recipients, and transconjugates. For each mating involving complementation studies, pGO1 and transposon-inactivated pGO1 derivatives were run as positive and negative controls, respectively.

Transposon insertion mutagenesis. Tn*917* insertions were generated in restriction-deficient strain RN1030 and were described in a previous publication (43). β-Galactosidase fusions were generated for this study by using Tn*917lac* as follows. The delivery vehicle for Tn*917lac* was plasmid pTV32ts, a temperature-sensitive replicon designed for creation of β-galactosidase fusions in *Bacillus subtilis* (52), which encoded resistance to chloramphenicol on the delivery vehicle and resistance to erythromycin on the transposon. The delivery plasmid was introduced into *S. aureus* RN450 by protoplast transformation by using previously described techniques (43), and pGO1 was introduced into the same strain by conjugation. The strain containing both plasmids was inoculated into BHI broth and grown overnight at the permissive temperature for pTV32ts replication (30°C). The strain was then taken through two cycles of 10:1 dilution and then incubated for 18 h in Mueller-Hinton broth plus 20 μg of erythromycin per ml at the nonpermissive temperature for replication (42°C). The bacteria were plated on Mueller-Hinton agar with erythromycin at a dilution that yielded single colonies, and individual colonies were then picked to agar containing chloramphenicol. Erythromycin-resistant, chloramphenicol-susceptible isolates were screened for the inability to transfer gentamicin (pGO1) and erythromycin (Tn*917lac*) into recipients, indicating strains with a transposon insert that inactivates conjugative transfer. The screening technique involved inoculation of 0.1 ml of donor and recipient colonies directly onto filters with a micropipet as previously described (43). Those yielding no transconjugants by this technique were confirmed as transfer inactivated by forcing donors and recipients through filters with a syringe as described above. Approximately 5 to 10% of erythromycin-resistant, chloramphenicol-susceptible colonies were inactivated for conjugative transfer. Tn*917lac* fusions oriented in the direction of transcription were localized to *trsI*, *trsK*, and *trsL* by PCR as described above.

Transduction and complementation studies. *S. aureus* transducing phage Φ11 was used to lyse strain 4220 containing pGO203C pGO171C, pGO324C, pGO325C, pGO311C, pGO338C, and pGO349C (see Fig. 3). Phage lysates were then used to infect *S. aureus* RN1030 (recombination deficient Φ11 lysogen) containing pGO1 with a Tn*917* insert that had inactivated its ability to transfer. All Tn*917* inserts had been generated in the RN1030 strain to facilitate genetic complementation without recombination (43). However, Tn*917lac* insertions had been generated in nonlysogenized, recombination-proficient strain RN450. To transduce plasmids into RN450, phage lysates were mixed with bacteria in a medium containing 8 mM sodium citrate as described by Kasatiya and Baldwin (18). Cells were selected on chloramphenicol and erythromycin as previously described (43), and appropriate transductants were confirmed by cetyltrimethylammonium bromide lysis and electrophoresis.

Nucleotide sequence accession number. The nucleotide sequence reported here (*trsA* through *trsN*) has been submitted to GenBank with accession number L11998.

RESULTS

Sequence analysis. The 13,612-base sequence of *S. aureus trs* is shown in Fig. 1. Some of the sequence of the insertion-like element that is directly repeated at each end of *trs* is included. The true sequence of *trs* begins and ends with the first base from the end of this element (43). The sequence of IS*431*/*257* has been determined at multiple different insertion sites, thus defining its ends (6, 10). Fourteen intact ORFs were identified by GCG program analysis. These ORFs, designated *trsA* through *trsN* (shown schematically in Fig. 2), range in size from 189 to 2,019 bp. *trsA* to *trsM* have the same direction of transcription, while *trsN* is transcribed from the opposite strand. There is one partial ORF at the 3′ end of the predominant coding strand, just beyond *trsM*, that extends for 150 bp before it is interrupted by the insertion element. Since it is unlikely to have a role in transfer, this ORF was not given a *trs* designation but is called ORF O′ and will not be considered further in this report. A summary of *trs* ORFs is shown in Table 2. All ORFs had near-consensus ribosomal binding sites (AGGAGGA, between −8 and −12 from the translational start site), and four, *trsA*, *trsD*, *trsL*, and *trsN*, had near-consensus promoter regions (TATAAT at −10 and TTGACA at −35) as predicted by the GCG PATTERN.DAT and SEARCH programs. These near-consensus ribosomal binding sites and promoter regions are similar to those previously identified for other staphylococcal genes (31). The putative promoter regions of *trsA* and *trsL* are strikingly similar, as shown in Fig. 3. The −35 regions of these predicted promoters also contain a five-base sequence, ACATG, that is directly repeated three times in succession. Similar five-base sequences are repeated three times from bases 414 to 428 on the same strand as *trsA* and from bases 429 to 414 on the opposite strand. The first triplet of five-base repeats has a T in place of the first A in the final

repeat, and the triplet on the opposite strand has the first and third A's replaced by T's. The triplet repeats from bases 414 to 428 are in position to provide another −35 region and, together with a near-consensus −10 sequence at base 450, provide a possible tandem promoter for *trsA*. The triplet repeats on the opposite strand overlap those of the −35 sequence for the *trsA* tandem promoter and provide the predicted −35 sequence for the *trsN* promoter.

Most of the predicted *trs* ORFs begin with an ATG translational start codon, except for two, *trsA*, which begins with TTG, and *trsJ*, which begins with GTG, both of which are known to be functional in *S. aureus* (31). The start codons for *trsD* and *trsK* overlap the end of the preceding ORF. The remaining *trs* ORFs have an average of 14 bases between the preceding stop codon and the following start codon, with two exceptions. *trsB* and *trsC* have 59 bases between them, and *trsK* and *trsL* have 75 bases between them. Sequence analysis of the regions between *trsB* and *trsC*, between *trsK* and *trsL*, preceding *trsA*, and following *trsM* identified sequences with dyad symmetry. The regions between *trsB* and *trsC* and following *trsM* were predicted by the GCG TERMINATOR search program to be rho-independent terminators, but the sequences showing dyad symmetry between *trsK* and *trsL* and 5′ to *trsA* were not. The palindromes 5′ to *trsA* and *trsL* are perfect 8- and 17-base inverted repeats, respectively. In addition, the entire palindrome 5′ to *trsA*, including the intervening six bases between the repeats, is identical to the internal eight bases and the intervening sequence of the palindrome 5′ to *trsL*, as shown in Fig. 3. No other terminator or repeated sequences were identified in *trs*.

***E. coli* S30 coupled transcription-translation studies.** By using various subclones of *trs*, 10 of the 14 predicted *trs*-encoded proteins were visualized by autoradiography. By comparing the labelled proteins in each lane to the vector control lane, *trs*-encoded proteins were identified, as shown in Fig. 4. Visualized proteins were approximately the predicted sizes for *trsA* (38 kDa), *trsB* (12 kDa), *trsC* (16 kDa), *trsD* (28 kDa), *trsE* (78 kDa), *trsF* (50 kDa), *trsG* (38 kDa), *trsL* (34 kDa), and *trsM* (16 kDa). *trsA* and *trsG* were not visualized as separate bands, since they are of the same approximate molecular weight. The protein product of *trsN* was also labelled and visualized with this system by using either pGO187 or the PCR product of *trsN* with its promoter (40) but is not shown in Fig. 4. The band identified as *trsL* in lane D appears to migrate to the same place in the gel as a vector protein in lane A. However, we believe that the band in lane A is the Tet protein that migrates at a molecular weight of 36,000 (15). This band should be missing in lanes B, C, and D because the *tet* gene was interrupted by cloning into the *Hin*dIII and *Bam*HI sites of the vector. Proteins corresponding to the predicted sizes of *trsH*, *trsI*, *trsJ*, and *trsK* were not clearly demonstrated, but a faint band corresponding to *trsH* may have been present. The predominate labelled proteins for vector pBR322 DNA (31.5 and 114 kDa) correspond to the predicted sizes of β-lactamase and its precursor form, respectively. The difference in the numbers of vector-encoded proteins seen in lane A with the vector alone versus the lanes containing clones is not clear, but the patterns shown were reproducible following multiple replications of the assay.

Complementation studies. Tn*917* or Tn*917lac* inserts into *trs* that abolished transfer were precisely mapped by PCR, and inserts into specific ORFs were then targeted for complementation studies to determine transcriptional organization within *trs*. As shown in Fig. 5, transposon inserts were localized to ORFs B (pGO1-83), C (pGO1-230), D (pGO1-106), E (pGO1-94), F (pGO1-15 and pGO1-86), I (pGO1-2P), K (pGO1-43), and L (pGO1-96). Various subclones of *trs* that contained successive deletions from the right end (beginning with *trsA*) were used for these experiments. These subclones are also shown in Fig. 5. At least 200 transconjugants were routinely seen if complementation of transfer genes was successful. If fewer than 10 transconjugantes were detected, the transposon insert was not considered to be fully complemented. Vector readthrough was felt to account for these results, since cloning of the fragment in the opposite orientation abolished complementation.

pGO203C, containing the entire region upstream of *trsA*, *trsA* to *trsH*, and part of *trsI*, was able to complement inserts into *trsB*, *trsC*, *trsD*, *trsE*, and *trsF*, and plasmid transfer occurred at frequencies seen for pGO1 (10^{-5} to 10^{-7}). pGO171C, however, with a deletion of the upstream region and translational start site of *trsA*, was unable to complement inserts into *trsB* and *trsC* but was able to complement inserts into *trsD*, *trsE*, and *trsF*. pGO324C, with a deletion of *trsA* and all of *trsB*, except for the last 25 bases, and pGO325C, with a deletion of *trsA*, *trsB*, and 90 bases of *trsC*, were both also able to complement inserts into *trsD*, *trsE*, and *trsF*. However, pGO338C, with a deletion of *trsA*, *trsB*, *trsC*, and the translational start site of *trsD*, was unable to complement any downstream inserts. pGO210C, containing the 3′ terminus of *trsI* and all of *trsJ*, *trsK*, *trsL*, and *trsM*, was able to complement inserts into *trsL* but not *trsK* or the 3′ terminus of *trsI*. pGO349C was able to complement an insert into *trsL* but not an insert into *trsK*. This subclone contained the left end of *trs* from the *Sph*I site, deleting the 5′ 240 bases of *trsK* but containing intact *trsL* and *trsM* and all sequences between *trsK* and *trsL*. Finally, pGO311, containing intact *trsH* and *trsI*, was unable to complement an insert into *trsI*. A summary and diagram of the results of the complementation studies are shown in Fig. 5. On the basis of these data, we concluded that three transcription units were identified by available transposon inserts. One contained *trsA* through *trsC*, another contained *trsD* through *trsK*, and a final one contained *trsL* and probably *trsM*, although there were no inserts into *trsM* to complement.

Northern analysis. Two probes were used as controls for Northern analysis, a probe for the gene that encodes gentamicin resistance (*aac-aph*) and a probe for the gene that encodes staphylococcal protein A (45). The gentamicin resistance gene probe (pGO137 [12]) served as a control for the amount of message encoded by a constitutively expressed gene on the same plasmid. The protein A gene probe

FIG. 1. Complete nucleotide sequence of *trs* (GenBank accession number L11998). Lowercase letters indicate insertion sequences that define the boundary of the *trs* sequence indicated by capital letters. The amino acid sequence of predicted *trs*-encoded proteins is above the corresponding DNA sequence. SD, Shine-Dalgarno region. Translational start and stop are indicated for each ORF, and putative promoter regions are designated −35 and −10. Tn*917* and Tn*197lac* approximate insertion sites (±50 bases) are as follows: pGO1-83, 1,630 (*trsB*); pGO1-230, 2,100 (*trsC*); pGO1-106, 2,356 (*trsD*); pGO1-94, 3,219 (*trsE*); pGO1-15, 5,681 (*trsF*); pGO1-86, 5,924 (*trsF*); pGO1-2P, 9,337 (*trsI*); pGO1-43, 11,216 (*trsK*); pGO1-96, 12,770 (*trsL*). The exact deletion sites of pGO200 that resulted in pGO200Δ1 (at bases 2,367 and 12,363) are designated by the symbol Δ.

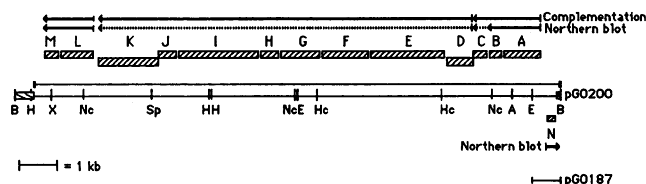


FIG. 2. Schematic representation of predicted *trs*-encoded ORFs, designated A to N, and units of transcription identified by complementation and Northern blot procedures. The entire pGO200 subclone of pGO1 is shown, including the restriction endonuclease cleavage sites used to create subclones. The region corresponding to the DNA sequence in Fig. 1 is represented by the line between the two dark boxes above pGO200. Lines with arrows above pGO200 indicate direction and units of transcription determined by complementation and Northern blot analysis. The top solid arrows represent the *trsA* through *trsC*, *trsD* through *trsK*, and *trsL-trsM* transcripts identified by complementation. The arrows directly below this represent the transcripts identified by Northern blot analysis and are as follows: the first solid arrow represents the 1,300-base *trsA-trsB* transcript seen with pGO187 as a probe, and the broken line above *trsC* represents the 1,800-base *trsA* through *trsC* transcript seen with *trsB* as a probe; the broken line above *trsD* through *trsK* represents the predicted-size transcript based on DNA sequence analysis but not identified by Northern blot analysis; the final solid line represents the 1,500-base *trsL-trsM* transcript identified with *trsL* and *trsM* as probes. Subclone pGO187 is also indicated. Restriction enzyme cleavage sites are abbreviated as follows, with the numbers in parentheses corresponding to nucleotide sequence numbers in Fig. 1: B, *Bgl*II (1, 14,409); E, *Eco*RI (766, 7,005); A, *Ava*I (1,234, 6,825); Nc, *Nco*I (1,804); Ac, *Acc*I (1,977); Hp, *Hph*I (2,291); Hc, *Hin*cII (3,169, 6,533); H, *Hin*dIII (9,162, 9,211); Sp, *Sph*I (10,716); X, *Xba*I (13,376). *trsD* and *trsK* are represented below the other ORFs, since their translational start sites overlap the translational stop sites of the preceding ORFs. The hatched boxes at the ends of pGO200 represent the insertion-like sequences that border *trs*.

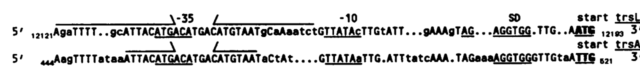


FIG. 3. Comparison of sequences 5′ to the predicted translational start sites for *trsL* and *trsA* (promoter 1). Capital letters indicate identical bases, lowercase letters indicate nonmatching bases, and ellipses indicate gaps introduced to maximize similarities between the two sequences. Predicted promoter regions are marked −35 and −10. SD, Shine-Dalgarno region. The numbers at the beginning and end correspond to the nucleotide sequence numbers in Fig. 1. The arrows indicate areas of dyad symmetry. The translational start sites for *trsL* and *trsA* are in boldface type.

served as a control for the amount of message present on the blot from a well-expressed chromosomally encoded gene. Both of these probes identified transcripts of the appropriate sizes (1,900 bases for the gentamicin resistance gene and 1,500 bp for the protein A gene [45]). As is evident from the Northern blot (Fig. 6), the highest level of message was detected with the protein A gene probe and the next highest level was detected with the gentamicin resistance gene probe.

Figure 6 shows the transcripts most easily seen by Northern blot analysis. The analysis was performed on eight separate occasions, and the results shown in Fig. 6 and presented below were consistently observed. Furthermore, with our RNA isolation technique, there were few background or rRNA signals so that we could attribute even faint bands to hybridizing mRNA species. The *trsN* probe hybridized to one abundant transcript of approximately 400 bases. Probe pGO187, containing the *trsN* gene and the first 244 bases of *trsA*, hybridized to two easily identified transcripts of approximately 1,280 and 400 bases, corresponding in size to a transcript containing *trsA* and *trsB* and one containing *trsN*. A band of approximately 1,800 bases was also seen with pGO187 as a probe but is not well visualized in Fig. 6. When *trsA* alone was used as a probe, faint bands of 1,280 and 1,800 bases were seen (data not shown). The *trsN* transcript was longer than the *trsN* ORF, presumably be-

TABLE 2. Summary of *trs* ORFs

ORF	Size (bp)	Protein size (Da)[a]	SD sequence and translational start[b] (5′→3′)	Promoter sequence(s) (5′→3′)
A	975	37,463	CAATAGAAAAGGTGGGTTGTAATTG	−35 (−35) −10 TTACATGACATGACATGTAATACTATGTTATAATTGA (P1)[c] TGTCATGACAAAAAAGTTAAAAAAGTTTTATAATTTA (P2)[c]
B	315	11,318	ATATAAGGAAGGTTGATTAATTATG	
C	408	15,476	GTTTTAAAAAGGAGTGAATTATATG	
D	684	26,766	AAAAGTAAGAAGGATTGGGTGATG	−35 −10 ATTTAGCAGAAGGCAGAAAGTTTACTTCTATAAAAGT
E	2,019	77,838	AGAACGAAGGGAGTTTATGATTATG	
F	1,281	50,187	TTTATGAATAGGAGGTAATCTCATG	
G	1,077	38,387	AATAGGAGGGAGGTAAAGTATCATG	
H	486	18,763	TTATGATAAGTAGGTGTAAAAAATG	
I	2,103	80,757	AGAATAGAAAGAGGTAATACTTATG	
J	465	17,437	CGATTAAAAGGAGGATAAGAAAGTG	
K	1,641	62,672	TAGAAGAAAGAAGTGATAAGGAATG	
L	918	33,908	TATTGAAAGTAGAGGTGGTTGAATG	−35 (−35) −10 ACATGACATGACATGTAATGCAAAATCTGTTATACTT
M	393	14,861	AAATAAAGAAAGGAAGATATTTATG	
N	189	6,993	ACAAAAAGGAGGTTATATTACTATG	−35 −10 TGACATGTCATGTGTAAATGTGGTATTGTGTAATTAA

[a] The size of each *trs* protein corresponds to the product of each *trs* ORF if the translational start and stop of the protein are as predicted.

[b] SD, Shine-Dalgarno sequence. The alternate Shine-Dalgarno sequence and translational starts for *TrsE* are indicated by lines over the corresponding sequences. The size of *trsE* was determined by using the first translational start.

[c] *trsA* has two potential promoter regions, designated P1 and P2.

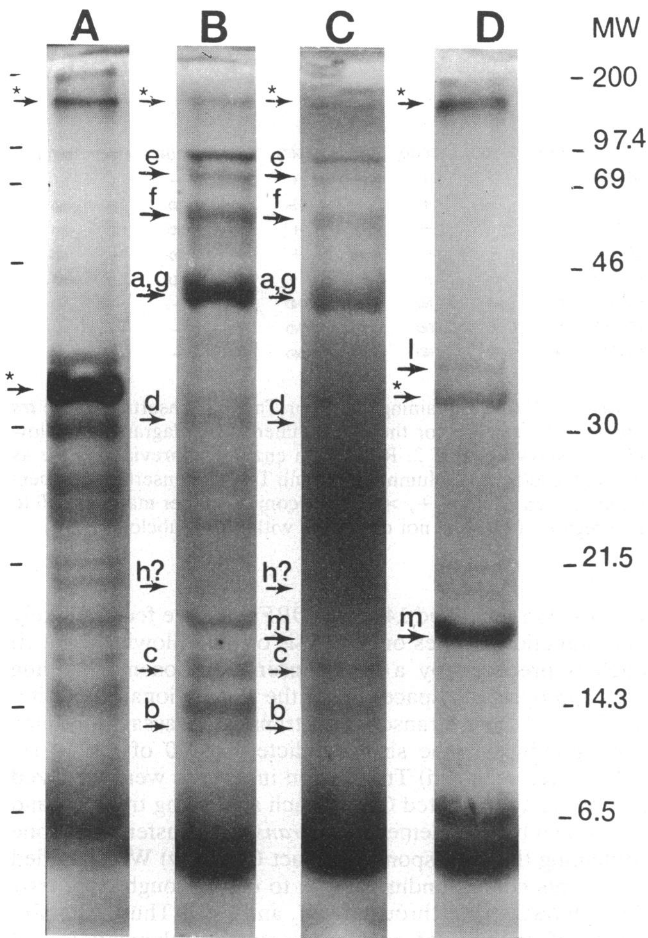


FIG. 4. Autoradiogram of *E. coli* S30 coupled transcription-translation studies. Lanes: A to D, labelled proteins produced by specific DNA used for the reaction; A, pBR322; B, pGO203C; C, pGO200; D, pGO210. Rainbow molecular weight markers (Amersham) were used to size proteins, and the migration of these bands is indicated by dashes and the corresponding molecular weights (MW). Arrows with letters indicate the *trs*-encoded proteins corresponding to the protein bands visualized. Arrows with asterisks indicate the β-lactamase protein (31.5 kDa) and its precursor form (114 kDa). The band above *trsE* (e) in lanes B and C did not correspond to any of the predicted *trs*-encoded proteins but may be a vector-encoded protein that is visualized faintly in lane A. A *trsD*-encoded protein (d in lanes B and C) was revealed by Western immunoblotting of the proteins produced in lanes B and C by using polyclonal antisera generated against the purified *trsD* gene product.

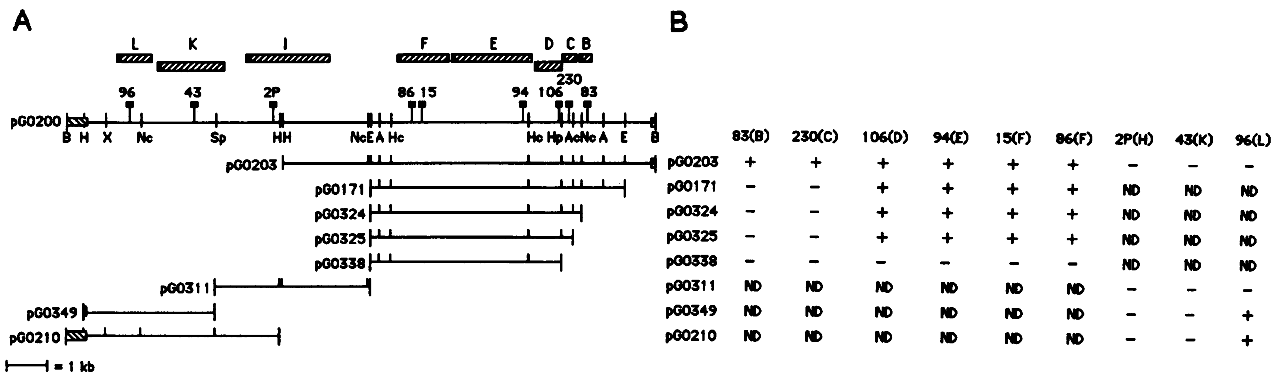
cause of run-off transcription due to the absence of terminator sequences. The *trsB* probe also identified transcripts of 1,800 and 1,280 bases, no 400-base transcript, and a possible transcript of 500 bases. A weak signal of approximately 1,800 bases was barely visible when the *trsC* probe was used. The *trsD*, *trsH*, *trsI*, and *trsJ* probes did not identify any specific transcript but demonstrated a smearing down the lane which may suggest the degradation of a single large transcript recognized by these probes (29). The *trsL* and *trsM* probes each identified a single transcript of approximately 1,500 bases that is easily visible in Fig. 6. Comparisons of the levels of the *trs*-encoded message between donor cells alone (pGO1/4220) and mating mixtures of donor and recipient cells (4220) did not reveal any increase in message in the mating mixture (data not shown).

Comparisons of *trs* to other transfer sequences. The predicted amino acid sequences of *trs* ORFs were compared to the predicted sequences of other ORFs in the GenBank data base by using the GCG TFASTA program. *trsK* showed significant homology to *traD* (GenBank accession number M29254), one of the genes in the *E. coli* F plasmid conjugative transfer operon (49); *virD4* (accession number X06045), a gene on the *Agrobacterium tumefaciens* Ti plasmid that is involved in the transfer of this plasmid into plants (32); and *traG* (accession number X54458), a gene on broad-host-range conjugative plasmids RP4 and R751 (53). *trsK* showed 22% identity and 46% similarity to *traD* with 23 gaps over 643 amino acids, 22% identity and 48% similarity to *virD4* with 19 gaps over 581 amino acids and 20.8% identity and 40.1% similarity to *traG*, with 10 gaps over 389 amino acids. Recently, Lessl et al. (24) compared the amino acid sequences encoded by *traD*, *virD4*, and *traG* and found sequences shared among all three proteins, most of which we also found to be shared by *trsK*. Of the most interest, however, was the shared nucleotide triphosphate (NTP)-binding motif seen among the protein products of gram-negative species that is highly similar in *trsK*. The type A consensus binding site (24) was not highly conserved for *traG* and *virD4*. However, *trsK* contained identical amino acid sequences over 9 of its 13 amino acids, SIVvtDps GEVYE (underlined capital letter indicate identity, and capital letters indicate similarity; bases 10,652 to 10,690 in Fig. 1), compared with the *traG* and *virD4* partial NTP type A sequences. In contrast, the type B consensus NTP-binding motif was highly conserved among *trsK*, *traD*, *traG*, and *virD4*. The sequence VdFLLDEW (bases 11,342 to 11,365 in Fig. 1) was identical or highly conserved over seven of eight amino acids with respect (i) to *virD4* (indicated as described above), (ii) to *traG* over seven of eight amino acids, and (iii) to *traD* over six of eight amino acids. The contribution of the NTP-binding motif of the proteins in gram-negative species to their function is unknown.

In addition, the predicted amino acid sequence related to that of *mobB*, of *trsC* was encoded on *S. aureus* plasmid pC221. We have previously shown that *mobA* and *mobB*, two overlapping ORFs, are essential for conjugative mobilization of this plasmid (33). *trsC* is 32% identical and 53% similar to the terminal 140 of the 233 amino acids of *mobB* of pC221 (GenBank accession number X02529 [34]), with four gaps. No additional significant homologies (>20% identity over ≥50% of the amino acid sequence) were found between predicted amino acid sequences of *trs* ORFs and sequences in the data base encoded by genes involved in conjugative transfer. In addition, because of the findings obtained with *trsK*, the predicted gene products of all *trs* ORFs were directly compared to the predicted amino acid sequences of all genes encoded in the Tra1 and Tra2 regions of RP4; no other significant homologies were found. Because of the similarities between gene products noted above, pC221 was introduced into pGO1-230, a transposon insert into *trsC* on pGO1 that inactivates transfer, and no complementation was seen. Finally, the entire *trs* region clone (pGO200) did not complement a *traG* mutation in RP4 when both were introduced into *E. coli* (46).

DISCUSSION

In an earlier study, the conjugative transfer region of staphylococcal plasmid pGO1 was localized by transposon insertion mutagenesis and by subcloning of a functional 14.4-kb *Bgl*II fragment on a separate staphylococcal replicon



	83(B)	230(C)	106(D)	94(E)	15(F)	86(F)	2P(H)	43(K)	96(L)
pGO203	+	+	+	+	+	+	−	−	−
pGO171	−	−	+	+	+	+	ND	ND	ND
pGO324	−	−	+	+	+	+	ND	ND	ND
pGO325	−	−	+	+	+	+	ND	ND	ND
pGO338	−	−	−	−	−	−	ND	ND	ND
pGO311	ND	ND	ND	ND	ND	ND	−	−	−
pGO349	ND	ND	ND	ND	ND	ND	−	−	+
pGO210	ND	ND	ND	ND	ND	ND	−	−	+

FIG. 5. Results of complementation studies. (A) Schematic representation of pGO200 containing Tn*917* or Tn*917lac* insertions. The *trs* ORF that corresponds to each insert is diagrammed above pGO200, and the subclones used for these experiments are diagrammed below pGO200. Only ORFs containing transposon inserts are included. All ORFs are shown in Fig. 2. Restriction enzyme abbreviations are as described in the legend to Fig. 2. (B) Subclones used for complementation are in the left-hand column. Tn*917* and Tn*917lac* insertion numbers and the *trs* ORF into which each inserted (in parentheses) are listed at the top of each column. +, >200 transconjugates per mating; −, 0 to 10 transconjugates per mating; ND, not determined since the corresponding region of DNA is not contained within the subclone.

(43). In this study, we sequenced the entire fragment and determined its transcriptional organization. We designated the 13.6-kb region *trs* (transfer in staphylococci) to distinguish it from the *tra* regions of plasmids from gram-negative species. We identified 14 intact ORFs that we feel are likely to be functional genes on the basis of the following data. (i) Each is preceded by a near-consensus ribosomal binding site, appropriately spaced from the translational start site. (ii) In an *E. coli* transcription-translation assay, proteins corresponding to the sizes predicted for 10 of the 14 *trs* ORFs were seen. (iii) Transposon insertions were localized to 8 of the 14 predicted ORFs, each abolishing transfer, and each could be complemented in *trans* for transfer by a clone containing the corresponding intact ORF. (iv) We identified transcripts corresponding in size to *trsA* through *trsB*, *trsA* through *trsC*, *trsL*, through *trsM*, and *trsN*. Thus, a combination of analysis of gene products, complementation of inactivated genes, and identification of transcripts of the appropriate sizes demonstrated a probable functional role for all of the predicted ORFs except *trsJ* and *trsK*. The precise role for each of these ORFs in transfer will have to be defined in future studies.

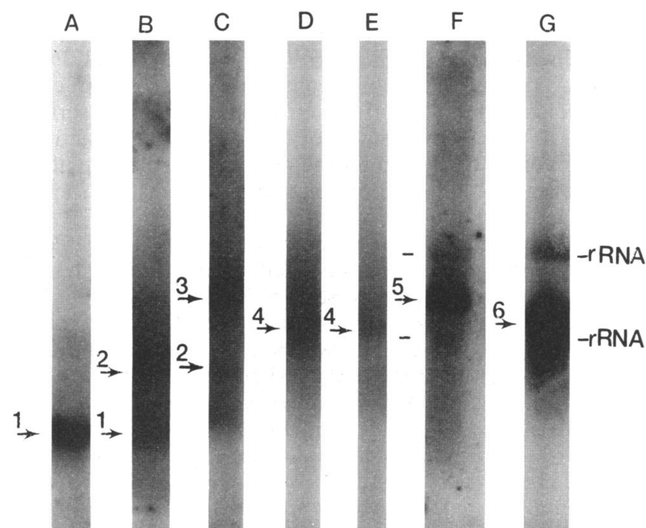


FIG. 6. Autoradiogram of Northern blot analysis. (A to G) Total cellular mRNA preparations of *S. aureus* pGO1/4220 separated through 1.2% agarose containing 2.2 M formaldehyde. DNA probes and relative exposure times: lane A, *trsN* PCR product, 23 h; B, pGO187 DNA, 23 h; C, *trsB* PCR product, 23 h; D, *trsL* PCR product, 23 h; E, *trsM* PCR product, 23 h; F, pGO137 DNA (gentamicin resistance gene probe [*aac-aph*]), 7 h; G, protein A gene probe DNA, 3 h. The specific radioactivity of each probe and the amount of RNA were the same for each lane. −, rRNA bands. Arrows, easily identified transcript. 1, 400-base *trsN* transcript; 2, 1,300-base *trsA-trsB* transcript; 3, 1,800-base *trsA-trsC* transcript; 4, 1,500-base *trsL-trsM* transcript; 5, 1,800-base gentamicin resistance gene transcript; 6, 1,500-base protein A gene transcript. Sizes of transcripts were determined by comparisons to RNA molecular weight standards (not shown). Northern blot procedures were repeated several times, and identical results were obtained each time. The band hybridizing below transcript 2 in lane C was not explainable by sequence or complementation data but was consistently seen.

Additional evidence that two predicted *trs*-encoded proteins not seen well in the *E. coli* transcription-translation assay are produced came from Western blot (immunoblot) analysis. Polyclonal antisera previously generated against purified gene products of *trsD* and *trsH* identified both proteins with the predicted molecular weights (28) among total cellular proteins from *S. aureus* RN4220 containing pGO1 (28) and a band corresponding in size to TrsD among the proteins produced by *E. coli* transcription-translation of pGO203C and pGO200 (29). Proteins corresponding to the predicted product of *trsI*, *trsJ*, or *trsK* were not seen. This could be due to the inability of these staphylococcal genes to be transcribed and translated in an *E. coli* system, to poor resolution of the proteins during electrophoresis, or to the sensitivity of these proteins to degradation by cellular proteases. Although the S30 extract system is OmpT protease deficient, other proteases could be present in the extract that degrades these proteins. This hypothesis is supported by the observed degradation of the protein encoded by *trsI* by cellular proteases (28).

We deduced transcriptional organization of the *trs* region from analysis of DNA sequence motifs, complementation for transfer of transposon inserts that abolished conjugation, and identification of mRNA transcripts on Northern blots. Three major complementation groups were identified. The

first included *trsA* through *trsC*, a predicted transcript length of approximately 1,800 bases; the second included *trsD* through *trsK*, producing a 9,900-base transcript; and the third included *trsL* and *trsM*, a 1,500-base transcript. Allowing for two mismatches from the consensus *E. coli* −10 and −35 sequences (TATAAT and TTGACA) and by comparison with known staphylococcal promoter sequences (31), near-consensus candidate promoter sequences were identified 5′ to *trsA*, *trsD*, and *trsL*, consistent with the complementation data. Secondary structures containing areas of dyad symmetry following *trsK* and *trsM* may serve to terminate transcription at those sites. There is no sequence that is an obvious transcriptional terminator between *trsC* and *trsD*; in fact, the translational stop of *trsC* and the start of *trsD* overlap. Rather, there is an untranslated 58-base gap between *trsB* and *trsC* that contains a predicted rho-independent terminator. Some of the Northern blot data are consistent with these sequence motifs. Transcripts of 1,800 bases, consistent with transcription of *trsA*, *trsB*, and *trsC*, and a fainter band of 1,300 bases, equal to the predicted transcription of only *trsA* and *trsB*, were seen when *trsB* was used as a probe. A probe (pGO187) containing *trsA* showed a 1,300-base major transcript with a faint band that may represent the 1,800-base transcript. The 500-base transcript identified by the *trsB* probe could not be explained from the sequence or complementation data but may be the result of message degradation or processing. Probing with *trsC* revealed only a very faint band that may be the 1,800-base transcript. These data are consistent with transcription of *trsA* and *trsB* at some stages of conjugation, with transcription of *trsA*, *trsB*, and *trsC* occurring at other times. Transcription of *trsC* could, therefore, be regulated by antitermination. Instability or low abundance of transcripts containing *trsC* may explain the difficulty in demonstrating a transcript by using this probe. Since there is no predicted terminator sequence downstream of *trsC*, *trsA* through *trsC* may at times be transcribed as *trsA* through *trsK*. However, since a construct removing sequences 5′ to *trsA* could complement transposon inserts downstream of *trsD* but not those in *trsB* or *trsC*, *trsD* through *trsK* transcription must predominate. No transcript corresponding to the large predicted complementation group *trsD* through *trsK* was seen when *trsD*, *trsH*, *trsI*, or *trsJ* was used as a probe in any of the Northern blot procedures. This may have been due to degradation of this long message in the process of RNA preparation or the message's inherent instability or low abundance.

We also identified regions that will be targeted for further study with respect to regulation of *trs*. DNA sequence analysis revealed identical −35 sequences in the predicted promoter regions for *trsA*, *trsL*, and *trsN* (Fig. 1 and Table 2). In addition, promoter sequences for *trsA* and *trsL* were contained within identical regions of dyad symmetry (Fig. 3). An abundant *trsN* transcript was seen by Northern blot analysis (Fig. 6), and a protein of the size predicted for TrsN was seen in the *E. coli* transcription-translation assay when only the *trsN* PCR product was used (40). We have preliminary evidence that the *trsN* gene product is at least one of the *trs* regulatory molecules and that one of its targets is the sequence immediately 5′ to *trsL* (40).

The organization and units of transcription of genes involved in conjugative transfer vary among other plasmid systems. On F and related plasmids from gram-negative organisms, transfer genes are clustered as a contiguous unit (49), as are the *trs* genes on pGO1. Even though the F system was the first and one of the best described, the organization of most of its transfer genes into a single major transcription unit appears to be uncommon. Broad-host-range IncP plasmids have two separate transfer regions, one that contains genes for surface exclusion and formation of mating aggregates (Tra2) and another that encodes mostly genes involving DNA transfer and metabolism (Tra1). Furthermore, the genes in Tra1 seem to be arranged in at least four individual transcription units (47) while a single primary promoter transcribes the Tra2 genes (23). Other conjugative plasmids from gram-negative organisms, such as those of the IncN, IncP10, IncH, and IncIα incompatibility groups, as well as *Agrobacterium* Ti plasmids (16, 27, 36, 42, 51), also have conjugative transfer functions segregated into two or more distinct regions that differ in transcriptional organization. Among other conjugative plasmids from gram-positive organisms, there also appear to be separate transfer regions. Enterococcal pheromone-responsive plasmids have individual regions that contain genes for DNA transfer and mating aggregation (pheromone response), and broad-host-range streptococcal plasmid pIP501 has two separate clusters of transfer genes with undefined functions (20, 48). Thus, *trs* seems to be unlike the most prevalent broad-host-range plasmids from gram-negative organisms and more similar to F in that 8 of the 14 transfer genes are within a single transcription unit and all transfer genes are clustered. However, since there are no surface appendages (26) and no aggregation substances (5) on staphylococcal cells that contain pGO1, most of the *trs* genes may be involved in DNA transfer rather than in mating aggregation. Therefore, *trs* may be more similar to the 10- to 15-kb gene clusters on broad-host-range plasmids from gram-negative species and pheromone-responsive plasmids from gram-positive species that function primarily in conjugal DNA metabolism and transfer.

Some additional evidence for the similarity of *trs* and some of the transfer genes from gram-negative organisms came from the comparison of predicted *trs* gene products with those in the data base. The amino acid similarity among the predicted products of the transfer genes of gram-negative species, including *virD4*, *traD*, and *traG*, has recently been noted by Lessl and colleagues (24). The *trsK* gene product is also related to the predicted products of these genes of gram-negative organisms and has a consensus sequence for a type B NTP-binding site in common with these genes. The *traD* gene product has been localized to the cell membrane of *E. coli* and has been proposed either to guide the DNA through the cell membrane or to link the relaxasome with other membrane proteins that serve to form a membrane pore (23). *traG* and *virD4* are grouped in operons that contain genes involved in DNA relaxation (47), suggesting that *traD*, *traG*, and *virD4* serve to link DNA transfer with single-strand nicking. However, no other *trs* genes have significant similarity to gram-negative conjugative transfer genes in the data base and there is no formal evidence that pGO1 moves from donor to recipient cells as a single strand. The only indirect evidence for single-strand transfer comes from experiments showing that pGO1 mobilization of small, nonconjugative plasmid pC221 from staphylococci requires pC221 relaxation and that relaxation involves two pC221-encoded genes, *mobA* and *mobB* (32). *mobA* encodes DNA-nicking activity, while *mobB* is, presumably, a single-strand binding protein. It is of interest, therefore, that the only other *trs* gene with a predicted product showing amino acid similarity to a transfer gene product was *trsC*, with homology to the pC221 *mobB* product. This further strengthens the

theory that single-strand transfer is the operative mechanism for pGO1.

While a number of studies have assessed conjugation systems in gram-negative bacteria, similar studies are infrequent for conjugative plasmids in gram-positive cocci. Given the growing importance of staphylococci as nosocomial pathogens, largely because of their acquisition of antimicrobial resistance genes, it seems important to characterize gene transfer among these organisms. This study provides the first complete DNA sequence and transcriptional analysis of gram-positive conjugative transfer genes and gives some indication that genetic organization differs in many ways from the organization reported for other transfer systems. It should serve as a basis for further studies of gene function and regulation.

ACKNOWLEDGMENTS

This study was supported by U.S. Public Health Service grant AI/GM21772 from the National Institute of Allergy and Infectious Diseases.

REFERENCES

1. **Archer, G. L., J. P. Coughter, and J. L. Johnston.** 1986. Plasmid encoded trimethoprim resistance in staphylococci. Antimicrob. Agents Chemother. **29:**733–740.
2. **Archer, G. L., D. R. Dietrick, and J. L. Johnston.** 1985. Molecular epidemiology of transmissible gentamicin resistance among coagulase-negative staphylococci in a cardiac surgery unit. J. Infect. Dis. **151:**243–251.
3. **Archer, G. L., and J. L. Johnston.** 1983. Self-transmissible plasmids in staphylococci that encode resistance to aminoglycosides. Antimicrob. Agents Chemother. **24:**70–77.
4. **Archer, G. L., and J. Scott.** 1991. Conjugative transfer genes in staphylococcal isolates from the United States. Antimicrob. Agents Chemother. **35:**2500–2504.
5. **Archer, G. L., and W. D. Thomas.** 1990. Conjugative transfer of antimicrobial resistance genes between staphylococci, p. 115–122. *In* P. Novick (ed.), Molecular biology of the staphylococci. VCH Publishers, New York.
6. **Barberis-Maino, L., B. Berger-Bächi, H. Weber, W. D. Beck, and F. J. Kayser.** 1987. IS*431*, a staphylococcal insertion sequence-like element related to IS*26* from *Proteus vulgaris*. Gene **59:**107–113.
7. **Bolivar, F., F. L. Rodriguez, P. J. Green, M. C. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Cross, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2:**95–113.
8. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41:**459.
9. **Bullock, W. O., J. M. Fernandez, and J. M. Short.** 1987. XL-1 Blue: a high efficiency plasmid transforming recA *Escherichia coli* strain with beta-galactoside selection. BioTechniques **4:**376–379.
10. **Byrne, M. E., M. T. Gillespie, and R. A. Skurray.** 1990. Molecular analysis of a gentamicin resistance transposon-like element on plasmids isolated from North American *Staphylococcus aureus* strains. Antimicrob. Agents Chemother. **34:**2106–2113.
11. **Forbes, B. A., and D. R. Schaberg.** 1983. Transfer of resistance plasmids from *Staphylococcus epidermidis* to *Staphylococcus aureus*: evidence for conjugative exchange of resistance. J. Bacteriol. **153:**627–634.
12. **Froggatt, J. W., J. L. Johnston, D. W. Galetto, and G. L. Archer.** 1989. Antimicrobial resistance in nosocomial isolates of *Staphylococcus haemolyticus*. Antimicrob. Agents Chemother. **33:**460–466.
13. **Galleto, D. W., J. L. Johnston, and G. L. Archer.** 1987. Molecular epidemiology of trimethoprim resistance among coagulase-negative staphylococci. Antimicrob. Agents Chemother. **31:**1683–1688.
14. **Goering, R. V., and E. A. Ruff.** 1983. Comparative analysis of conjugative plasmids mediating gentamicin resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **24:**450–452.
15. **Hickman, R. K., and S. B. Levy.** 1988. Evidence the TET protein functions as a multimer in the inner membrane of *Escherichia coli*. J. Bacteriol. **170:**1715–1720.
16. **Holsters, M. B. Silva, F. Van Vliet, C. Genetello, M. De Block, P. Dhaese, A. Depicker, D. Inze, G. Engler, R. Villarroel, F. Van Montagu, and J. Schell.** 1980. The functional organization of the nopaline *A. tumefaciens* plasmid pTiC58. Plasmid **3:**212–230.
17. **Jaffe, H. W., H. M. Sweeney, C. Nathan, R. A. Weinstein, S. A. Kabins, and S. Cohen.** 1980. Identity and interspecific transfer of gentamicin-resistance plasmids in *Staphylococcus aureus* and *Staphylococcus epidermidis*. J. Infect. Dis. **141:**738–747.
18. **Kasatiya, S. S., and J. Baldwin.** 1967. Nature and the determinant of tetracycline resistance in *Staphylococcus aureus*. Can. J. Microbiol. **13:**1079–1086.
19. **Kornblum, J. S., S. J. Projan, S. L. Moghazeh, and R. P. Novick.** 1988. A rapid method to quantitate non-labelled RNA species in bacterial cells. Gene **63:**75–85.
20. **Krah, E. R., and F. L. Macrina.** 1989. Genetic analysis of the conjugal transfer determinants encoded by the streptococcal broad-host-range plasmid pIP501. J. Bacteriol. **171:**6005–6012.
21. **Kreiswirth, B. N., S. Lofdahl, M. J. Betley, M. O'Reilly, P. M. Schlievert, M. S. Bergdoll, and R. P. Novick.** 1983. The toxic shock exotoxin structural gene is not detectably transmitted by a prophage. Nature (London) **305:**709–712.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature (London) **227:**680–685.
23. **Lessl, M., D. Balzer, W. Pansegrau, and E. Lanka.** 1992. Sequence similarities between the RP4 Tra2 and the Ti VirB region strongly support the conjugation model for T-DNA transfer. J. Biol. Chem. **267:**20471–20480.
24. **Lessl, M., W. Pansegrau, and E. Lanka.** 1992. Relationship of DNA-transfer-systems: essential transfer factors of plasmids RP4, Ti and F share common sequences. Nucleic Acids Res. **20:**6099–6100.
25. **McDonnell, R. W., H. M. Sweeney, and S. Cohen.** 1983. Conjugational transfer of gentamicin resistance plasmids intra- and interspecifically in *Staphylococcus aureus* and *Staphylococcus epidermidis*. Antimicrob. Agents Chemother. **23:**151–160.
26. **Mikesell, T. S.** 1988. Observations on the mechanism of gene transfer in gram-positive bacteria. M.S. thesis. Eastern Michigan University, Ypsilanti.
27. **Moore, R. J., and V. Krishnapillai.** 1982. Tn*7* and Tn*501* insertions into *Pseudomonas aeruginosa* plasmid R91-5: mapping of two transfer regions. J. Bacteriol. **149:**276–283.
28. **Morton, T. M.** 1992. Characterization of the staphylococcal conjugative gene complex. Ph.D. thesis. Medical College of Virginia, Virginia Commonwealth University, Richmond.
29. **Morton, T. M.** Unpublished data.
30. **Novick, R.** 1967. Properties of a high frequency transducing phage in *Staphylococcus aureus*. Virology **33:**155–166.
31. **Novick, R. P.** 1990. The staphylococcus as a molecular genetic system, p. 25–26. *In* R. P. Novick (ed.), Molecular biology of the staphylococci. VCH Publishers, New York.
32. **Porter, S. G., M. F. Yanofsky, and E. W. Nester.** 1987. Molecular characterization of the *virD* operon from *Agrobacterium tumefaciens*. Nucleic Acids Res. **15:**7503–7517.
33. **Projan, S. J., and G. L. Archer.** 1989. Mobilization of the relaxable *Staphylococcus aureus* plasmid pC221 by the conjugative plasmid pGO1 involves three pC221 loci. J. Bacteriol. **171:**1841–1845.
34. **Projan, S. J., J. Kornblum, S. L. Moghazeh, I. Edelman, M. L. Gennaro, and R. P. Novick.** 1985. Comparative sequence and functional analysis of pT181 and pC221, cognate plasmid replicons from *Staphylococcus aureus*. Mol. Gen. Genet. **199:**452–464.
35. **Ranellin, D. M., C. L. Jones, M. B. Johns, G. J. Mussery, and S. Khan.** 1985. Molecular cloning of staphylococcal enterotoxin B gene in *Escherichia coli* and *Staphylococcus aureus*. Proc. Natl.

Acad. Sci. USA **82**:5850–5854.

36. **Rees, C. E. D., D. E. Bradley, and B. M. Wilkins.** 1987. Organization and regulation of the conjugation genes of $IncI_1$ plasmid ColIb-P9. Plasmid **18**:223–236.
37. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed., p. 1.25–1.28. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
38. **Schaberg, D. R., D. H. Culver, and R. P. Gaynes.** 1991. Major trends in the microbial etiology of nosocomial infection. Am. J. Med. **91**(Suppl. 3B):725–755.
39. **Schenk, S., and R. A. Laddaga.** 1992. Improved method for electroporation of *Staphylococcus aureus*. FEMS Microbiol. Lett. **94**:133–138.
40. **Sharma, V. K., T. M. Morton, J. L. Johnston, and G. L. Archer.** Unpublished data.
41. **Shaw, J. H., and D. B. Clewell.** 1985. Complete nucleotide sequence of macrolide-lincosamide-streptogramin B-resistance transposon Tn*917* in *Streptococcus faecalis*. J. Bacteriol. **164**: 782–796.
42. **Taylor, D. E., E. C. Brose, S. Khan, and W. Yan.** 1985. Mapping transfer regions within incompatibility group H1 plasmid R27. J. Bacteriol. **162**:1221–1226.
43. **Thomas, W. D., and G. L. Archer.** 1989. Identification and cloning of the conjugative transfer region of *Staphylococcus aureus* plasmid pGO1. J. Bacteriol. **171**:684–691.
44. **Townsend, D. E., N. Ashdown, S. Bolton, and W. B. Grubb.** 1985. The use of cetyltrimethylammonium bromide for the rapid isolation from *Staphylococcus aureus* of relaxable and non-relaxed plasmid DNA suitable for *in vitro* manipulation. Lett. Appl. Microbiol. **1**:87–94.
45. **Vandenesch, F., J. Kornblum, and R. P. Novick.** 1991. A temporal signal, independent of *agr*, is required for *hla* but not *spa* transcription in *Staphylococcus aureus*. J. Bacteriol. **173**: 6313–6320.
46. **Waters, V. L., and D. G. Guiney.** Unpublished data.
47. **Waters, V. L., B. Strack, W. Pansegrau, E. Lanka, and D. G. Guiney.** 1992. Mutational analysis of essential IncPα plasmid transfer genes *traF* and *traG* and involvement of *traF* in phage sensitivity. J. Bacteriol. **174**:6666–6673.
48. **Weaver, K. E., and D. B. Clewell.** 1988. Regulation of the pAD1 sex pheromone response in *Enterococcus faecalis*: construction and characterization of *lacZ* transcription fusions in a key control region of the plasmid. J. Bacteriol. **170**:4343–4352.
49. **Willets, N., and R. Skurray.** 1987. Structure and function of the F factor and mechanism of conjugation, p. 1110–1133. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
50. **Winans, S. C., and G. C. Walker.** 1985. Conjugal transfer system of the IncN plasmid pKM101. J. Bacteriol. **161**:402–410.
51. **Winter, R. B., and L. Gold.** 1983. Overproduction of bacteriophage QB maturation (A_2) protein leads to cell lysis. Cell **33**:877–885.
52. **Youngman, P.** 1987. Plasmid vectors for recovering and exploiting Tn*917* transpositions in *Bacillus* and other gram-positive bacteria, p. 79–103. *In* K. G. Hardy (ed.), Plasmids: a practical approach. IRL Press, Oxford.
53. **Ziegelin, G., W. Pansegrau, B. Strack, D. Balzer, M. Kroger, V. Kruft, and E. Lanka.** 1991. Nucleotide sequence and organization of genes flanking the transfer origin of promiscuous plasmid RP4. J. DNA Sequencing Mapping **1**:303–327.