JOURNAL OF BACTERIOLOGY, Apr. 1993, p. 2451–2454 Vol. 175, No. 8
0021-9193/93/082451-04$02.00/0
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Role of the *recA*-Related Gene Adjacent to the *recA* Gene in *Pseudomonas aeruginosa*

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Received 21 September 1992/Accepted 12 February 1993

The region adjacent to the 3′ end of the *recA* gene is indispensable for normal cell division in a *rec-2* strain of *Pseudomonas aeruginosa* when the *recA* gene is highly expressed. A putative protein encoded by this region may play a regulatory role(s) in *recA* function.

The *recA* gene of *Escherichia coli* has been extensively studied, and its major contributions to homologous recombination and the SOS system have been described in detail (see reference 11 for a review). Recently, the *recA* homologs of divergent bacterial species have been isolated and characterized (see reference 6 for a review). However, for species other than *E. coli*, we know little of the roles of the *recA* gene and related genes in their regulatory network.

We have recently cloned the *rec* gene of *Pseudomonas aeruginosa*; this gene is located near the *pyoAP41-argF-argG* region on the PAO chromosome. By molecular genetic analysis, we clearly showed that it is the homolog of *E. coli recA* in function and structure (8). When the cloned *P. aeruginosa rec* gene was introduced into *recA13* and *recA56* mutants of *E. coli*, it restored defects associated with the *rec* mutations, such as sensitivity to methyl methanesulfonate and UV irradiation and deficiency of recombination in these strains. Furthermore, the amino acid sequence of the *P. aeruginosa* Rec protein showed extensive homology to that of the RecA protein of *E. coli*. We demonstrated that several *rec* mutations in *P. aeruginosa* (*rec-1*, *rec-2*, *rec-102*, *rec-301*, and *rec-302*) (2, 4, 10) were all complemented with the *Pseudomonas recA*$^+$ clone and that these mutations are allelic to the *recA* gene. Among these *rec* mutant strains, the *rec-2* strain PAO2003 behaved unlike other strains, since one of the *recA*$^+$ plasmids lacking the 1.2-kb *Sal*I fragment adjacent to the 3′ end of the *recA* gene (pMY2101) failed to transform PAO2003 (8) (Fig. 1). With a plasmid completely lacking the *recA* gene, PAO2003 was transformed normally. These results may suggest that PAO2003 carries one or more defects besides *recA* that have something to do with the *recA* gene.

To define the region necessary for sustaining PAO2003 as RecA$^+$, I constructed a series of plasmids carrying the *recA*$^+$ gene and its downstream region with several types of deletions in it. First, the 2.5-kb *Sma*I-*Xho*I fragment (Fig. 1) of pMY21, through pYSS9 (8), was inserted between the unique *Sma*I and *Xho*I sites of pKT240, a high-copy-number plasmid derived from RSF1010 and used as a shuttle vector between *P. aeruginosa* and *E. coli* (1). As the resultant plasmid, pYSX24, yielded *rec*$^+$ transformants of PAO2003 (data not shown), deletants of pYSX24 were further constructed by using the restriction sites *Stu*I, *Eco*RV, and *Nco*I, and these plasmids (pYSX24ΔS, pYSX24ΔRV, pYSX24ΔN1, pYSX24ΔN2, and pYSX24ΔN3) were introduced into PAO2003 by transformation (Fig. 1 and Table 1). While all of these plasmids yielded about the same number of transformants in PAO2001 (*recA*$^+$) and PAO2002 (*rec-1*), all of them except pYSX24ΔS, which carries the *Sma*I-*Stu*I fragment, failed to transform PAO2003, suggesting that the region from the first *Nco*I site to the *Stu*I site contains a sequence essential for keeping the *rec-2* strain viable in the presence of a highly expressed *recA*$^+$ gene.

To better understand the function of the segment adjacent to the *recA* gene, experiments were designed to determine whether (i) this region must be downstream of the *recA* gene or whether it is functional in *trans* and (ii) whether a primary sequence is required as a *cis* element or whether it must be transcribed (and functions as RNA) or translated to exhibit the function. To evaluate these possibilities, PAO2003 was transformed in advance with a *recA* deletion plasmid, pYKS24 or pYSK24, which carries the *Kpn*I-*Stu*I fragment in either direction, against the kanamycin resistance (Km^r) promoter of pKT240. The effects of these resident plasmids were compared by measuring the transformation frequencies of the plasmid-borne *recA*$^+$ gene on the compatible plasmid pRK404-*recA*$^+$ (Fig. 1). The *recA* gene on pRK404 is constitutively transcribed from the *lac* promoter of the vector

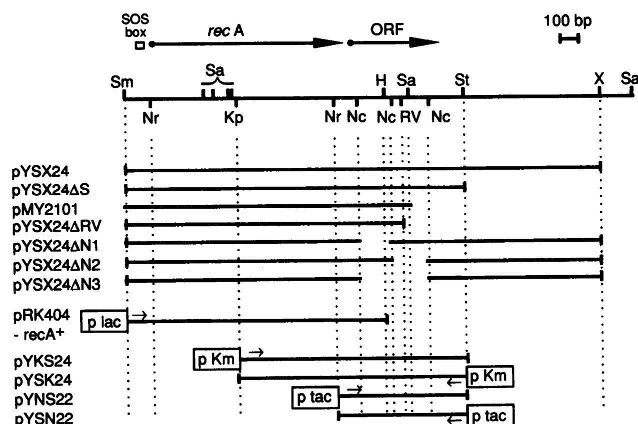

FIG. 1. Plasmids used in this study, with the restriction map of the *recA* gene and its downstream region. The plasmids, pYSX24 and the deletion derivatives, contain the inserts in the Km^r gene of pKT240 oriented in the opposite direction to the Km^r gene. The vector plasmid used to construct pMY2101, pYNS22, and pYSN22 was pMMB22 (1), which carries the *lacI*^{q} gene in the backbone of pKT240. pMY2101 has additional fragments outside this region (see reference 8). In pRK404-*recA*$^+$, the *Sma*I-*Hin*dIII fragment carrying the *recA* gene was inserted into the unique *Hin*dIII site downstream of the *lac* promoter of pRK404, a derivative plasmid of RK2 (3). Abbreviations: ORF, open reading frame; H, *Hin*dIII; Kp, *Kpn*I; Nc, *Nco*I; Nr, *Nru*I; RV, *Eco*RV; Sa, *Sal*I; Sm, *Sma*I; St, *Stu*I.

TABLE 1. Determination of the region necessary for complementing the *rec-2* mutation with plasmid-borne *recA*$^+$ with various 3′ adjacent segments

Host (genotype)	No. of Cb^r transformants with plasmid[a]:					
	pYSX24ΔS	pYSX24ΔRV	pMY2101	pYSX24ΔN1	pYSX24ΔN2	pYSX24ΔN3
PAO2001 (*recA*$^+$)	3.4×10^3	2.6×10^3	2.3×10^3	1.9×10^3	2.0×10^3	1.5×10^3
PAO2002 (*rec-1*)	1.8×10^3	1.7×10^3	2.1×10^3	1.2×10^3	1.5×10^3	1.1×10^3
PAO2003 (*rec-2*)	2.0×10^3	0[b]	0	0[b]	1[b]	1[b]

[a] The numbers of carbenicillin-resistant (Cb^r) transformants were compared for *P. aeruginosa* strains of various *rec* backgrounds. Each plasmid was introduced into PAO2001, and then the plasmid DNA was prepared for the transformation experiments. Bacteria were transformed with about 10 ng of each plasmid DNA by the method of Sano and Kageyama (7). The number of colonies on YT plates (8) containing 250 μg of carbenicillin per ml was counted after incubation at 37°C for 24 h.

[b] A small number of minute colonies appeared after a long incubation period (52 h).

plasmid. As shown in Table 2 (experiment 1), transformants were obtained only with pYKS24, in which the *Kpn*I-*Stu*I fragment is transcribed from the Km^r promoter in the same direction as the *recA* gene on the chromosome. However, the strain bearing the vector itself or pYSK24, in which the same segment is present in the opposite direction, could not produce transformants. This result suggests that the segment need not be located adjacent to the downstream region of the *recA* gene but that it must be transcribed to be functional.

In the second experiment whose results are shown in Table 2, PAO2003 carried the *Nru*I-*Stu*I fragment (downstream of *recA*) under the control of the *lacI*^{q} gene on the vector plasmid pMMB22 (1) and was thus inducible by IPTG (isopropyl-β-D-thiogalactopyranoside). In the absence of IPTG, transformants with pRK404-*recA*$^+$ DNA were obtained with all of the *rec-2* strains carrying either pMMB22, pYNS22, or pYSN22 but not with the plasmidless strain. Since pKT240 was not effective in producing transformants (experiment 1), it appears that the effect of pMMB22 is due to its *lacI*^{q} gene, which controls *recA*$^+$ expression. Among these transformants, the strain carrying pYNS22 and pRK404-*recA*$^+$ could grow normally after addition of IPTG, but the other two strains carrying pMMB22 or pYSN22 and pRK404-*recA*$^+$ produced long, filamentous cells. In these cells, however, the SOS response did not seem to be induced, as judged on the basis of pyocin production (data not shown). Similarly, PAO2003 bearing pYNS22 was transformed with pRK404-*recA*$^+$ DNA in the presence of IPTG (Table 2). In pYNS22, the *Nru*I-*Stu*I segment is transcribed from the *tac* promoter in the same direction as the *recA* gene under the induced conditions. These results indicate that the *rec-2* strain is viable even when the *recA* gene is present, as long as its expression is repressed by the *lacI* repressor, but once the *recA* gene is highly expressed, transcription of the *Nru*I-*Stu*I segment in the correct direction is indispensable for normal cell division in this mutant strain. I designated the putative gene on this segment *recX*.

To examine whether this region encodes a protein responsible for this phenomenon, the nucleotide sequence was determined by a method described previously (8). As shown in Fig. 2A, I identified an open reading frame, immediately following the *recA* gene, which encodes a protein with a molecular weight of 17,400. The amino acid sequence of this putative protein shows some similarity to those of the resolvases of certain transposons, such as Tn*501* and Tn*21*, although the DNA-binding motif is not conserved (Fig. 2B). Horn and Ohman (5) reported methyl methanesulfonate-inducible transcripts of 1.2 and 1.4 kb in the region between *Bam*HI (upstream of *Sma*I) and *Xho*I; both of these transcripts were transcribed in the same direction, with the larger one transcribed through the *Hin*dIII site (Fig. 1). The 1.2-kb transcript was sufficient to produce the RecA protein (5, 8), and the role of the 1.4-kb transcript remained uncertain. Supposing that transcription starts at a point or points between the SOS box and the putative ribosome-binding site for the *recA* gene and terminates at the putative transcription terminators shown in Fig. 2A, the lengths of the transcripts are about 1.1 and 1.5 to 1.6 kb, respectively, in accord with the observations of Horn and Ohman (5). Both transcripts were not found in the *rec-2* strain but appeared after introduction of the *recA* gene with the *Bam*HI-*Hin*dIII fragment on the low-copy-number plasmid, although no RecA protein was detectable (5). Thus, the 1.4-kb transcript may encode a putative 17,400-molecular-weight protein which is not produced in the *rec-2* strain, probably because of a polar mutation in *recA* or some defect(s) in *recX* (the gene for the 17,400-molecular-weight protein) along with the *recA* mutation. The latter possibility seems more likely because Horn and Ohman (5) have shown that the phenotype conferred by a *recA*::Tn*501* polar mutation is quite different from that

TABLE 2. Complementation of the *rec-2* mutation by expression of the *trans*-acting downstream region of *recA* in the *recA*$^+$ background

Presence of IPTG in culture medium	No. of Tc^r transformants with pRK404-*recA*$^+$ for indicated resident plasmid[a]						
	Expt 1			Expt 2			
	pKT240[b]	pYKS24	pYSK24	None	pMMB22	pYNS22	pYSN22
No	2	4.0×10^3	3	0	1.5×10^3	1.1×10^3	1.7×10^3
Yes	NT	NT	NT	1	1	1.4×10^3	2

[a] PAO2003 was transformed in advance with the plasmids listed. Resultant strains carrying one of these plasmids were transformed with pRK404-*recA*$^+$ DNA. About 25 or 40 ng of plasmid DNA prepared from PAO2002(pRK404-*recA*$^+$) was used for experiment 1 or 2, respectively. Transformation was performed as described in Table 1, footnote *a*, except that bacteria were cultured with or without 5 mM IPTG for about three generations before competent cells were prepared and selection was done on plates containing 100 μg of tetracycline per ml in the presence or absence of 5 mM IPTG. Tc^r, tetracycline resistant; NT, not tested.

[b] With the same DNA preparation, 3.8×10^3 transformants were obtained with PAO2001 as a recipient strain.

A.

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                                             NruI
GAAATCGGTTCGGTACTGGAGAAGACCATTCGCGACCAGTTGCTGGCCAAGAGCGGCCCGGTGAAGGCCGACGCCGAAGAAGTGGCTGAC  1170
E  I  G  S  V  L  E  K  T  I  R  D  Q  L  L  A  K  S  G  P  V  K  A  D  A  E  E  V  A  D
                                                                              NcoI
GCCGAAGCCGATTGAGGCCAATGGCGATCGTGCTCGATACGCCCGTCGCCGTGCGGCGGGCGGCCATGGATCTGCTGGCGCGCCGTGAGC  1260
A  E  A  D  *        M  A  I  V  L  D  T  P  V  A  V  R  R  A  A  M  D  L  L  A  R  R  E  H

ACGGGCGCGCCGAGCTTTCCCGCAAGTTGCGCCAGCGTGGCGCGTCTGCCGAGTTGATCGATCCTGCCCTCGACCGCCTGGCCGAGGAAG  1350
 G  R  A  E  L  S  R  K  L  R  Q  R  G  A  S  A  E  L  I  D  P  A  L  D  R  L  A  E  E  G
                                   HindIII                         NcoI
GTCTGCTCGACGAATCCCGCTATCTCGAAAGCTTCATCGCCAGTCGCGCCCGTAGCGGCCATGGGCCGTTGCGCATCCGTGAGGAACTGG  1440
 L  L  D  E  S  R  Y  L  E  S  F  I  A  S  R  A  R  S  G  H  G  P  L  R  I  R  E  E  L  A
                             EcoRV                        SalI
CGCAGCGCGGTCTGCCGCGCGCCGATATCGAAAGGGCGCTGGGTGCCTGCGAGGTCGACTGGAGTGCGCAATTGCGTGAGGTCTGGCGCC  1530
 Q  R  G  L  P  R  A  D  I  E  R  A  L  G  A  C  E  V  D  W  S  A  Q  L  R  E  V  W  R  R
                                                                                 NcoI
GCAAGTTCGCCCGCCTGCCGCAGGACGCCAGGGAAAAGGCCCAGCAGGCGCGTGTCCTGGCGTACCGGGGTTACTCCATGGAGTCGATCA  1620
 K  F  A  R  L  P  Q  D  A  R  E  K  A  Q  Q  A  R  V  L  A  Y  R  G  Y  S  M  E  S  I  M

TGCGACTGTTGAACGGCCGCAGCGACGATTGAGCGCGTGCCGGAGCGGCAGCTTCAGTCCCAGTTTTCCGGCAGGTTGATGTAGTCCAGC  1710
 R  L  L  N  G  R  S  D  D  *

AATTCCCGCAGCCGCCCCTGGTCGCGCCCGTTGAACACGAAGGCGAGGCGGGTCAGGTTGCGGAACTCCGGTTCGTCCTGTTCCTGCTCG  1800
     StuI
CTGTAGGCCTGCTGCTGGAAGCCGCCGCTCAGGCAGAGGCTGGCGAAGTGTTCGTGCAGGTGCGCCAGCGCGGCTTCGTTCAGGGCGTGG  1890

TTCAGGCGAATCACGAAAGTGCCTTTCACCAGCGGCTGGAGTGGAAGTTGCGGTAGAACTGGGCGATTTCCTTCACCGCGTCTTCGGCCG  1980

AATGCACCAGGCGCATCAGGCGCATGTCGGCCGGCAGGATATAGTGATTCTCCAGCAACTGTTCCTGCATGAACTCCAGGGCGTGTTCCC  2070

AGTAGCGGCCGCCCGGCTGGTCGAGCAGCACGATCGGCACCAGCGGACTCTTGCCGGTCTGTACCAGGGTCAGCACTTCCAGTGCCTCGT  2160

CGAGGGTGCCGAAGCCGCCGGGGCAGAGCACCAGGGCGTCGGCTTCCTTGACGAAGAACACGTTGCGCAGGAAGAAAAAGTGGAACGACA  2250

GCAGGTTGCCGCTGCCGTCCACCGTATGGTTGGCGTGCTGCTCGAAGGGCAGGGTGATGTTGAAGCCCAGGCTGTTCTCCAGCCCGGCGC  2340

CTTCGTGCCGGCGGCCATGATGCCGCCGCCGGCGCCGGTGATCACCATCAGGTCGTAGCGGGCCAGTTCCTCGCCCACGTTGCGCGCCAG  2430
                                                                                XhoI
GGCATAGACCGGATGTTCGACCGGCGTGCGCGCCGAGCCGAACACGGTGACCTTGCGTCGCCGCTTGAACTGCTCGAGGACGCTGAAGGC  2520

ATGCTCCATCTCGCGCAGGGTCTGCAGCATGATCTTGGCGTCCCAGCGGTTGCGGTCGGCCTGGGCCATGCGCGTCACGGTGAAGAGCAT  2610
                                                                     SalI
CTCGCGGTAGAGCGCGAGATTGGGGCTGTCGCCGGGAGCCGCAAGGGCCGCCAGTTCGTCGAC  2673
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B.

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Tn501  M··QGHRIGYVRVSSFDQNPERQLEQTQVSKVFTDKASGKDTQRPQLEAL
ORF    MAIVLDTPVAVRRAAMDLLARREHGRAELSRKLRQRGASAELIDPALDRL
Tn21   MT··GQRIGYIRVSTFDQNPERQLEGVKVDRAFSDKASGKDVKRPQLEAL

Tn501  LSFVREGDTVVVHS·MDRLARNLDDLRRLVQKLTQRGVRIEFLKEGLVFT
ORF    AEEGLLDESRYLESFIASRARSGHGPLRIREELAQRGLPRADI·ERALGA
Tn21   ISFARTGDTVVVHS·MDRLARNLDDLRRIVQTLTQRGVHIEFVKEHLSFT

Tn501  GEDSPMANLMLSVMGAFAEFERALIRERQREGITLAKQRGAYRG·RKKAL
ORF    CEVDWSAQLREVWRRKFARLPQDA·REKAQQGRFL·····AYRGYSMESI
Tn21   GEDSPMANLMLSVMGAFAEFERALIRERQREGIALAKQRGAYRG·RKKSL

Tn501  SDEQAATLRQRATAGEPKAQLAREFNISRETLYQYLRTDD
ORF    SRLLNG·····························RSDD
Tn21   SSERIAELRQRVEAGEQKTKLAREFGISRETLYQYLRTDQ
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FIG. 2. (A) Nucleotide sequence of the 3′ end of the *recA* gene and its downstream region. The sequence from the 5′ end to the *Hin*dIII site was reported previously, and the nucleotide numbers are those of the previous report (8). Arrows indicate the putative transcription terminators. The putative ribosome-binding site is underlined. Asterisks indicate stop codons. (B) The amino acid sequence of the protein encoded by the open reading frame (ORF) adjacent to the *recA* gene is aligned with those of the resolvases of Tn*501* and Tn*21* (9). Identical and similar amino acids are indicated with vertical lines and dots, respectively. The DNA-binding domain is indicated by dashed underlining.

conferred by a *rec-2* mutation in *P. aeruginosa*. Involvement of this protein in the regulatory network of the *recA* gene is apparent because the *rec-2* strain is not viable without the expression of this gene when the *recA* gene is highly expressed, either present on the high-copy-number plasmids or transcribed from a strong promoter such as the *lac* promoter. However, it is not yet clear whether it controls the expression of the *recA* gene or interacts with the RecA protein itself.

Nucleotide sequence accession number. The nucleotide sequence in Fig. 2A has been deposited in the DDBJ, EMBL, and GenBank DNA data bases under accession number D13090.

I am grateful to M. Shimaji-Murayama for DNA sequencing and to M. Kageyama and T. Shinomiya for encouragement throughout the work and for critical reading of the manuscript.

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