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 PA02003 behaved unlike other strains, since one of the recA* plasmids lacking the 1.2-kb Sall fragment adjacent to the 3' end of the recA gene (pMY2101) failed to transform PA02003 (8) (Fig. 1). With a plasmid completely lacking the recA gene, PA02003 was transformed normally. These results may suggest that PA02003 carries one or more defects besides recA that have something to do with the recA gene.

To define the region necessary for sustaining PA02003 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 Smal-XhoI fragment (Fig. 1) of pMY21, through pYSS9 (8), was inserted between the unique Smal and XhoI sites of pKT240, a high-copy-number plasmid derived from RSFI010 and used as a shuttle vector between P. aeruginosa and E. coli (1). As the resultant plasmid, pYXS24, yielded rec* transformants of PA02003 (data not shown), deletants of pYXS24 were further constructed by using the restriction sites Sall, EcoRV, and NcoI, and these plasmids (pYXS24AS, pYXS24ARV, pYXS24AN1, pYXS24AN2, and pYXS24AN3) were introduced into PA02003 by transformation (Fig. 1 and Table 1). While all of these plasmids yielded about the same number of transformants in PA02001 (recA*) and PA02002 (rec-l), all of them except pYXS24AS, which carries the Smal-Sall fragment, failed to transform PA02003, suggesting that the region from the first NcoI site to the Sall site contains a sequence essential for keeping the recA* gene intact. 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 KpnI-StuI fragment in either direction, against the kanamycin resistance (Km*) 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, pYXS24 and the deletion derivatives, contain the inserts in the Km' gene of pKT240 oriented in the opposite direction to the Km' gene. The vector plasmid used to construct pMY2101, pYNS22, and pYNS22 was pMMB22 (1), which carries the lac promoter in the backbone of pKT240. pMY2101 has additional fragments outside this region (see reference 8). In pRK404-recA', the Smal-HindIII fragment carrying the recA gene was inserted into the unique HindIII site downstream of the lac promoter of pRK404, a derivative plasmid of RK2 (3). Abbreviations: ORF, open reading frame; H, HindIII; Kp, KpnI; Nr, NcoI; Nc, Nci; NcI; Nt; RV, EcoRV; Saal; Sm, Sall; St, StuI.](http://jb.asm.org/Downloaded from http://jb.asm.org/)
plasmid. As shown in Table 2 (experiment 1), transformants were obtained only with pYKS24, in which the KpnI-StuI fragment is transcribed from the Km' 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, PA02003 carried the NruI-StuI fragment (downstream of recA) under the control of the lacPq 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 pK2T240 was not effective in producing transformants (experiment 1), it appears that the effect of pMMB22 is due to its lacPq 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, PA02003 bearing pYNS22 was transformed with pRK404-recA+ DNA in the presence of IPTG (Table 2). In pYNS22, the NruI-StuI segment is transcribed from the lac 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 NruI-StuI segment in the corrected 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 Tn501 and Tn21, 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 BamHI (upstream of Smal) and XhoI; both of these transcripts were transcribed in the same direction, with the larger one transcribed through the HindIII 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 BamHI-HindIII 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::Tn501 polar mutation is quite different from that

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**TABLE 1. Determination of the region necessary for complementing the rec-2 mutation with plasmid-borne recA**

<table>
<thead>
<tr>
<th>Host (genotype)</th>
<th>No. of Ch+ transformants with plasmid*</th>
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<tbody>
<tr>
<td>PA02001 (recA+)</td>
<td>pYXS24AS: 3.4 x 10³ pYXS24ARV: 2.6 x 10² pMY2101: 2.3 x 10⁴ pYXS24AN1: 1.9 x 10³ pYXS24AN2: 2.0 x 10⁵ pYXS24AN3: 1.5 x 10³</td>
</tr>
<tr>
<td>PA02002 (rec-1)</td>
<td>pYXS24AS: 1.8 x 10³ pYXS24ARV: 1.7 x 10³ pMY2101: 2.1 x 10⁴ pYXS24AN1: 1.2 x 10³ pYXS24AN2: 1.5 x 10³ pYXS24AN3: 1.1 x 10³</td>
</tr>
</tbody>
</table>

* The number of carbenicillin-resistant (Ch+) transformants were compared for P. aeruginosa strains of various rec backgrounds. Each plasmid was introduced into PA02001, 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.

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

<table>
<thead>
<tr>
<th>Presence of IPTG in culture medium</th>
<th>Exp 1</th>
<th>Exp 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pK2T240</td>
<td>pYKS24</td>
</tr>
<tr>
<td>No</td>
<td>2</td>
<td>4.0 x 10³</td>
</tr>
<tr>
<td>Yes</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

* PA02003 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 PA02002 (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', tetracycline resistant; NT, not tested.

* With the same DNA preparation, 3.8 x 10⁷ transformants were obtained with PA02001 as a recipient strain.
A.

**NOTES**

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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 HindIII 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 Tn501 and Tn21 (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.

**REFERENCES**


