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Regulation of Pyocin Genes in *Pseudomonas aeruginosa* by Positive (*prtN*) and Negative (*prtR*) Regulatory Genes

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Most strains of *Pseudomonas aeruginosa* produce various types of bacteriocins (pyocins), namely, R-, F-, and S-type pyocins. The production of all types of pyocins was shown to be regulated by positive (*prtN*) and negative (*prtR*) regulatory genes. The *prtN* gene activates the expression of various pyocin genes, probably by the interaction of its product with the DNA sequences conserved in the 5′ noncoding regions of the pyocin genes. The *prtR* gene represses the expression of the *prtN* gene, and its product, predicted from the nucleotide sequence, has a structure characteristic of phage repressors and seems to be inactivated by the RecA protein activated by DNA damage. A model for the regulation of the pyocin genes is proposed.

The production of pyocins in *Pseudomonas aeruginosa* is inducible by treatments that cause damage to DNA and is dependent on the *recA* gene (19, 24). Determination of the nucleotide sequence of the *recA* gene in *P. aeruginosa* revealed that a sequence homologous to the SOS box, the binding site for the LexA protein of *Escherichia coli*, was present in its 5′ noncoding region (19). These results suggested that the production of pyocins may be regulated by a system similar to the SOS system in *E. coli*, in which various SOS genes and colicin genes are activated by cleavage of the LexA repressor catalyzed by activated RecA protein (28).

However, the following recent findings suggested the possibility that the inducible production of pyocins in *P. aeruginosa* is regulated by a mechanism different from that regulating the production of colicins in *E. coli*. (i) A pyocin R2-deficient mutant having a lesion in the *prtN* gene (24) was subsequently found also to be defective in the production of pyocins F2 and S2. (ii) No SOS box-like sequence could be found in the 5′ noncoding regions of the pyocin AP41, S1, and S2 genes (20, 21). Instead, another conserved sequence was found in their 5′ noncoding regions (21). These data suggest that a gene(s) distinct from the *lexA*-like gene is involved in the regulation of the pyocin genes.

We show in this work that *prtN* is an activator gene for various pyocin genes and that the conserved sequences mentioned above are responsible for the activation of various pyocin genes by the *prtN* gene product. In the course of this study, we identified another regulatory gene (*prtR*), which represses the expression of the *prtN* gene. We propose a model for the regulation of the pyocin genes by the *prtN*, *prtR*, and *recA* genes.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *P. aeruginosa* strains used were PAO3182 (24), PAO3375 (*prtN375*) (25), and PAO3628 (*recA301*) (19). The *E. coli* strains used were C600 (1), JM109 (29), and AB1157Δ*recA* (a gift from H. Ogawa and T. Ogawa). The following plasmids were used as cloning vectors: pKT230 (2), pKT240 (3), pRK404 (6), pUC18 and pUC19 (29), pUC118 and pUC119 (27), pACYC184 (5), and pJLA503 (23). pYS211 carries the pyocin AP41 gene (*pyoAP41*) in pACYC184. pYSS9H carries the *recA* gene of *P. aeruginosa* in pUC18 (19).

Media. *P. aeruginosa* and *E. coli* cells were grown in NB (24) and YT (29), respectively. Bacteria were plated on NA plates (24) or on YT plates with 1.5% agar. Antibiotics were used at the following concentrations: for *P. aeruginosa*, carbenicillin at 500 μg/ml and streptomycin at 250 μg/ml; and for *E. coli*, carbenicillin at 100 μg/ml, streptomycin at 50 μg/ml, tetracycline at 40 μg/ml, and chloramphenicol at 30 μg/ml.

Manipulation of plasmid DNA. The techniques used to manipulate plasmid DNA were essentially those of Maniatis et al. (15). Plasmid DNA was prepared by the boiling method (11). DNA cloning was performed with *E. coli*, and appropriate clones were transferred to *P. aeruginosa* by transformation when necessary (17).

Construction of recombinant plasmids. The recombinant plasmids (see Fig. 1) were constructed as follows. The 8-kb *Hin*dIII fragment carrying the *prtN* gene (25) was recloned in the *Hin*dIII site of pKT230 (pHKM2). As the *prtN* gene was shown to be located on the 4.3-kb *Xho*I fragment, the *Xho*I fragment was inserted into the *Sal*I site of pUC18 (pTUM8). The 1.2-kb *Hin*cII (*Sal*I)-*Hin*cII fragment isolated from pTUM8 was recloned in the *Hin*cII site of pUC18 in both orientations (pTUM81 and pTUM82). The inserts in these pUC18 clones were cut out by digestion with *Hin*dIII and *Bam*HI and recloned between the *Hin*dIII and *Bam*HI sites of pKT230 (pHKM101, pHKM102, and pHKM103). pHKM111 and pHKM1112 were obtained from pHKM101 by deletion with *Bgl*II and *Bam*HI and with *Xho*I and *Sal*I, respectively. pHKM114 and pHKM115 were obtained from pHKM102 and pHKM103, respectively, by deletion with *Bgl*II and *Bam*HI. pHKM117 was obtained from a derivative of pHKM2 by deletion with *Sal*I and *Hin*dIII. pHKM118 was obtained from pHKM101 and pHKM103 by recombination at the *Bgl*I site.

Assay of pyocin activity. The production of pyocins was detected by the plate method described previously (24). Quantitative measurement of pyocin activity in liquid cultures was done by the serial dilution method with appropriate indicator strains sensitive to the pyocins to be assayed.

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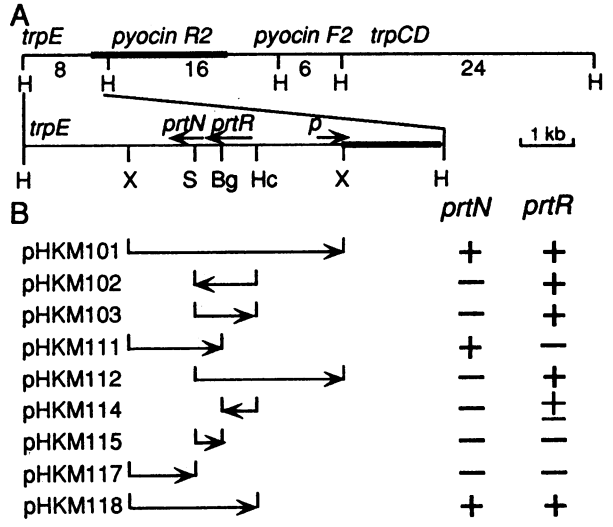

FIG. 1. Localization of the *prtN* and *prtR* genes. (A) Restriction enzyme map. The upper map shows the *Hin*dIII sites in the region containing the *prtN* and *prtR* genes and the gene clusters of pyocins R2 and F2 (14a, 25). The thick line indicates the location of the pyocin R2 gene cluster. Numbers refer to the sizes of *Hin*dIII fragments in kilobases. The lower map shows an enlargement of the 8-kb *Hin*dIII fragment. Arrows indicate the orientations of the *prtR* and *prtN* genes determined by sequencing and of a promoter (*p*) that is activated in the presence of the *prtN* gene (see the text). Abbreviations: Bg, *Bgl*II; H, *Hin*dIII; Hc, *Hin*cII; S, *Sal*I; X, *Xho*I. (B) Mapping of the *prtN* and *prtR* genes. Various subfragments of the 8-kb *Hin*dIII fragment were cloned in pKT230 as described in Materials and Methods. The activity of the *prtN* gene was assayed on the basis of the production of pyocins R2, S2, and F2 in PAO3375 (*prtN375*). The activity of the *prtR* gene was assayed on the basis of the repression of pyocin production in PAO3182 (R2, S2, and F2 positive). Arrows indicate the direction of the promoter for the kanamycin gene on the vector (pKT230) (2). + and −, presence and absence of *prtN* or *prtR* gene activity, respectively; ±, weak activity.

The indicator strains used were *P. aeruginosa* PML14(R2^s), P1516d(S1^{s}S2^s), NIH13(F2^s), NIH3(S2^s), ZD283(S1^s), and PAO3092(AP41^s).

DNA sequencing. Nucleotide sequences were determined by the modified dideoxy termination method with denatured double-stranded DNA (10).

Nucleotide sequence accession number. The nucleotide sequence data have been deposited in the DDBJ, EMBL, and GenBank DNA data bases under the accession number D12706.

RESULTS

Cloning of *prtN* and *prtR* genes. *P. aeruginosa* PAO strains produce three pyocins, R2, F2, and S2 (13, 14). Pyocins R2 and F2 are bacteriophage tail-like pyocins with different morphologies, and pyocin S2 is a proteinase-sensitive S-type pyocin. The pyocin R2-deficient mutant PAO3375 (*prtN*) described previously (24) was also defective in the production of pyocins F2 and S2. This result suggested that the *prtN* gene may be a common regulatory gene for different types of pyocins. Previous genetic analyses (24, 25) indicated that the *prtN* gene was located close to but outside the cluster of the structural genes for pyocin R2 (Fig. 1A). To define the locus of the *prtN* gene, we cloned subfragments of the 8-kb *Hin*dIII fragment into pKT230 and introduced them into PAO3375. A recombinant plasmid carrying the 4.3-kb *Xho*I fragment (pHKM101) rescued both the pyocin R2 and the pyocin S2 deficiencies of PAO3375 (Fig. 1B). Further deletion analyses localized the *prtN* gene to the 1.8-kb *Xho*I-*Bgl*II region (pHKM111 in Fig. 1B).

We noticed during these analyses that pHKM111 restored the production of the three pyocins (R2, F2, and S2) to nearly wild-type levels but that pHKM101 restored pyocin production to only 1/10 to 1/100 the wild-type levels. This result suggested the presence of a gene or a region in pHKM101 that repressed pyocin production. To define this region, we introduced various subfragments cloned in pKT230 into PAO3182, which was proficient in pyocin R2, F2, and S2 production. The results shown in Fig. 1B indicated that the 1.2-kb *Sal*I-*Hin*cII region was sufficient to repress the produciton of pyocins. The production of pyocins R2, F2, and S2 was repressed by a factor of 100 in the presence of pHKM102 and pHKM103. As the nucleotide sequence shown below indicated the presence of a repressor-like gene in this region, we named it *prtR*.

So far, two other S-type pyocins, pyocins AP41 and S1, have been characterized (18, 20, 21). The production of these pyocins was also under the control of the *prtN* and *prtR* genes, as indicated below.

Structures of the *prtN* and *prtR* genes. The nucleotide sequence of the 2.4-kb *Xho*I-*Hin*cIII region was determined (Fig. 2; note that the orientation in this figure is reverse of that in Fig. 1). Two open reading frames consistent with the locations of the *prtN* and *prtR* genes shown in Fig. 1 were found.

The predicted *prtR* gene product is composed of 256 amino acid residues and has a structure characteristic of phage and LexA repressors. Striking homology to the φ80 cI repressor was found. When the amino acid sequence of the predicted PrtR protein was aligned with those of the φ80 cI repressor (9) and the LexA repressor (12) (Fig. 3), 98 amino acid residues were found to be identical between the PrtR protein and the φ80 repressor and 60 residues were found to be identical between the PrtR protein and the LexA repressor. Three characteristic features of known repressor proteins involved in the SOS regulation of genes are conserved in the putative PrtR protein. (i) The sequence between residues 27 and 46 has the structure of helix-turn-helix DNA-binding proteins. The scores calculated by use of the weight matrix, which was proposed by Dodd and Egan (7) to test the probability that a sequence is a helix-turn-helix motif, had a high standard deviation, 5.3. (ii) The Ala-Gly sequence in the LexA and phage repressors (Cys-Gly in the φ80 cI repressor) to be cleaved by the activated RecA protein (9, 12, 22) is found in tandem at residue 121. (iii) The Gly-Asn-Ser-Met sequence near residue 160 is homologous to the sequence conserved among phage and LexA repressors and proposed to be involved in the cleavage of these repressors (26).

The predicted *prtN* gene product is composed of 104 amino acid residues. We could not detect significant homology to any known protein sequences by a computer search of protein and nucleic acid data bases.

Correspondence between the functional genes and the open reading frames was verified by the following deletion analyses. A frameshift mutation generated in the 5′ end of the presumed *prtR* gene by filling in of the *Xho*II site at nucleotide 69 abolished *prtR* gene activity. This result suggests that the initiation codon of *prtR* is the ATG at nucleotide 53, although no apparent Shine-Dalgano sequence could be found upstream from this ATG codon. The minimal

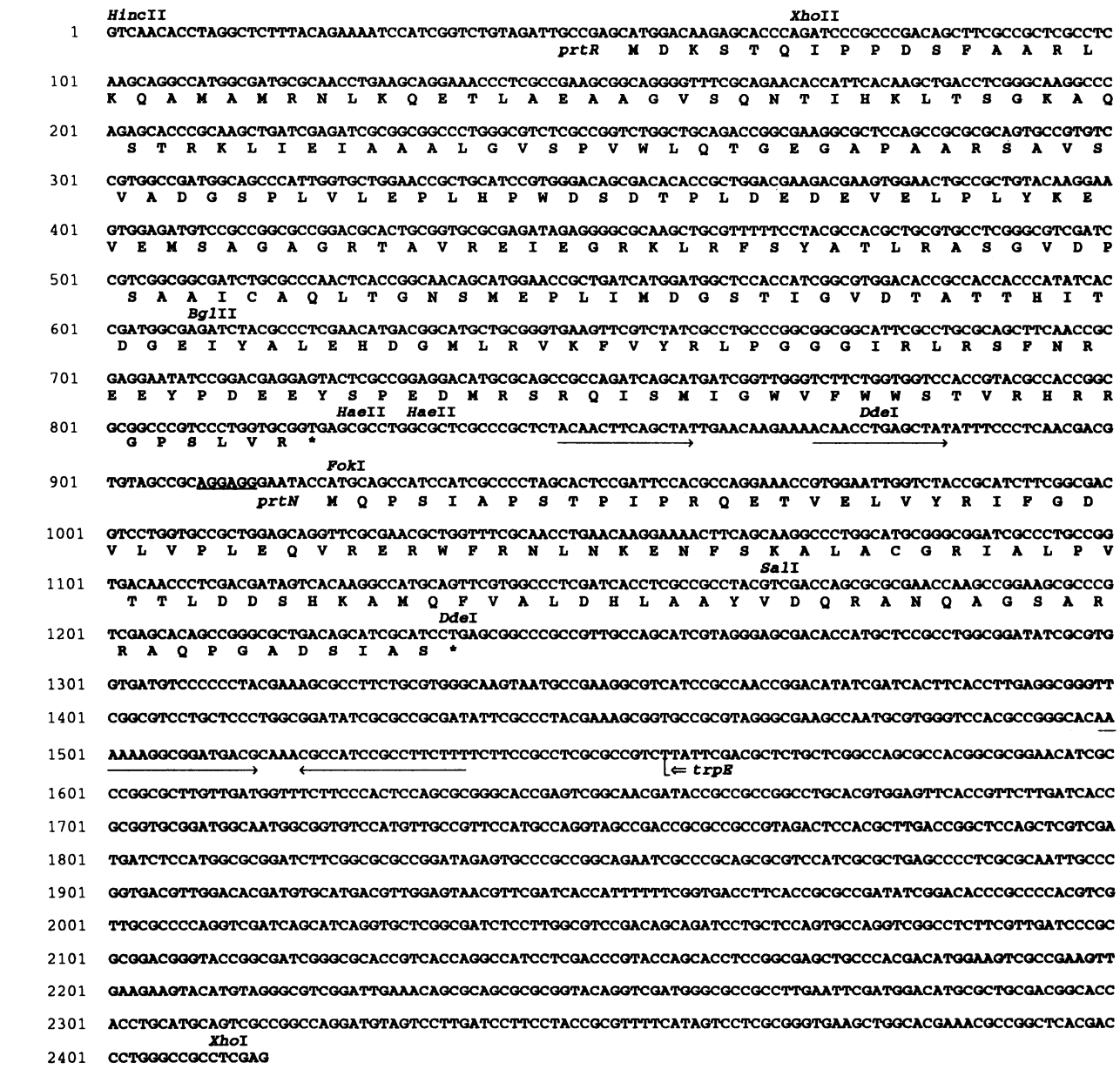

FIG. 2. Nucleotide sequences of the *prtR* and *prtN* genes and the deduced amino acid sequences of their products. The nucleotide sequence of the 2.4-kb *Hin*cII-*Xho*I fragment is shown. Note that the orientation is opposite that of the map shown in Fig. 1. The predicted amino acid sequences of the products of *prtN* and *prtR* are shown. The arrows at nucleotides 846 and 871 show direct repeats with a consensus sequence of ACAACNTNAGCTAT. The underlined sequence is the putative Shine-Dalgarno sequence for the *prtN*gene. The arrows at nucleotides 1499 and 1520 indicate inverted repeats that might form a hairpin-loop structure. The sequence from nucleotide 1599 is part of the coding region of the *trpE* gene, which is transcribed in the opposite direction.

region sufficient for functional *prtN* gene activity was defined by two *Dde*I sites at nucleotides 876 and 1234, since this region had *prtN* gene activity when cloned behind an exogenous promoter.

Requirement for *prtN*, *prtR*, and *recA* for the inducible production of pyocins. The experiment shown in Fig. 4 indicates that *prtN*, *prtR*, and *recA* are required for the inducible production of pyocin AP41. The cloned *prtN* and *prtR* genes and the *P. aeruginosa recA* gene were introduced in various combinations into a *recA*-deficient *E. coli* strain (AB1157Δ*recA*) carrying a plasmid with the *pyoAP41* gene (pYS211). In *E. coli* cells harboring pYS211 alone, pyocin AP41 was produced only poorly (row 1). Neither the presence of the *Pseudomonas recA* gene nor irradiation with UV restored the production of pyocin AP41. Introduction of the *prtN* gene into these cells resulted in the constitutive production of pyocin AP41 independently of UV irradiation and the presence of the *Pseudomonas recA* gene (row 3). Whereas the introduction of the *prtR* gene did not repress the production of pyocin AP41 at the basal level (row 2), it

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                10        20        30        40        50        60
PrtR   MDKSTQIPDDSF-AARLKQAM-AMRNLKQETLAEAAGV-SQNTIHKLTSGKAQSTRK-LIEIAAALGVSP
ϕ80CI          SSI-SERIKFLL-AREGLKQRDLAEALST-SPQTVNNWIKRDALS-REAAQQISEKFGYSL
LexA   MKALTARQQEVFDLIRDHISQTGMPPTRAE-IAQRLGFRSPNAAEEHL--KALA-RKGVIEIVSGASRGI
                                    Helix----Turn----Helix

          70        80        90       100       110       120       130
PrtR   VWLQTGEGAPAARSAVSVADGSPLVLEPLHPWDSDTPLDEDEVELPLYKEVEMSAGAGRTAVREIEGRKL
ϕ80CI  DWLLNGEGSPKKDLESNIPPESEWG--TVDAWDKNTPLPDDEVEVPFLKDIEFACGDGRVHDEDHNGFKL
LexA   ----------------------------------RLLNEEEEGLPLVGRV--AAGEPLLAQQHIEGHYN
                                                      ↑
         140       150       160       170       180       190
PrtR   RFSYATLRASG--VDPSAAIC-AQ----LTGNSMEPL-IMDGSTIGVDTATTHITDGEIYALEHDGMLRV
ϕ80CI  RFSKATLRRVGANSDGSGVLCFPA-----SGDSMEPV-IPDGATVAVDTGNKRVIDGELYAINQGDLKRI
LexA   ------------VDPSLFKPNADFLLRVSGMSMKDIGIMDGDLLAVHKTND-VRNGQVVVARIDDEVTV

      200       210       220       230       240       250   256
PrtR   KFVYRLPGGGIRLRSFNREEYPDEEYSPEDMRSRQISMIGWVFNWSTVRHRRGPSLVR
ϕ80CI  KQLYRKPGGKILIRSINR-DYDDEEADEAD-----VEIIGFVFWYSVLRYRR
LexA   KRLKK-QGNKVELLPEN-SEF---KPIVVDL--RQQSFTIEGLAVGVI--RNGDWL
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FIG. 3. Alignment of the amino acid sequence of PrtR with those of the ϕ80 *c*I and LexA repressors. The *prtR* gene product amino acid sequence deduced from the nucleotide sequence is aligned with those of the *c*I repressor of phage ϕ80 (9) and the LexA repressor (12). Amino acid residues identical between the PrtR protein and the ϕ80 or the LexA repressor are shaded. Gaps are shown with dashes.

suppressed the enhanced pyocin production mediated by the *prtN* gene (row 4). Only in the presence of all three genes, *prtN*, *prtR*, and *recA*, did the production of pyocin AP41 become inducible by UV irradiation.

The same sets of *Pseudomonas* genes were also introduced into a *recA*-proficient *E. coli* strain (C600). The results were essentially the same as those obtained with the *recA*-deficient strain harboring the *Pseudomonas recA* gene. This result indicates that the *E. coli recA* gene can be replaced with the *Pseudomonas recA* gene for the inducible production of pyocins, as suggested previously (19).

***prtN* activates the expression of pyocin genes.** We next examined whether the *prtN* gene activates the transcription of the *pyoAP41* gene. For this purpose, an *Nde*I site (CATATG) was generated at the start codon of the *prtN* gene by in vitro mutagenesis, and the DNA fragment from this *Nde*I site to the *Dde*I site located at the end of the coding region of the *prtN* gene was inserted downstream of the dual promoters (p_R and p_L) of pJLA503. The resultant plasmid (pJNB3) was transferred to AB1157Δ*recA* that had been transformed with pYS211 (pyocin AP41). As the temperature-sensitive repressor gene (*clts*857) was included in pJLA503, the expression of *prtN* was activated by raising the incubation temperature from 30 to 42°C. Activation of the *prtN* gene resulted in the synthesis of mRNA for pyocin AP41 within 30 min and a subsequent increase in pyocin activity (Fig. 5), indicating that the *prtN* gene activates the transcription of *pyoAP41*.

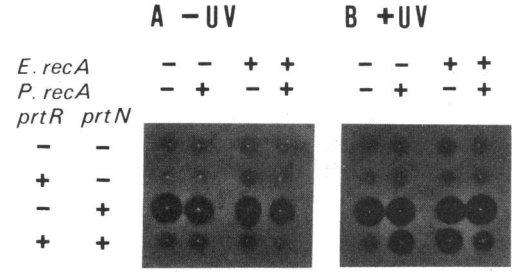


FIG. 4. Effects of *prtN* and *prtR* genes on the inducible expression of the pyocin AP41 gene in *E. coli*. The DNA fragments carrying *prtR* (*Sal*I-*Hin*cIII), *prtN* (*Xho*I-*Bgl*II), and *prtR* plus *prtN* (*Xho*I-*Hin*cII) were recloned in pRK404 between its *Hin*dIII and *Bam*HI sites. These clones and the vector plasmid were transferred to *E. coli* AB1157 Δ*recA* and C600 (*recA*$^+$) carrying the *pyoAP41* gene (pYS211) (20) and the *P. aeruginosa recA* gene (pYSS9H) (19). The production of pyocin AP41 was assayed on plates without (A) and with (B) UV irradiation. The results shown in the two left columns of panels A and B were those obtained in AB1157 Δ*recA* and the results shown in the two right columns were those obtained in C600.

Role of the P box in the activation of pyocin genes by *prtN*. Since the activation of pyocin production by the *prtN* gene has been observed for all pyocins so far examined, common sequences are expected to be responsible for activation by the *prtN* gene. A comparison of the nucleotide sequences of S-type pyocins AP41, S1, and S2 (21) revealed highly conserved sequences (P box) of about 50 bp and located upstream of their coding regions. A homologous sequence was also found near the pyocin R2 gene cluster at the position shown by an arrow (approximately 600 bp from the right *Xho*I site) in Fig. 1A. As shown in Fig. 6, the consensus motif of the P box, ATTGnn(n)GTnn(n), is repeated twice in the P box-like sequence of pyocin R2, while the motif is repeated four times in the case of S-type pyocin genes (21). The role of the P box in the activation of pyocin production by the *prtN* gene was analyzed for the pyocin S1 gene (*pyoS1*). The 2,280-bp *Bal*I fragment, including the *pyoS1* gene with its flanking region (21), was recloned in pUC119 in the direction opposite that of p_{lac}. Various deletions from the 5′ end were constructed by use of exonuclease III, and the activation of pyocin production by the *prtN* gene was examined (Fig. 7). The activation of pyocin S1 production by the *prtN* gene was retained in deletion Δ69 carrying the whole P box but not in deletion Δ17 lacking the entire P box. As we could not obtain a deletion ending within the P box by exonuclease III digestion, such a deletion was made by use of the *Bst*XI site. The *prtN* gene did not activate pyocin production by the mutant from which the DNA upstream from this site had been deleted. This deletion did not affect the basal expression of the pyocin gene in the absence of *prtN*, however. The basal level of pyocin production was

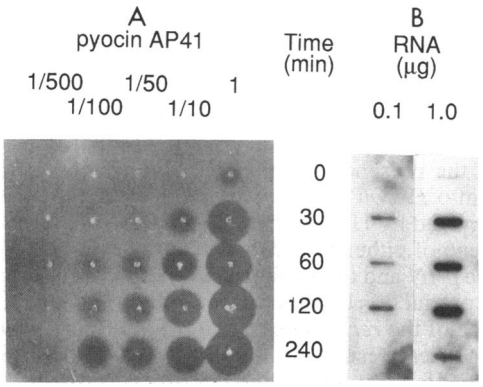
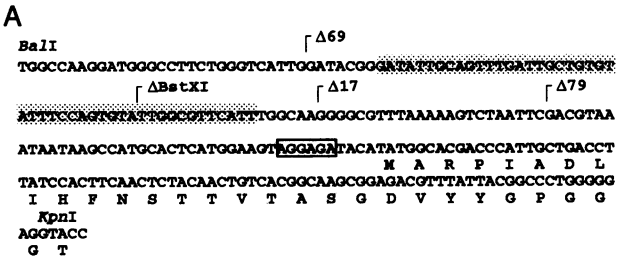


FIG. 5. Activation of the transcription of *pyoAP41* by *prtN*. The *prtN* gene was cloned into pJLA503 under the control of the p_L and p_R promoters as follows. An *Nde*I site was generated at the start codon of the *prtN* gene by site-directed mutagenesis. The DNA fragment from this *Nde*I site to the *Dde*I site located at the stop codon of the *prtN* gene was cloned between the *Nde*I and *Bam*HI sites of pJLA503 (pJNB3). pJNB3 was transferred to C600 carrying pYS211 (pyocin AP41). Cells were grown at 30°C, and the incubation temperature was raised to 42°C. RNA was prepared at 0, 30, 60, 120 and 240 min thereafter. RNA (0.1 or 1.0 μg) was slot blotted onto a nitrocellulose filter, and pyocin mRNA was quantitated by hybridization with a ^{32}P-labeled probe (2,058-bp *Afl*II-*Eco*RI fragment [20]). (A) Titration of pyocin AP41 activity. Various dilutions of the culture were spotted on a lawn of indicator bacteria. (B) Detection of mRNA specific for pyocin AP41.



	- prt N	+ prtN	+ prtN / - prtN
Control	10^2	10^4	10^2
Δ69	10^2	10^4	10^2
Δ*Bst*XI	10^2	10^2	1
Δ17	10^2	10^2	1
Δ79	10	10	1

FIG. 7. Role of the P box sequence in the activation of *pyoS1* by *prtN*. The 2,280-bp *Bal*I fragment carrying the *pyoS1* gene and its flanking sequence (21) was recloned in pUC119 in the direction opposite that of p_{lac}. Deletions from the 5′ end were constructed by digestion with exonuclease III. In addition, a deletion lacking the region upstream of *Bst*XI was constructed. The deletion plasmids were introduced into C600 carrying pKT230 or pHKM111 (*prtN*), and the production of pyocin S1 in liquid culture was examined. (A) Sequence near the P box and the 5′-end position of each deletion. The P box sequence is shaded, and the putative Shine-Dalgarno sequence of the *pyoS1* gene is boxed. (B) Activity of pyocin S2 (in units).

reduced by further deletions, such as Δ79. These results strongly suggest that the P box is responsible for the activation of the promoters of the pyocin genes by the *prtN* gene but is not crucial for promoter activity itself. The activation of the pyocin R2 gene cluster by *prtN* may also be mediated by the P box-like sequence shown in Fig. 6. The DNA fragment containing this sequence had promoter activity towards the pyocin R2 gene cluster (Fig. 1A) when cloned in promoter-probe vector pKT240. Exonuclease III deletion analysis strongly suggested that the P box-like sequence of pyocin R2 is also responsible for the activation of this promoter (data not shown).

***prtR* represses the expression of the *prtN* gene.** The *prtR* gene cloned in multicopy plasmid vector pKT230 repressed pyocin production in *P. aeruginosa* strains proficient in pyocin production (Fig. 1). As the predicted *prtR* gene product has a structure characteristic of repressor proteins, the *prtR* gene product was thought to regulate pyocin genes at the transcriptional level. The following three possibilities were considered. (i) The PrtR protein represses the expression of various pyocin genes directly. (ii) The PrtR protein represses the expression of the *prtN* gene, which is necessary for the activation of pyocin genes. (iii) The PrtR protein interferes with the interaction of the PrtN protein with pyocin gene regulatory sequences.

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AP41   GATATTGTAGTTTG.TTGTCGTGCATCGCTCGTGCATCACCGTTTATT
S1     GATATTGCAGTTTGATTGCTGTGTATTTCCAGTGTATTGGCGTTCATT
S2     GATATTGAAGTT.GATTGCAGTGTATTGCCGATGCATTGGGGCTTATT
R2     CCTATTGCAGTG.GATTGTCGAGAAGGCATTGC
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FIG. 6. P box sequences in 5′ noncoding regions of pyocin genes. Sequences conserved in the 5′ noncoding regions of the pyocin AP41, S1, and S2 genes (P box) (21) are shown. A sequence homologous to the P box and found in the region near the pyocin R2 gene cluster is also shown.

The data shown in Fig. 4 indicated that the *prtR* gene did not affect the basal level of expression of pyocin AP41 in the absence of the *prtN* gene but repressed the pyocin production activated by the *prtN*gene. These results indicate that the *prtR* gene represses the *prtN* gene or interferes with the interaction between the PrtN protein and its recognition site. The latter possibility was excluded, because the *prtR* gene did not repress the production of pyocin AP41 activated by the promoterless *prtN* gene transcribed by the exogenous phage promoter (data not shown).

To test the possibility that the *prtR* gene represses the expression of the *prtN* gene, we examined the effect of the *prtR* gene on the promoter of the *prtN* gene by using promoter-probe vector pKT240. Recombinant plasmid pTUM82#45 carrying the DNA segment from nucleotide 587 to the *Sal*I site at nucleotide 1166 (Fig. 2) was digested with *Fok*I, whose recognition site is CATCC at position 930 and whose cleavage site is at position 917. After end filling and *Eco*RI digestion, the fragment containing the sequence from nucleotides 587 to 916 was inserted between the *Eco*RI and *Hpa*I sites of pKT240 so that the insert lay just upstream of the promoterless streptomycin resistance gene (3). The data shown in Fig. 8 indicated that this fragment had promoter activity that was repressed by the presence of the *prtR* gene. As this fragment contained part of the coding region of the *prtR* gene, the following deletion was constructed. Digestion of the fragment with *Hae*II and religation generated an *Eco*47III site (AGCGCT) just downstream of the coding region of the *prtR* gene. With this site, the fragment from nucleotides 833 to 916 was inserted into the *Hpa*I site of pKT240. This fragment also had promoter activity that was repressed by *prtR* (Fig 8). We concluded, therefore, that the *prtR* gene repressed the promoter activity of the *prtN* gene.

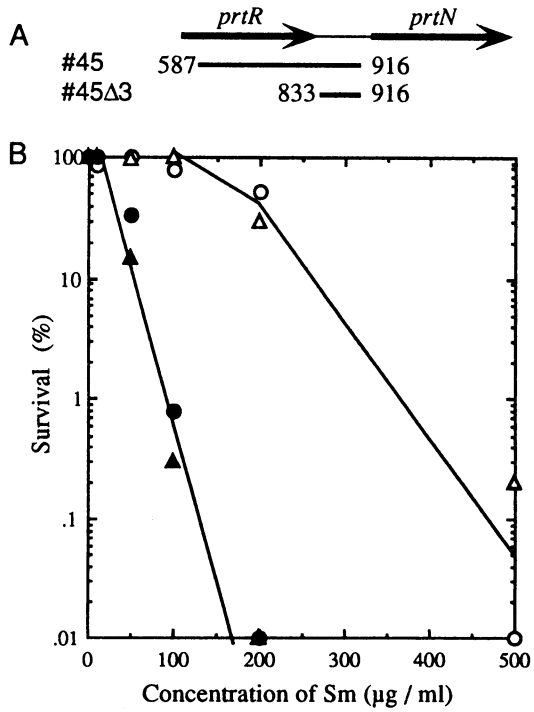

FIG. 8. Repression of *prtN* promoter activity by *prtR*. The DNA fragments containing the 5′ noncoding region of the *prtN* gene were cloned into pKT240 just upstream of the promoterless streptomycin resistance gene, and promoter activity was assayed by measuring the resistance of the transformants to streptomycin in the presence or absence of the *prtR* gene. (A) Location of the DNA fragments used upstream of the *prtN* gene. The fragments containing the promoter region of the *prtN* gene were constructed as described in the text. (B) Effect of *prtR* on promoter activity. The above-mentioned DNA fragments were inserted in the *Hpa*I site of pKT240, located just upstream of its streptomycin resistance gene. The sequence containing the *prtR* gene (from nucleotides 1 to 1033 in Fig. 2) was inserted between the *Eco*RI and *Hin*dIII sites of pBR322. The resistance of C600 transformants to streptomycin was assayed by measuring survival on plates containing various concentrations of streptomycin. Symbols: ○ and ● #45; △ and ▲, #45Δ3; ○ and △, absence of *prtR*; ● and ▲, presence of *prtR*.

Direct repeats of 14 nucleotides with the consensus sequence ACAACNTNAGCTAT and found in this region (Fig. 2) may be binding sites of the PrtR protein.

Inactivation of *prtR* function is dependent on *recA*. In wild-type strains of *P. aeruginosa*, the induction of pyocin production is dependent on treatments that damage DNA and on the presence of the *recA* gene. Pyocin genes introduced into *E. coli* strains were expressed poorly and were not activated by UV irradiation, even in the presence of the *recA* gene of either *E. coli* or *P. aeruginosa* (Fig. 4). When both the *prtN* and the *prtR* genes were present in these cells, pyocin production became inducible by UV irradiation in the presence of the *recA* gene of either *E. coli* or *P. aeruginosa* (Fig. 4). These results strongly suggest that the PrtR protein is inactivated by the activated RecA protein after UV irradiation.

DISCUSSION

We present genetic evidence that the production of various pyocins is regulated by two regulatory genes, *prtN* and *prtR*, in addition to the *recA* gene. The main conclusions are as follows. (i) The *prtN* gene is an activator gene whose product activates pyocin genes, probably by binding to the sequences conserved in their 5′ noncoding regions (P box). (ii) The *prtR* gene is a repressor gene whose product represses the expression of the *prtN* gene. (iii) The PrtR repressor must be inactivated by the activated RecA protein, resulting in the induction of pyocin production. A model for the regulation of pyocin production deduced from these results is shown in Fig. 9.

In *E. coli*, the expression of colicin genes is directly repressed by the LexA protein, a common repressor of various SOS-responsive genes (8, 28). A striking feature of the regulation of pyocin genes in *P. aeruginosa* proposed in Fig. 9 is the participation of a positive regulator (*prtN*) in their expression. Therefore, the regulatory system of pyocin genes is more similar to that of lambdoid phages, in which positive regulatory *cro* genes and negative regulatory *c*I repressor genes are involved. In fact, striking homology was found between the PrtR protein and the *c*I repressors of φ80 phage or other bacteriophages. However, we could not detect any homology between the PrtN protein and the Cro proteins of φ80 phage or other bacteriophages. The PrtN protein may be a novel type of positive regulator different

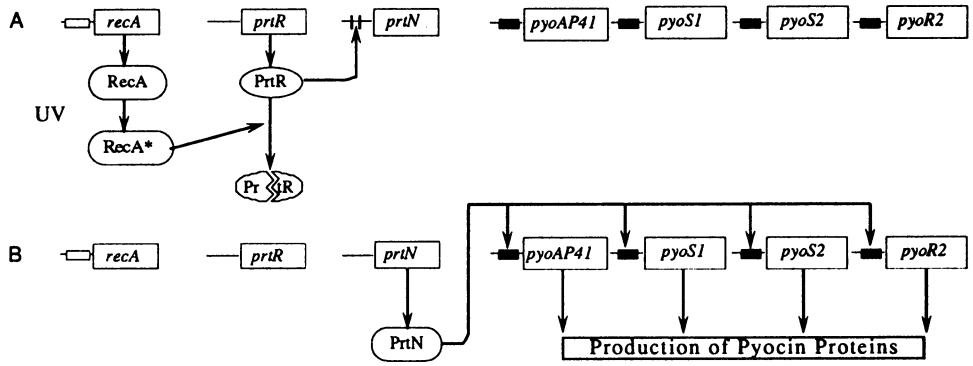

FIG. 9. Model for the regulation of pyocin genes in *P. aeruginosa*. (A) The expression of the positive regulator gene, *prtN*, is repressed by the PrtR repressor under noninducing conditions. Following UV irradiation or other treatments that cause damage in DNA, the PrtR repressor is cleaved by the activated RecA protein. (B) The *prtN* gene is expressed after the breakdown of the PrtR repressor, and the PrtN protein activates the expression of various pyocin genes. P boxes and SOS boxes are represented by black and white boxes, respectively.

from Cro-like proteins or a novel transcription initiation factor.

As previously shown, the *recA* gene of *P. aeruginosa* has a sequence homologous to the SOS box of *E. coli* in its 5′ noncoding region, and its expression, like that of the *E. coli recA* gene (19), is inducible by DNA-damaging agents (16, 16a). This fact suggests that *P. aeruginosa* has a homolog of the *lexA* gene whose product binds to the SOS box-like sequence. A *lexA*-like gene in *P. aeruginosa* has been cloned (4), but its sequence has not yet been determined. The PrtR protein predicted from the nucleotide sequence has characteristic features of phage and LexA repressors. However, it seems unlikely that the *prtR* gene is a homolog of the *lexA*-like gene in *P. aeruginosa*. If it were, the SOS box-like sequence would be expected to be present in the 5′ noncoding region of the *prtN* gene, but we could not find such a sequence in this region. We presume, therefore, that the *prtR* gene is distinct from the *lexA*-like gene in *P. aeruginosa*.

This study showed that pyocin genes are regulated by the *prtN* and *prtR* genes. The *recA* gene of *P. aeruginosa* seems to be regulated by the *lexA*-like gene. It would be interesting to test whether the other SOS genes in *P. aeruginosa* are regulated by the *prtN-prtR* system or by the *lexA*-like gene.

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