

Cis-acting, orientation-dependent, positive control system activates pheromone-inducible conjugation functions at distances greater than 10 kilobases upstream from its target in *Enterococcus faecalis*

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ABSTRACT The *prgB* gene encodes the surface protein, Asc10, which mediates cell aggregation, resulting in high-frequency conjugative transfer of the pheromone-inducible tetracycline-resistance plasmid pCF10 in *Enterococcus faecalis*. Messenger RNA analysis by Northern blot hybridization and primer extension indicates that *prgB* transcription is pheromone-inducible and monocistronic. Previous transposon mutagenesis and sequencing analysis of a 12-kilobase (kb) region of pCF10 indicated that several genes including *prgR* and *prgS* are required to activate expression of *prgB*. The distance (3–4 kb) between these regulatory genes and *prgB* suggested that the activation might function in trans. To test this, a promoterless *lacZ* gene fusion to *prgB* was constructed and cloned without some or all of the regulatory genes. Several restriction fragments of the regulatory region were cloned in a higher copy-number plasmid, and numerous complementation studies were carried out in *E. faecalis*. Complementation in trans was not observed in any of these experiments. However, when the regulatory region and target genes were cloned in different sites of the same plasmid, separated by as much as 12 kb, activation of *prgB* was observed. Interestingly, this activation occurred only when the regions were cloned in the same relative orientation in which they exist on wild-type pCF10. These results suggest that one or more regulatory molecules may bind to an upstream cis-acting site and track along the DNA to reach a target site to activate *prgB* transcription.

High-frequency conjugal transfer of certain *Enterococcus faecalis* plasmids in liquid mating is induced by small hydrophobic peptide pheromones excreted by recipient cells (1–3). Exposure of donor cells carrying the 58-kilobase (kb) tetracycline-resistance plasmid pCF10 to the heptapeptide pheromone, cCF10, initiates a complex response involving activation of the expression of a number of plasmid-encoded genes whose products are required for the formation of mating aggregates and the physical transfer of the plasmid DNA (2).

Previously, Tn917 insertional mutagenesis analysis showed that a 25- to 30-kb region of pCF10 encodes the pheromone-inducible conjugation system; at least 10 kb of this region is involved in positive and negative regulation of the pheromone response (4). Molecular cloning, Tn5 mutagenesis, and DNA sequencing revealed that the 7.5-kb *EcoRI* c fragment and the adjacent 4.5-kb *EcoRI* e fragment of pCF10 encode several positive control genes and two structural genes, *prgA* and *prgB*, encoding the surface antigens Sec10 and Asc10, respectively (Fig. 1A) (5, 6). Asc10 is a 150-kDa surface adhesin (aggregation substance) mediating the formation of cell aggregates (7). Sec10 is a 130-kDa surface protein involved in surface (entry) exclusion, which prevents donor–donor mating (8). Negative control, encoded

by genes mapping to the left of the *EcoRI* c fragment, can be inactivated physiologically by addition of pheromone or eliminated genetically by either deletion or insertional mutagenesis of the negative control region of pCF10 (Fig. 1A) (4). In either case, the positive regulation system then activates the expression of genes involved in aggregation and plasmid transfer between donor and recipient cells. Genes involved in plasmid DNA transfer between aggregated cells are believed to be located to the right of the *prgB* gene (6).

Several Tn5 insertions in the region containing *prgR* and *prgS* abolished the expression of *prgB* but had no effect on the expression of *prgA*, which is located between this regulatory region and *prgB*. Furthermore, Tn5 insertions in *prgA* did not affect the activation of *prgB* by this upstream positive control region (5, 6). The fact that this regulatory system functions not only at a distance of 3–4 kb from the target gene, *prgB*, in the case of wild-type pCF10 but also at the distance of 8–9 kb in the case of Tn5 insertion mutants of *prgA*, suggested that *prgR* and *prgS* may encode trans-acting regulatory molecules that activate the expression of *prgB*.

Tn5 insertions in the *prgX* gene appeared to be lethal in an *E. faecalis* background, since the transfer of plasmids carrying these insertions into *E. faecalis* resulted in severe deletions or rearrangements in the plasmid DNA (6). A gene (69 bp) to the right of *prgX* [previously designated as open reading frame 3 (6)], henceforth called *prgQ*, appears to encode a pheromone inhibitor that specifically and competitively inhibits cCF10 activity (unpublished data). As yet it has not been determined whether *prgQ* may have an additional role in control of *prgB* expression. We also have no genetic information about the putative gene product encoded by *prgT* because of the lack of Tn5 insertions in the gene, but DNA sequencing showed that there is some similarity (25–30% residue identity) between the *prgT* gene product and the *hutP* gene product of *Bacillus subtilis* (6). The latter product is required to activate transcription of genes involved in utilization of histidine (9), suggesting that the *prgT* gene product could be a DNA-binding protein involved in the positive regulation of the *prgB* expression along with the *prgR* and *prgS* gene products. The predicted structures of the *prgX*, *prgR*, or *prgS* gene products have no significant homology with previously sequenced regulatory proteins from prokaryotes or eukaryotes (6).

In this communication we present the results of mRNA analysis indicating that *prgB* is transcribed in a pheromone-inducible fashion as a monocistronic message. To investigate the mechanism of this positive regulatory system further, we constructed a transcriptional fusion in the *prgB* structural gene with a promoterless *lacZ* gene (Fig. 1B) and performed complementation analysis providing a number of different

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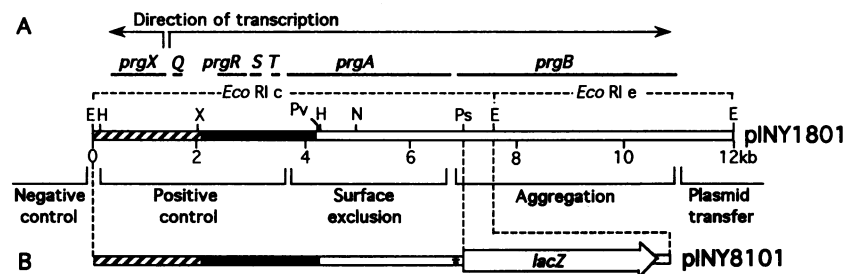


FIG. 1. (A) Physical map of the *EcoRI* c and e fragments of pCF10. Direction of transcription of each gene and relevant restriction enzyme sites in *EcoRI* c (7.5 kb) fragment are indicated as well as fragments used to construct plasmids pINY8401 (*Xba* I–*Hind*III fragment shown as a solid bar), pINY8402 (*EcoRI*–*Pvu* II fragment shown as solid and crosshatched bars) and pINY8403 (*EcoRI*–*Xba* I fragment shown as a crosshatched bar). E, *EcoRI*; H, *Hind*III; N, *Nsi* I; Ps, *Pst* I; Pv, *Pvu* II; X, *Xba* I. The *Hind*III, *Nsi* I, and *Pvu* II sites are located 589, 1231, and 573 bp downstream from the 5' end of the *prgA* structural gene, respectively. The *Xba* I site is located 168 bp downstream from the 3' end of the *prgQ* structural gene and 316 bp upstream from the 5' end of the *prgR* structural gene. Plasmid pINY1801 (5) contains the *EcoRI* c and e fragments in the *EcoRI* site of pWM402 (11). (B) Construction of the *prgB*–*lacZ* fusion. A promoterless *lacZ* gene is inserted into the *Pst* I site in the same orientation as *prgB*. The start site of the *prgB* transcript is shown with an asterisk. Plasmid pINY8101 contains this fragment in the *EcoRI* site of pWM402.

segments of the regulatory region either in trans or in cis to the *prgB*–*lacZ* fusion. We present data indicating that, despite the distance (3–4 kb) between the regulatory genes and the *prgB* gene, the positive regulatory system for the activation of the *prgB* gene works only in cis and in an orientation-dependent manner. We also demonstrate that other sequences to the left of the *prgR* gene are also involved in positive regulation, in addition to the gene products of the *prgR*–*prgS*–*prgT* region.

MATERIALS AND METHODS

Bacterial Strains, Reagents, and DNA Manipulation. *Escherichia coli* DH5 α (Bethesda Research Laboratories) strains were grown in Luria–Bertani broth and *E. faecalis* OG1RF (4) strains were grown in M9–YE (8) medium. The concentrations of antibiotics in selective media were tetracycline (10 μ g/ml), chloramphenicol (15 μ g/ml), and kanamycin (25 μ g/ml) in *E. coli* culture medium and 150 μ g/ml in broth and 750 μ g/ml in plates for *E. faecalis* culture).

Plasmid DNA was isolated from *E. coli* and *E. faecalis* as described (6). All restriction fragments used in the construction of plasmids were separated by agarose gel electrophoresis, and the appropriate DNA bands were excised from the gel and purified with GeneClean (Bio 101, La Jolla, CA) 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 to facilitate the cloning processes (10).

Strain and Plasmid Constructions. The shuttle vectors used for plasmid constructions were pWM401 and its derivative pWM402 (see Fig. 5, line a) (11) derived from the *E. coli* plasmid pACYC184 and the streptococcal plasmid pIP501. Plasmid pUT2100 is a derivative of pTV54 (12) in which *Sma* I and *Bam*HI sites were inserted in front of the ribosomal binding site of the promoterless *lacZ* gene (personal communication, S. Zahler of Cornell University). The *prgB*–*lacZ* transcriptional fusion was constructed by cloning the 3.3-kb *Sma* I fragment of the *lacZ* gene of pUT2100 into the blunted *Pst* I site of *EcoRI* c fragment of pCF10 on the shuttle vector pWM402 (Fig. 1B). This *Pst* I site is located 222 base pairs (bp) downstream from the 5' end of the *prgB* structural gene (6). Plasmid pUC4–Omega–Km2 (13) contains an *omega* element (14) and the *aphA3* kanamycin-resistance gene (15). A 2.2-kb *Sma* I fragment of pUC4–Omega–Km2 containing the *omega*–Km2 element was inserted into the blunted *Nsi* I site of the *prgA* gene to stop the transcription and the translation of *prgA* (see Fig. 4). A high-copy-number streptococcal plasmid pDL414 (16) was used to clone several

different restriction fragments of the upstream regulatory region. Transformation of *E. coli* and *E. faecalis* with the constructed plasmids was done by electroporation (17). Plasmid content of the transformants was confirmed by restriction enzyme digestion of plasmid preparations, analyzed by agarose gel electrophoresis.

Isolation and Analysis of RNA. Total cellular RNA was extracted from *E. faecalis* as described by Shaw and Clewell (18) and treated with RNase-free DNase (19). The overnight culture was diluted 1:3 with fresh M9–YE [M9–YE containing cCF10 was used for induction of OG1RF(pCF10)] and incubated for an additional 90 min before the extraction. RNA (Northern) blot analysis and primer extensions were performed as described (19). For Northern blot analysis, concentration of RNA was calculated from A_{260} and A_{280} , and equivalent quantities (30–40 μ g) of RNA were loaded. The blot for *prgB* mRNA was hybridized with a nick-translated plasmid probe pINY4429 (6), which carries an internal fragment of the *prgB* structural gene. Oligonucleotides used as a probe (17-mer) for Northern blot analysis of *prgA* mRNA or as a primer (35-mer) for primer extension were synthesized by using an Applied Biosystems 391 automated synthesizer and end-labeled (19).

β -Galactosidase Assays. β -Galactosidase activity was assayed as detailed by Miller (20) with the following modifications. The overnight culture was diluted 1:4 with fresh M9–YE and grown for an additional hour. Assays were conducted with 0.8 ml of the culture and incubated for 20 min.

RESULTS

Analysis of the *prgB* mRNA. To determine the sizes of the *prgA* and *prgB* transcripts and whether the transcription was inducible by pheromone, Northern blot analysis was performed. A hybridizing 2.8-kb mRNA was seen in RNA prepared from strains OG1RF(pCF10, uninduced), OG1RF(pCF10, induced), and OG1RF(pINY1801) by using a *prgA* probe (Fig. 2A). With a *prgB* probe, a hybridizing 4.1-kb mRNA was seen in RNA prepared from strains OG1RF(pCF10, induced) and OG1RF(pINY1801) (Fig. 2B), showing that a distinct transcript was associated with each open reading frame. The results also showed that transcription of *prgA* is constitutive, whereas transcription of *prgB* is inducible by pheromone in wild-type pCF10. In pINY1801, *prgB* was transcribed constitutively, consistent with previous results showing that the aggregation substance was produced constitutively in OG1RF(pINY1801) (7). The sizes of these transcripts in relation to those of their open reading frames (2667 bp for *prgA* and 3923 bp for *prgB*) indicate that both

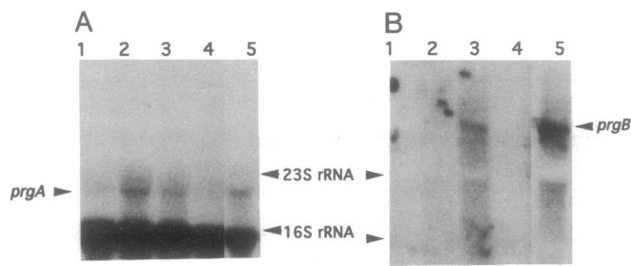


FIG. 2. Northern blot analysis of *prgA* mRNA and *prgB* mRNA production. The lanes contain RNA from the following *E. faecalis* strains: 1, OG1RF; 2, OG1RF(pCF10, uninduced); 3, OG1RF(pCF10, induced); 4, OG1RF(pWM402); and 5, OG1RF(pINY1801). (A) Blot probed with an oligonucleotide complementary to the 5' terminus of *prgA*. (B) Blot probed with a plasmid that carries an internal fragment of the *prgB* structural gene. Lane 5 in B is the same blot as the other lanes but exposed for a shorter period of time.

transcripts are monocistronic. However, it is possible that a large transcript could be made and processed to generate distinct sizes of smaller transcripts.

To confirm that the *prgB* transcript starts at the same site both in OG1RF(pCF10) and OG1RF(pINY1801) and to locate the promoter region more precisely, primer extension analysis was done with a synthetic oligonucleotide complementary to the DNA sequence located between 38 and 74 bases downstream of the 5' end of the *prgB* coding sequence (6). The same transcriptional start site was identified in both OG1RF(pCF10, induced) and OG1RF(pINY1801) (Fig. 3). Interestingly, the -35 region falls into the inverted repeat (IR) sequence that was thought to be involved in the termination of the *prgA* transcription, suggesting that the same IR sequence may be involved in the termination of *prgA* transcription as well as the initiation of *prgB* transcription. A similar arrangement was observed in *Streptococcus pyogenes*, where the -35 region of the promoter of the M6 protein structural gene overlaps the inverted repeat of the transcriptional terminator of the upstream gene *mry* (13).

Trans-Activation of *prgB*. Previous studies demonstrated that the insertions of Tn5 in the region containing *prgR* and *prgS*, which is located 3–4 kb upstream of the 5' end of *prgB*, abolishes *prgB* expression (5, 6). We wished to examine whether *prgR* and *prgS* could restore expression of *prgB* in trans. First, we inserted a promoterless *lacZ* gene as a reporter into the *prgB* structural gene to facilitate complementation assays. To examine complementation of *prgR*, *prgS*, and *prgB* in the *E. faecalis* background, we deleted the *prgR* and *prgS* region by subcloning the 6.6-kb *Hind*III–

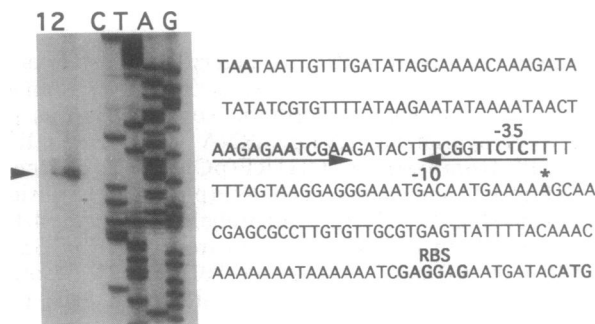


FIG. 3. Primer extension analysis of *prgB* mRNA. The lanes contain RNA from the following *E. faecalis* strains: 1, OG1RF(pCF10, induced); and 2, OG1RF(pINY1801). The approximate start site of transcription (*), the -10 and -35 boxes of the putative promoter, and the putative ribosomal binding sequence (RBS) are indicated. A 30-bp interrupted dyad symmetric element is indicated by thick horizontal arrows below the sequence.

*Eco*RI fragment of pINY8101 (Fig. 1B) containing 80% of the 3' end of the *prgA* structural gene and the *prgB-lacZ* fusion into pWM402 in an *E. coli* background. As expected, the expression of β -galactosidase activity seen in strain OG1RF(pINY8101) (Fig. 4, line b) was completely eliminated in strain OG1RF(pINY8110) by the deletion of the upstream regulatory region (Fig. 4, line d).

Several different restriction fragments containing portions of this regulatory region were cloned on the high-copy-number streptococcal plasmid pDL414, generating plasmids pINY8401, pINY8402 and pINY8403 (Fig. 4, lines e, f, and h). The introduction of pINY8401 containing *prgR*, *prgS*, and *prgT* in trans with pINY8110 did not restore β -galactosidase activity as shown in Fig. 4, line e. This result suggested several possibilities as follows: (i) that one or more additional upstream gene products such as *prgX* or *prgQ* may also be involved directly in the activation of *prgB* expression, in the activation of *prgR* and *prgS*, or both; (ii) that the gene products of *prgR* and *prgS* are actually required for the expression of *prgX* or *prgQ*, which in turn activates the expression of *prgB*; and (iii) that although the *Xba* I site used in the construction of pINY8401 is located considerably upstream (316 bp) from the 5' end of the *prgR* structural gene, this construct may not provide the full promoter elements of *prgR*, thus preventing *prgR* expression. To examine these possibilities, we cloned the intact upstream regulatory region carried on the 4.2-kb *Eco*RI–*Pvu* II fragment of the *Eco*RI c fragment of pCF10 to construct pINY8402 and introduced the region in trans into strain OG1RF(pINY8110). However, no complementation activity was detected (Fig. 4, line f). This result indicated that the region with *prgR*, *prgS*, and *prgT* may function in cis to activate *prgB* transcription. However, β -galactosidase activity was also abolished in OG1RF(pINY8109) (Fig. 4, line g), where only the 2.1-kb *Eco*RI–*Xba* I fragment encoding *prgX* and *prgQ* was deleted from pINY8101, leaving the region with *prgR*, *prgS*, and *prgT* intact with the *prgB-lacZ* fusion. Neither the introduction of *prgX* and *prgQ* on pINY8403 nor the entire upstream regulatory region containing *prgX*, *prgQ*, *prgR*, *prgS*, and *prgT* on pINY8402 restored β -galactosidase activity in trans (Fig. 4, lines h and i). In the latter construct, about 5–10% of the transformants formed blue colonies on X-Gal (5-bromo-4-chloro-3-indolyl β -D-galactoside) plates after a 48-hr incubation period, indicating that β -galactosidase was expressed. Restriction enzyme digestions of plasmid preparations of

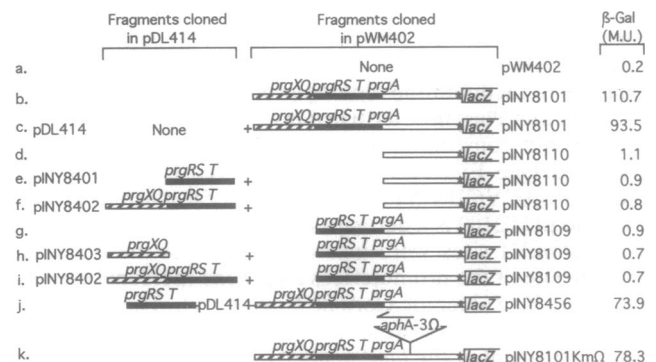


FIG. 4. Trans-activation of *prgB*. Each bar contains the following genes: open bar, 80% of the 3' end of the *prgA* structural gene and the *prgB-lacZ* fusion; cross-hatched bar, *prgX* and *prgQ*; and solid bar, *prgR*, *prgS*, *prgT*, and 20% of the 5' end of the *prgA* structural gene. The open triangle above pINY8101Km Ω indicates the site and the orientation of the insertion of the *omega*-Km2 element. Restriction enzyme sites used to construct each plasmid are *Eco*RI site of pWM402 for pINY8101 and pINY8101Km Ω ; *Eco*RV site of pWM402 for pINY8110 and pINY8109; *Hinc*II site of pDL414 for pINY8401 and pINY8403; and *Eco*RI and *Hinc*II sites of pDL414 for pINY8402.

these β -galactosidase-positive transformants showed that a recombinant plasmid was generated by homologous recombination of pINY8109 and pINY8402 (Fig. 4, line j). In the colonies showing no β -galactosidase activity, no recombinant plasmid DNA was detected. These data indicated that the upstream positive regulatory region has to be located in cis with *prgB* and that one or more elements encoded by the 2.1-kb *EcoRI*-*Xba* I fragment of the *EcoRI* c fragment of pCF10 (the *prgX*-*prgQ* region) are also required for the expression of *prgB*.

Cis-Activation of *prgB*. To confirm that the upstream regulatory region containing *prgX*, *prgQ*, *prgR*, *prgS*, and *prgT* functions in cis for the activation of *prgB*, we cloned both the 4.2-kb *EcoRI*-*Pvu* II fragment of the *EcoRI* c fragment of pCF10 and the *prgB*-*lacZ* fusion represented by the 6.6-kb *Hind*III-*EcoRI* fragment of pINY8101 into the same vector using several different restriction enzyme sites. Gene activation was only observed when the regulatory region was in the same relative orientation with respect to *prgB* as in wild-type pCF10 (in Fig. 5, compare line c with d and line e with f). The level of β -galactosidase activity increased as the distance between the two regions decreased (in Fig. 5, compare lines c, e, and g), but it was clear that the positive regulatory system functions even when the two regions are separated by as much as 12 kb (Fig. 5, line c) as long as they are in cis and in the proper orientation. Because plasmids are circular, the regulatory region in pINY8115 (Fig. 5, line d) is only about 5 kb from the *prgB* promoter region (this is closer than the corresponding distance in pINY8118 shown in Fig. 5, line e), if the relative orientation of the two regions is not considered. The fact that pINY8115 (Fig. 5, line d) does not express β -galactosidase activity further supports orientation dependence of the regulatory system.

Effects of the Introduction of a Transcriptional Terminator Between the Regulatory Region and *prgB*. To test whether the activation of the transcription of the *prgB* gene occurs by a transcriptional read-through of the *prgA* gene, the *omega* fragment, a transcriptional terminator which also possesses translational stop codons in all reading frames (15), was inserted into the middle of the *prgA* gene to construct plasmid pINY8101Km Ω (Fig. 4, line k). The result that the insertion of the *omega* element into the gene upstream of *prgB*, which would cause premature termination of the transcription of *prgA*, could not abolish the expression of the *prgB*-*lacZ*

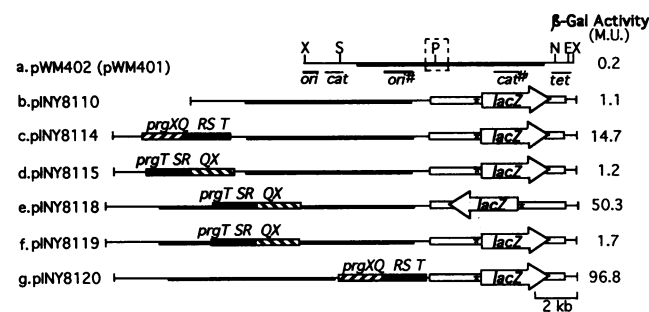


FIG. 5. Cis-activation of *prgB*. The shuttle vector plasmids pWM401 and pWM402 are shown in the line a. The streptococcal plasmid DNA is indicated as a darker line, whereas pACYC184 DNA is indicated as a lighter line. The locations of the origins of replication and the antibiotic resistance genes and the restriction enzyme sites used in cloning are also indicated. E, *EcoRV*; N, *Nru* I; P, *Pst* I; S, *Sca* I; X, *Xba* I. The 1.0-kb DNA fragment including the *Pst* I site shown in a dotted box was deleted in pWM402 (12). The open bar contains 80% of the 3' end of the *prgA* structural gene and the *prgB*-*lacZ* fusion along with the start site of the *prgB* transcript (*). The cross-hatched/solid bar contains the entire upstream positive regulatory region including *prgX*, *prgQ*, *prgR*, *prgS*, *prgT*, and 20% of the 5' end of the *prgA* structural gene.

fusion, as shown in Fig. 4, line k, indicates that the initiation of the transcription of *prgB* occurs at its own promoter region near the 5' end of the structural gene of *prgB*. The lower level of the β -galactosidase activity of this construct (Fig. 4, line k) compared with that of the wild type (Fig. 4, line b) is probably due to the additional distance generated by the insertion of the 2.2-kb DNA containing the *omega* element between the regulatory region and the *prgB* gene. The same effect was observed in the constructs where the regulatory region was cloned in cis to the *prgB*-*lacZ* fusion (Fig. 5, lines c and e).

DISCUSSION

In this report, we present evidence that the *prgB* gene encoding aggregation substance, Asc10, is positively regulated at the transcriptional level in cis by the upstream regulatory region containing *prgX*, *prgQ*, *prgR*, *prgS*, and *prgT*. A previous study (5) showing that Tn5 insertions in *prgA* did not affect the expression of *prgB* suggested that *prgB* is transcribed from its own promoter region because Tn5 insertions are known to be polar (21). The present data obtained from RNA analysis further support that *prgA* and *prgB* are transcribed independently. Results obtained from complementation analysis with various constructs of the regulatory region and the *prgB*-*lacZ* fusion clearly indicated that despite the distance (3–5 kb) between the two regions, the positive control system functions not only in cis but also in an orientation-dependent manner to activate the expression of the *prgB* gene. Recent Northern blot analysis of the *prgQ* region showed that the same transcript of the *prgQ* gene as the wild-type transcript was made in all of the constructs, whether the regulatory region encoding *prgX*, *prgQ*, *prgR*, *prgS*, and *prgT* was cloned in cis to *prgB*-*lacZ* (pINY8114 and pINY8115; Fig. 5, lines c and d) or was cloned separately in plasmid pINY8402 (Fig. 4, lines f and i) (data not shown). This result indicates that the lack of the complementation in the constructs listed in Fig. 4 is not because of the lack of the expression of the regulatory region in those constructs. The fact that strain OG1RF(pINY8109), where the 2.1-kb *EcoRI*-*Xba* I fragment (the *prgX*-*prgQ* region) was deleted, could not be activated in trans even with the entire upstream regulatory region (Fig. 4, line i) indicates (i) that in addition to the *prgR*-*prgS*-*prgT* region, one or more regulatory elements located further upstream from the *prgR* gene are required for the activation of *prgB*; and (ii) that one or more of these additional regulatory elements function in cis. These other regulatory elements may include a cis-acting site (presumably a DNA-binding site for one or more regulatory molecules) and possibly the gene product of *prgQ* or *prgX*. However, it is possible that *prgQ* or *prgX* is not involved in the activation of *prgB*. Instead, the 2.1-kb *EcoRI*-*Xba* I fragment may provide a promoter element for expression of the *prgR*-*prgS*-*prgT* region and the gene products of *prgR*, *prgS*, and *prgT* may function in cis to activate *prgB*.

The lack of similarity between the *prgR* and *prgS* gene products and other regulatory proteins suggested that a molecular mechanism different from previously analyzed gene regulation systems may be involved in the positive regulation of the pheromone-inducible plasmid transfer (6). This notion is supported by the present findings that the positive regulation system functions only in cis and in an orientation-dependent manner at 3–5 kb upstream from the target gene in the wild-type pCF10.

In several vertebrate genes, orientation-dependent 5' regulatory elements have been observed, but they are also shown to be position dependent and located in the intron closest to the promoter region (22). Most enhancers in eukaryotes and prokaryotes function either upstream or downstream of their target genes and are independent of orientation (23). The formation of DNA-DNA looping inter-

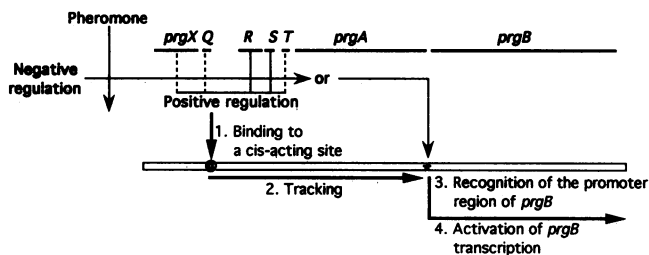


FIG. 6. Model for the regulation of the expression of *prgB*. The asterisk represents the promoter region of *prgB*, and "@" indicates a putative cis-acting site that may interact with one or more of the positive regulatory elements.

action between upstream regulatory sequences and the promoter region of a target gene via regulatory proteins has been shown to be involved in many cis-acting regulatory systems (24). However, the orientation-specific enhancement by an upstream activating sequence acting at such a large distance (up to 12 kb) from its target is difficult to explain by a looping model, since the flexibility of the DNA molecule should allow the two sites to align in either orientation.

The number of regulatory elements of the upstream regulatory region involved in this complicated positive transcriptional regulation of *prgB* and the way in which they interact with one another are unclear at this time. The simplest model which fits best with the currently available data is that one or more regulatory gene products may bind to an upstream cis-acting site and track in a specific direction to reach a target site to activate transcription of *prgB* (Fig. 6) (25). Theoretically, the transcription of *prgB* could be initiated at a point far upstream from its start codon—e.g., in the *prgQ* region—with antitermination occurring at the 3' end of *prgA* in the absence of a functional negative control system. In this case, some sort of RNA processing would generate the distinct sizes of *prgA* and *prgB* mRNAs, which were shown by Northern blot analysis, as well as the 5' end of the *prgB* mRNA identified by primer extension. This system would also have to be capable of reading through Tn5 insertions isolated throughout *prgA* as well as through several kilobases of both *E. coli* and streptococcal vector sequences in several of the constructs shown in Fig. 5. Alternatively, the tracking could occur by a mechanism other than transcription. In this type of model, the regulatory factors would move along the DNA, presumably from the region upstream of *prgR*, bind to a target site near the 5'-end of *prgB*, and initiate transcription at the experimentally determined start site. The fact that the insertion of the *omega* element, an efficient transcriptional terminator, in *prgA* could not abolish the activation of the *prgB* gene (Fig. 4) strongly supports the latter model. Negative regulatory elements encoded by genes located to the left of the *EcoRI* c fragment of pCF10 may repress either the expression or the function of the positive regulatory ele-

ments. In either case, an addition of pheromone relieves negative regulation, allowing activation of *prgB* by the positive control system. In the bacteriophage T4 system, a DNA-tracking mechanism is required for the transcriptional regulation of the late genes (26), providing a precedent for the model presented in Fig. 6.

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