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Transcriptional Analysis of a Region of the *Enterococcus faecalis* Plasmid pCF10 Involved in Positive Regulation of Conjugative Transfer Functions

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The *prgB* gene encodes aggregation substance (Asc10) which is essential for transfer of the pheromone-inducible conjugative plasmid pCF10 in *Enterococcus faecalis*. The *prgQ* and *prgS* regions, located 4 kb upstream of *prgB*, are required for the expression of *prgB*. Complementation studies indicated that the *prgQ* region functions in *cis* and in an orientation-dependent manner relative to the *prgB* gene (J. W. Chung and G. M. Dunny, Proc. Natl. Acad. Sci. USA 89:9020–9024, 1992). Analysis of transcriptional fusions in this study, using a promoterless *lacZ* gene in several locations between *prgQ* and *prgB*, confirmed that the *prgQ* region does not carry a promoter for the expression of *prgB* and that *prgB* does not comprise an operon with *prgA* (which encodes the surface exclusion protein Sec10), the gene immediately upstream from *prgB*. Northern (RNA) blot analysis demonstrated that two distinct transcripts (Q_S RNA and Q_L RNA), much larger than the *prgQ* gene, were expressed in the *prgQ* region. Q_S RNA was produced constitutively, whereas Q_L RNA was produced inducibly by pheromone. The lack of any other open reading frame in Q_L RNA and significant sequence complementarity between the 3′ end of Q_L RNA and the promoter region of *prgB* suggested that the functional products of the *prgQ* region might be RNA molecules rather than proteins. A mutation in *prgS* completely abolished the production of Q_L RNA. A model for transcriptional activation of *prgB* is presented.

When *Enterococcus faecalis* cells carrying certain conjugative plasmids such as pCF10 (58 kb) are mixed in broth with *E. faecalis* recipient cells, visible aggregation of the cells in the mating mixture occurs, accompanied by high-frequency plasmid transfer (9, 11, 15). The efficient transfer of pCF10 is inducible by a heptapeptide, cCF10, which is one of several chromosomally encoded peptide pheromones secreted by recipient cells (13, 20). Upon induction, donors synthesize a 150-kDa surface protein called aggregation substance (Asc10), which facilitates formation of mating aggregates (24).

Previous studies of pCF10 involving transposon mutagenesis, subcloning, Western blot (immunoblot) analysis, and DNA sequencing identified the structural gene (*prgB* [*prg*; pheromone-responsive gene]) encoding Asc10 and an upstream regulatory region required for the expression of Asc10, both of which are located on the contiguous *Eco*RI C and E fragments of pCF10 (18). *E. faecalis* cells carrying the cloned *Eco*RI C and E fragments produce Asc10 constitutively if the positive-control region located 3 to 6 kb upstream from the *prgB* gene is not disrupted (5). Genes responsible for signal transduction and negative control of plasmid transfer functions of pCF10 are located adjacent to the positive-control genes on the side opposite *prgB* (5, 6).

Complementation studies demonstrated that the positive-control system functions in a novel *cis*-acting, orientation-dependent fashion to activate transcription of *prgB* (8). Primer extension and Northern (RNA) blot analyses of the *prgB* message indicated that it is monocistronic (8). In addition, expression of *prgB* was not abolished by insertion of a transcriptional terminator or insertion of Tn*5* between the regulatory region and *prgB* (8, 18). These results suggested that the activation mechanism might involve tracking of positive regulatory molecules along the DNA to the *prgB* promoter region rather than initiation of a long transcript from the regulatory region that reads through to *prgB* and is processed to the mRNA species detected by physical analysis (8). When the positive-control region was sequenced, a number of open reading frames (ORFs) were found, which were given the gene designations *prgQ*, *-R*, *-S*, and *-T*; these genes are depicted in Fig. 1 of the accompanying article (7). It has been determined recently that *prgQ* encodes a secreted peptide product, iCF10, that can act as a competitive inhibitor of the pheromone (22); it is likely that iCF10 is involved in prevention of self-induction by donors, and it is not known whether iCF10 plays any direct role in positive regulation. In addition to these putative regulatory genes, the *prgA* gene encoding the Sec10 surface exclusion protein is located between the 3′ end of *prgT* and the 5′ end of *prgB* (18). The genetic data reported in the accompanying article (7) indicated that Tn*5* insertional mutagenesis of the putative *prgQ* promoter region or of the *prgS* gene abolished positive control of *prgB*, whereas *prgR* and *prgT* were not required for *prgB* expression. Interestingly, there were some insertions 3′ to *prgQ* or 3′ to *prgS* that also abolished positive control. It was hypothesized that the insertions downstream from *prgS* might affect message stability, whereas the effects of the insertions in the region 3′ to the ORF corresponding to *prgQ* could be explained if RNA products were the functional positive regulatory molecules encoded by this region of pCF10. In this article, we present transcriptional analysis of the positive-control region. In addition, a molecular model for the positive regulatory system is presented.

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TABLE 1. Plasmids used in this study

Plasmid(s)	Description	Reference(s) or source
pBR322	Amp^r Tc^r; 4.36-kb *E. coli* vector	Gibco/BRL
pCF10	Tc^r; 58-kb conjugative plasmid	14
pDL414	Km^r; 5.7-kb multicopy streptococcal plasmid	19
pINY1801	*Eco*RI C and E fragments of pCF10 cloned in the natural orientation into *Eco*RI site of pWM402	17
pINY1825	*Eco*RI C fragment of pCF10 cloned into *Eco*RI site of pWM402	17
pINY4501 and -4560	pINY1801::Tn*5* derivatives	17, 18
pINY8101	3.3-kb *Sma*I fragment of pUT2100 carrying *lacZ* cloned into *Pst*I site of pINY1825	7, 8
pINY8109	2.2-kb *Eco*RI-*Xba*I fragment of pINY8101 cloned into *Eco*RV site of pWM402	7, 8
pINY8301 and -8329	Plasmids containing deletions in the structural gene of *prgX* and its putative promoter region	7
pINY8402	4.2-kb *Eco*RI-*Pvu*II fragment of pINY1825 ligated with 5.3-kb *Eco*RI-*Hin*cII fragment of pDL41	8
pINY8503 and -8508	pINY8301::Tn*5* derivatives	7
pUT2100	Cm^r Ery^r; 16.8-kb *Bacillus* plasmid with temperature-sensitive replicon containing Tn*917*::*lacZ*	8
pWM402	Cm^r Tc^r (*E. coli*), Cm^r (*E. faecalis*); 11.5-kb shuttle vector	30

MATERIALS AND METHODS

Bacterial strains, media, and reagents. *Escherichia coli* DH5α (Gibco/Bethesda Research Laboratories [Gibco/BRL], Gaithersburg, Md.) was grown in LB medium (26), and *E. faecalis* strains OG1RF (14) and INY3000 (a cell wall mutant defective as a conjugal recipient; 2, 28) were grown in M9-YE medium (12). The concentrations of antibiotics in selective media were as follows: ampicillin, 125 μg/ml; chloramphenicol, 15 μg/ml; kanamycin, 200 μg/ml in broth medium and 750 μg/ml in agar medium; and tetracycline, 10 μg/ml. All antibiotics were purchased from Sigma Chemical Co. Restriction enzymes, DNA polymerase Klenow fragment, RNase-free DNase, T4 polynucleotide kinase, and T4 DNA ligase were from Gibco/BRL or Promega (Madison, Wis.). Sequenase and sequencing reagents were from United States Biochemical Corp. (Indianapolis, Ind.). [α-^{35}S]dATP, and [γ-^{32}P]ATP were acquired from Amersham (Arlington Heights, Ill.). Oligonucleotides were synthesized with an Applied Biosystems 391 automated synthesizer.

Plasmid and strain constructions. Plasmids used in this study are listed in Table 1. Standard recombinant DNA techniques (1, 26) were used with the following modifications. All restriction fragments used in the construction of plasmids were separated by agarose gel electrophoresis, and the appropriate DNA bands were excised and purified by using a Geneclean II kit (Bio 101, La Jolla, Calif.) as recommended by the supplier. When necessary, DNA fragments were treated with Klenow enzyme to generate blunt ends and/or were dephosphorylated with calf intestinal phosphatase (Promega) to facilitate the cloning processes.

Transformation of *E. coli* and *E. faecalis* with the constructed plasmids was done by electroporation as described elsewhere (8). The plasmid composition of transformants was confirmed by restriction enzyme digestion and analyzed by agarose gel electrophoresis.

Northern blot, Western blot, and primer extension analyses. Extraction of total cellular RNA, Northern blot analysis and primer extensions were performed as described previously (1), with the modifications noted elsewhere (8). For Northern blot analysis, a duplicate gel was stained in ethidium bromide solution (0.5 μg/ml) and the intensities of 16S rRNA and 23S rRNA bands in each lane were compared to confirm that equivalent quantities (30 to 40 μg) of RNA were loaded. RNA was separated by using a 1.5% agarose–formaldehyde gel. Prehybridization and hybridization of transferred nitrocellulose blots were performed as described previously (1).

Approximately 50 μg of total cellular RNA was used for a primer extension. The oligonucleotide used in the primer extension was also used as a primer for a sequencing reaction of the plasmid DNA containing the template of the RNA transcript to be tested. Extended products were separated by an 8% polyacrylamide gel electrophoresis. Reverse transcriptase and RNasin were purchased from Promega.

The probes used in Northern hybridization reactions were as follows: *prgQ*, R01999-17 (5′-GCCCCCTAGGATTGCCG-3′) and R02273-17 (5′-GCGT GCTTGTTACTTGG-3′); *prgR*, R02510-17 (5′-GCGTTCTGTTGTGCCG-3′) and R02657-17 (5′-CGGAAGTTCCGAGCAGC-3′); and *prgS*, R02909-17 (5′-CCTTCGTGTCCGTGTGC-3′). The primer used for primer extension analysis of the *prgQ* transcript was R01877-31 (5′-TTTTTTAGAGTGGTTTTCATTTA CACCCCTCC-3′). The experimental data described in the text indicates the positions of the pCF10 sequences complementary to these oligonucleotides. The 5′-end labeling of oligonucleotides was done with [γ-^{32}P]ATP as described elsewhere (1). Extraction of surface proteins and Western blot analysis were performed as described previously (24).

Nucleotide sequence accession number. The sequence of the region of pCF10 described in this article is deposited in GenBank under accession number M64978.

RESULTS

Transcriptional analysis of the *prgQ*, *-R*, and *-S* region. Northern blot analysis of *prgQ*, *-R*, and *-S* was performed in order to determine transcript identity, size, and inducibility by pheromone. Total cellular RNA was extracted from strains OG1RF, OG1RF(pCF10) (uninduced), OG1RF(pCF10) (induced), OG1RF(pWM402), and OG1RF(pINY1801) and hybridized with an end-labeled oligonucleotide probe corresponding to each regulatory gene. For *prgQ* and *prgS*, primer extension experiments were also performed to determine the start sites of transcription.

***prgQ*.** Two different sizes of transcripts were detected by Northern blot hybridization in the *prgQ* region, using a single probe (Fig. 1A). A transcript of about 400 nucleotides, Q_{SHORT} RNA (Q_S RNA), was detected constitutively with RNA extracted from OG1RF(pCF10) and OG1RF (pINY1801). An additional transcript of about 530 nucleotides, Q_{LONG} RNA (Q_L RNA), was also detected in RNA extracted from OG1RF(pCF10) induced with pheromone and OG1RF(pINY1801). This longer transcript was detected only in the presence of pheromone in OG1RF carrying pCF10. However, like *prgB* mRNA, Q_L was produced constitutively in OG1RF(pINY1801), indicating that in the absence of the negative regulatory region, transcription of Q_L RNA is independent of pheromone induction.

Interestingly, the sizes of Q_S and Q_L RNAs were much larger than the size of the ORF of *prgQ* (69 bp). In addition to *prgQ*, another short ORF (ORFq; 18 amino acids) with a potential ribosomal binding site could be found 223 bp downstream from the stop codon of *prgQ* (see Fig. 5). Tn*5* insertion in ORFq (pINY4518; Fig. 2), however, did not abolish expression of *prgB* (7), indicating that ORFq is not involved in activation of *prgB*. The abundance of translational stop codons in all three possible reading frames (data not shown) and the lack of other ORFs with potential ribosomal binding sites in this region make it unlikely that any polypeptides, other than the inhibitor peptide, iCF10, are translated from Q_S or Q_L RNA. This suggests that these RNA molecules might be directly involved in the activation of *prgB*.

Two inverted repeat sequences (IRSs) are located downstream from *prgQ* (Fig. 2). The first IRS (IRS1) resembles a typical factor-independent transcriptional terminator (4) which could form a stable stem-loop structure ($\Delta G = -31.8$ kcal) because of its high G+C content. The second IRS (IRS2) is located approximately 130 bp downstream from IRS1 and could form a less stable stem-loop structure ($\Delta G = -15.2$

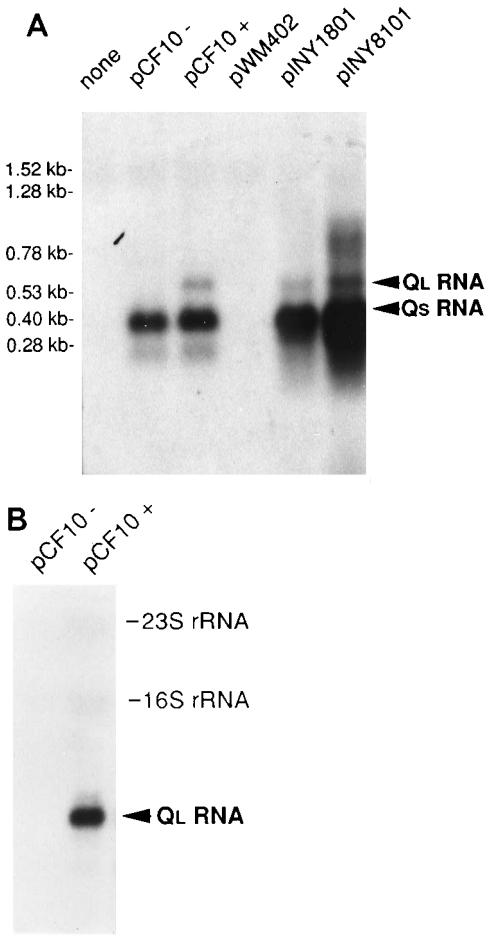


FIG. 1. Northern blot analysis of the *prgQ* region. The plasmid content of each OG1RF strain from which RNA was extracted is indicated above each lane. pCF10− and pCF10+ indicate that cells were not induced (−) or were induced (+) with pheromone cCF10 prior to the extraction. The positions of the small-molecular-size RNA standards (Gibco/BRL) are indicated to the left of the blot. The blot was probed with R01999-17 (A) or with R02273-17 (B). The calculation of the size of the transcript shown in panel B was based on its migration in the gel relative to both rRNA standards and the small-molecular-size standards described above; only the positions of the rRNAs are shown in the photograph. The locations of the pCF10 sequences complementary to the probes used to detect these RNAs are shown below in Fig. 2.

kcal). The fact that the distances between the putative promoter region of *prgQ* and these IRSs corresponded to the sizes of these two RNA species suggested that the transcription of Q_S and Q_L RNA might start at the same site but terminate at two different positions (Q_S RNA at IRS1 and Q_L RNA at IRS2).

To identify the transcriptional start sites of both Q_S and Q_L RNA, a primer extension experiment was performed with RNA extracted from OG1RF(pCF10) (uninduced), OG1RF (pCF10) (induced), and OG1RF(pINY1801). Identical extended product was detected in each lane (no additional signal increased in size by ~130 nucleotides was seen in Q_L-containing reactions), suggesting that both Q_S and Q_L RNA were transcribed from the same start site. This experiment was repeated several times with different RNA preparations from all strains; in most cases, the A residue indicated in Fig. 2 was the apparent start site. A second, and usually less intense, signal was also observed at the T residue 1 base upstream. Occasionally, we observed some variation in the ratio of the two signals, with the T residue being predominant as is seen in the pINY1801 mRNA shown in Fig. 2. Because computer analysis predicts that the Q RNA species would contain extensive secondary structure in regions >100 nucleotides from the 5′ end, it was necessary to use a primer very close to the start; this may have contributed to this slight variation in the observed start site. In spite of this problem, these results indicated that the difference between Q_S and Q_L RNA was very likely in the 3′ end of the transcripts. In addition, this experimentally determined *prgQ* transcriptional start site agrees with that suggested by analysis of the sequence and is consistent with the observed phenotypes of mutant plasmids containing Tn*5* insertions in a region described in the accompanying article (7) that was postulated to be the *prgQ* promoter. The presence of two different stem-loop structures in the nucleotide sequence 3′ from *prgQ* and the distance (approximately 130 nucleotides) between these two stem-loop structures support the hypothesis that these structures are found at the 3′ ends of Q_L and Q_S RNAs. Northern blot analysis was carried out using a probe (Fig. 2) complementary to the region between IRS1 and IRS2. Only Q_L RNA was detected in RNA prepared from induced cells carrying pCF10 (Fig. 1B), indicating that transcription of Q_S RNA can be extended through IRS1 in OG1RF(pCF10) in the presence of pheromone.

RNA extracted from OG1RF (pINY8101; Fig. 1A) was also tested by Northern blot analysis to determine whether the absence of the whole *prgB* gene and/or the presence of the *lacZ* gene had any effects on the transcription of the Q_L RNA. As shown in Fig. 4, Q_L RNA was produced normally in OG1RF cells carrying pINY8101.

***prgR* and *prgS*.** Previous sequencing analysis suggested that *prgR* and *prgS* might form an operon (18). To test this hypothesis, Northern blot analysis was performed with oligonucleotide probes to three different portions of the *prgR* and *prgS* region. No transcript could be detected for the *prgR* region (data not shown), using RNA samples obtained from several independent preparations and two different probes to *prgR* (Fig. 3). With the same RNA samples, however, a transcript of approximately 400 nucleotides was detected from the *prgS* region and was produced constitutively both in OG1RF(pCF10) and OG1RF(pINY1801); OG1RF(pINY1801) or pheromone-induced OG1RF(pCF10) did appear to produce a higher level of this mRNA.

These data suggested the following possibilities: (i) *prgR* is not transcribed in *E. faecalis*; (ii) *prgR* is transcribed independently of *prgS*, but the half-life of *prgR* mRNA is much shorter than that of *prgS* mRNA, resulting in an undetectable *prgR* transcript by the methods used in this study; or (iii) *prgR* and *prgS* form an operon, but the 5′ portion of mRNA encoding *prgR* is degraded much faster than that of *prgS*. Primer extension analysis of *prgS* mRNA (data not shown) suggested that the transcript might initiate 30 to 35 bp downstream from the putative TTG start codon of the *prgS* structural gene (18), indicating that the functional product of *prgS* might not be the protein predicted by DNA sequence analysis. However, as noted below in the Discussion, there is efficient processing of the primary *prgS* transcript which would have precluded detection of the actual initiation point of the *prgS* message.

RNA extracted from OG1RF(pINY8101) and OG1RF (pINY8109) (in which *prgX* and *prgQ* were deleted from pINY8101) was also examined by Northern blotting to determine whether the deletions of *prgB* (pINY8101) and *prgQ* (pINY8109) or the presence of *lacZ* (pINY8101) has any effect on the transcription of the *prgS* region. As shown in Fig. 3,

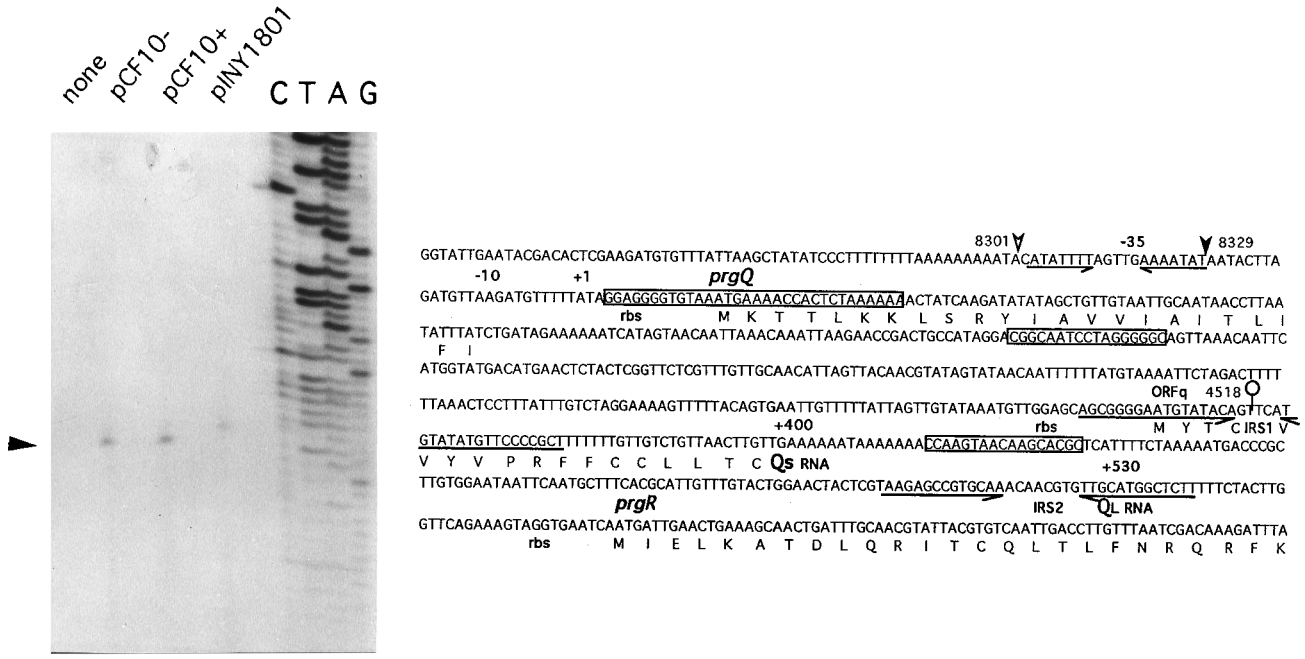


FIG. 2. Primer extension analysis of *prgQ* mRNA. Each lane contains RNA extracted from *E. faecalis* strains containing plasmids indicated above the gel. Sequences around the *prgQ* region are shown. The approximate start site of transcription (+1), the −10 and −35 boxes of the putative promoter region, and ribosomal binding sites (rbs) are indicated. IRSs are indicated by inverted horizontal arrows below the sequence. Oligonucleotides complementary to the probe used in primer extension analysis (R01877-31 indicated in the boxed sequence in the second row of sequence from the top) and Northern blot analysis (R01999-17, box in third row from the top; R02273-17, box in the sixth row from the top) are shown. The locations of the estimated 3′ ends of Q_S and Q_L RNA transcripts are also indicated. ORFs for *prgQ* and ORFq are shown with corresponding rbs. The locations of deletion endpoints (▽) and Tn*5* insertion sites (⚲) of pINY8301, -8329 and -4518 are indicated; open symbols indicate that the construct expressed Asc10, while closed symbols indicate that Asc10 was not expressed by the construct.

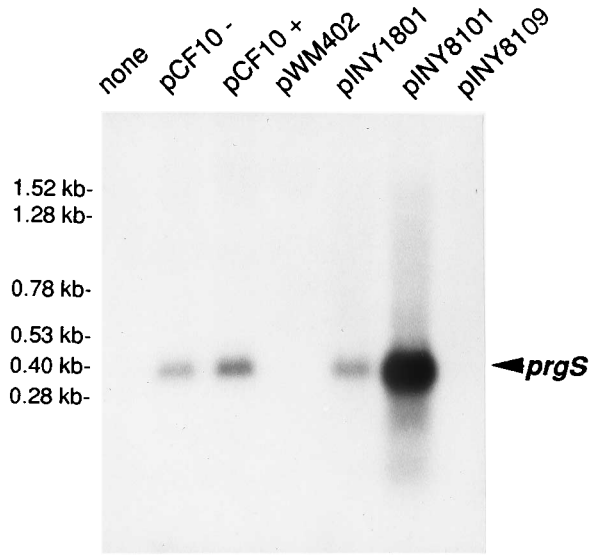
OG1RF(pINY8101) produced the *prgS* transcript. OG1RF cells carrying pINY8109, however, did not produce the transcript of *prgS*. This result indicates that the *prgQ* region is essential for the transcription of *prgS*, suggesting that either the inhibitor peptide (iCF10), Q_S RNA, or a *cis*-acting DNA sequence may be required for the transcription of *prgS*. Q_L RNA may not be required for the transcription of *prgS*, since significant *prgS* transcription occurs constitutively, whereas Q_L RNA transcription is found only in pheromone-induced OG1RF (pCF10).

Effect of mutation in *prgS* on the expression of Q_L RNA. Although earlier studies demonstrated that *prgS* was essential for the expression of *prgB* (18), its function has been unknown. One possibility is that the gene product of *prgS* might be involved in the production of Q_L RNA, perhaps by causing antitermination of the transcription of Q_S RNA or by stabilizing Q_L. To test this possibility, Northern blot analysis was performed to examine the production of Q_L RNA in strain OG1RF carrying a plasmid with a Tn*5* insertion in *prgS* (pINY4501). As shown in Fig. 4, only Q_S RNA was detected with a probe (R01999-17) which normally detects both Q_S and Q_L RNAs, indicating that *prgS* is required for the production of Q_L RNA. The negative-control system might interfere with the function of the gene product of *prgS*, thus preventing synthesis or stabilization of Q_L RNA and the subsequent transcription of *prgB*.

DISCUSSION

In the accompanying article (7), a genetic analysis utilizing deletions and transposon insertions in the positive-control region of the pCF10 conjugative transfer genes indicated that the *prgQ* and *prgS* genes, as well as sequences 3′ to the ORFs corresponding to these genes, were required for activation of the *prgB* gene encoding the aggregation protein Asc10. Phenotypic analyses of certain regulatory mutants suggested three interesting features of the system, including the following: (i) one or more RNA products transcribed from the pCF10 DNA 3′ from the ORF corresponding to *prgQ* might be involved in positive regulation, (ii) mRNA processing, especially of the *prgS* message, might affect expression of these genes, and (iii) at least some Tn*5* insertions in the regulatory region might generate outward reading promoters that could affect the expression of genes downstream from the insertion and complicate determination of the operon structure of these genes.

This article presents the results of physical analyses of the mRNA species transcribed from the positive-control region in pheromone-induced or uninduced cells carrying both the positive- and negative-control genes of wild-type pCF10, as well as analysis of mRNA encoded by cells carrying various cloned regions of pCF10. The most significant result of this study is the identification of RNA species called Q_S and Q_L, which appear to be transcribed from the same *prgQ* promoter (Fig. 1 and 2). Both of these RNAs appear to be identical at the 5′ end but extend well beyond the 69-bp ORF corresponding to *prgQ*, which encodes the inhibitor peptide, iCF10 (22). On the basis of Northern blot analyses, we estimate the 3′ end of each of these RNAs to be in the vicinity of an IRS, IRS1 for Q_S and IRS2 for Q_L (Fig. 2). Further analysis showed that in wild-type donor cells, Q_L was inducible by pheromone, as was the *prgB* target gene, whereas Q_S was produced constitutively (Fig. 1). Interestingly, disruption of Q_S also abolished expression of the constitutively transcribed *prgS* (Fig. 3) and *prgA* (7) genes,



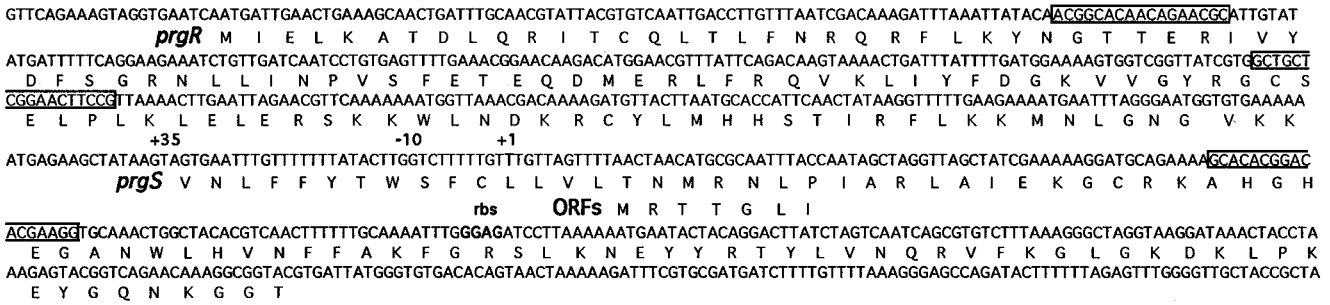


FIG. 3. Northern blot analysis of the *prgR* and *prgS* region. The plasmid content of each OG1RF strain from which RNA was extracted is indicated above the lane. pCF10− and pCF10+ indicates that cells were not induced (−) or were induced (+) with pheromone cCF10 prior to the extraction. The positions of the small-molecular-size RNA standards (Gibco/BRL) are indicated to the left of the blot. The oligonucleotides complementary to the sequences of the probes (R02510-17, box in top row of sequence; R02657-17, box in third and fourth rows; R02909-17, box in fourth and fifth rows) used to detect each transcript are shown.

while insertion into *prgS*, eliminated Q_L (Fig. 4). The simplest interpretation of these data is that in wild-type donors grown in the absence of pheromone, the Q_S transcript is produced constitutively and either terminates in the region of IRS1 or is rapidly generated from processing of a longer product. According to this model, this RNA species is required for transcription of *prgS* and *prgA*. In the presence of pheromone, the negative-control system is abolished, presumably allowing for either antitermination of the Q message at IRS1 or stabilization of the larger Q_L message; in either case, the product of *prgS* is apparently required. Recently, we have found that introduction of a nonsense codon in *prgS* abolishes positive control, suggesting that the functional product of *prgS* is a protein (3). In view of this result, along with the transcriptional analysis of *prgS* described in this article (Fig. 3 and text) and the effects of Tn*5* insertions 3′ from *prgS* presented in the accompanying article, it is very likely that the *prgS* message is subject to specific processing. In any case, the presence of a significant amount of Q_L in the cell would allow for expression of *prgB*. Interestingly, the Q_L message is the only product of the positive-control region that is inducible by pheromone, and the available evidence suggests that the control mechanism does not involve regulation of the initiation of *prgQ* transcription. It is also noteworthy that a regulatory RNA molecule was shown

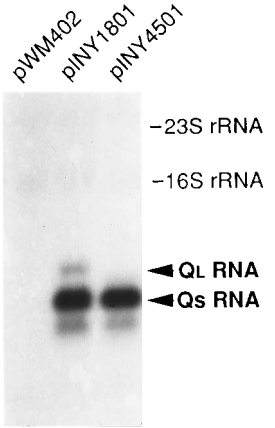


FIG. 4. Effect of a mutation in *prgS* on the expression of Q_L RNA. Northern blot analysis was performed to examine the effect of a mutation in *prgS* on the expression of Q_L RNA. The plasmid content of each OG1RF strain from which RNA was extracted is indicated above each lane. The oligonucleotide complementary to the sequences of the probe (R01999-17) is shown in Fig. 2.

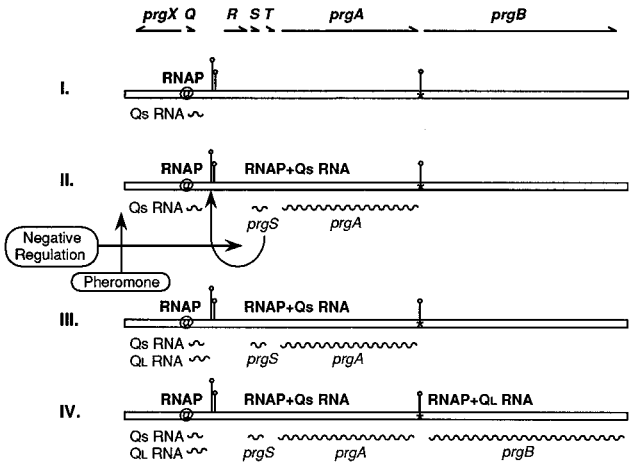


FIG. 5. Model for the regulation of the expression of *prgB*. (I) RNAP transcribes Q_S RNA. (II) RNAP and Q_S RNA complex transcribes *prgS* and *prgA*. (III) PrgS causes antitermination and allows Q_L RNA to be transcribed. (IV) RNAP and Q_L RNA complex transcribes *prgB*. The putative promoter region of *prgQ* (@), the promoter region of *prgB* (*), and stem-loop structures (♀) are shown.

to be involved in the production of staphylococcal virulence factors (23).

On the basis of evidence obtained in the pCF10 system to date, the following model can be proposed to explain how the upstream positive regulatory elements might function in *cis* and in an orientation-dependent manner in order to activate the expression of *prgS*, *-A*, and *-B*. Since Q_S RNA appears to be required in *cis* for the expression of *prgS* and *prgA*, it can be envisioned that upon completion of its transcription, Q_S RNA immediately binds to a chromosomally encoded host factor, for example, an RNA polymerase (RNAP) subunit, thus modifying RNAP in a way that it would reduce dissociation from the DNA template and induce tracking in the direction of *prgA* and *prgB* (Fig. 5). The RNAP and Q_S RNA complex might then recognize promoters of *prgS* and *prgA* and initiate transcription of these genes but fail to recognize the promoter of *prgB*. In the presence of pheromone or by deletion of the negative-control region, the gene product of *prgS* could allow production of Q_L RNA. Like Q_S RNA, Q_L RNA, which is an extended product of Q_S RNA, might also associate with RNAP. Because of the sequence homology between the end of Q_L RNA and the promoter region of *prgB* (Fig. 6), Q_L RNA could be involved in recognition of the *prgB* promoter region, allowing RNAP to initiate transcription of the *prgB* gene. Alternatively, the Q_S and Q_L RNAs might bind to pCF10 DNA near their own promoter region, probably with other unidentified gene products, and track along DNA in a specific orientation and recognize promoters of target genes, allowing RNAP to bind and initiate transcription.

Genetic analysis of the pheromone-inducible conjugative hemolysin plasmid pAD1, involving Tn*917*::*lacZ* mutagenesis, suggested that *asa1*, encoding aggregation substance of pAD1, might be activated by an antitermination and transcriptional readthrough of the *iad* gene (16, 25, 29). The *iad* gene encodes the inhibitor iAD1 of pAD1 and is positioned in a location similar to that of *prgQ* of pCF10 (10, 22). Transcriptional analysis demonstrated that transcription of *asa1* was initiated near the 5′ end of its structural gene (16). Further studies indicated that the positive regulator in the pAD1 system, TraE1, appears to function in *trans* to activate the *asa1* gene (21, 27). These data, combined with the result that DNA sequence and deduced amino acid sequence analysis of the positive-control regions of pCF10 and pAD1 showed little homology, suggest that different positive regulatory mechanisms might be involved in these two plasmids.

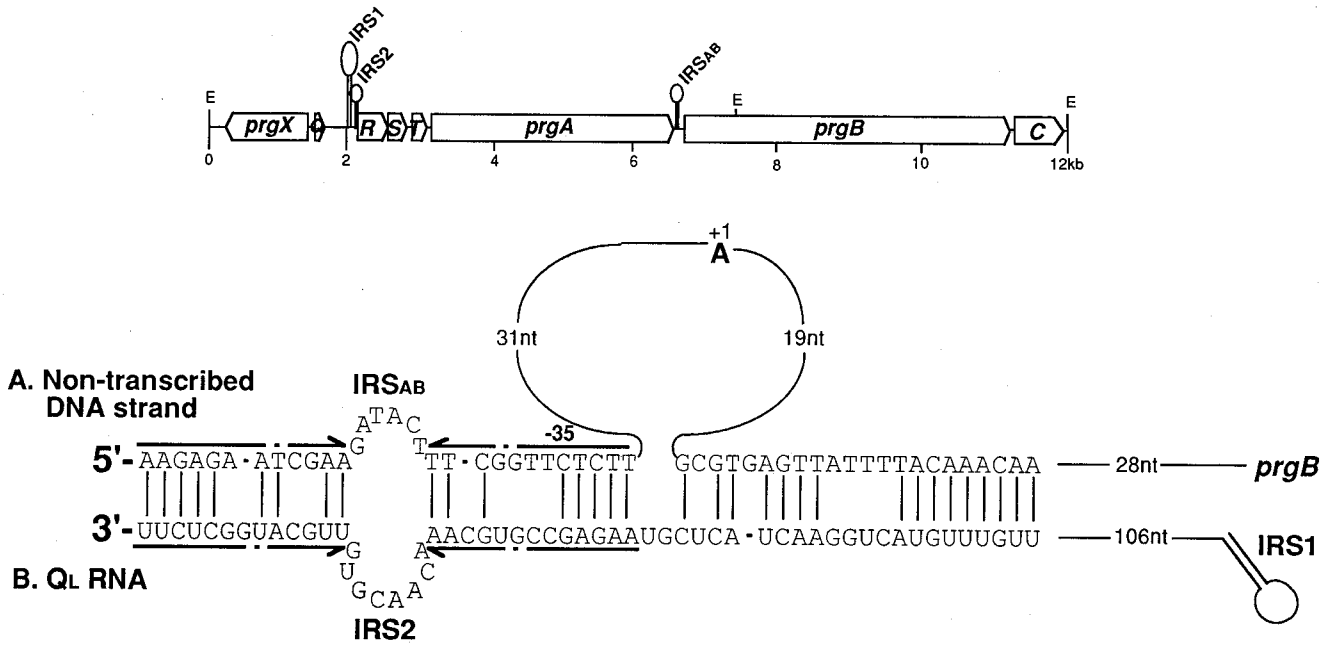


FIG. 6. Sequence homology between the 3′ end of Q_L RNA and the promoter region of *prgB*. Genetic organization of the pCF10 *Eco*RI C and E fragments is shown on top. The nontranscribed promoter region of *prgB* is shown as it aligns with the 3′ end of Q_L RNA. IRSs in the region are indicated. The +1 represents the 5′ end of the *prgB* transcript. The −35 region of the *prgB* promoter is also indicated.

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