Sequence and Expression of the Escherichia coli recR Locus
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The Escherichia coli RecR protein participates in a recombinational DNA repair process. Its gene is located in a region of chromosome that extends from 502 to 509 kilobases on the physical map and that contains apt, dnaX, orf12-recR, htpG, and adk. Most, if not all, of these are involved in nucleic acid metabolism. The orf12-recR reading frames consist of 935 base pairs and overlap by one nucleotide, with the 3' A of the orf12 termination codon forming the 5' nucleotide of the recR initiation codon. The orf12-recR promoter was located upstream of orf12 by sequence analysis, promoter cloning, and S1 nuclease protection analysis. The start point of transcription was determined by primer extension. The transcript 5' end contained a long, apparently untranslated region of 199 nucleotides. Absence of a detectable promoter region for recR and the overlap of the orf12 and recR reading frames suggest that translation of recR is coupled to that of orf12. By maxicell analysis, it was determined that both orf12 and recR are translated.

Escherichia coli recR mutants were identified by Mahdi and Lloyd (20) as derivatives of a recB sbeB sbeC strain which became recombination deficient and UV sensitive. recR mutations reduced recombination after conjugation or transduction in a recBC sbeBC background but had little effect in a recBC" sbeBC" background (20). recR mutations also increased UV sensitivity, but in both recBC sbeBC and recBC" sbeBC" strains. It was concluded, therefore, that the RecR product participates in a recombinational repair pathway (20). The fact that recR mutations decreased recombination proficiency and UV repair when combined as recB recR but not as recF recR double mutants indicated that recR is part of the RecF pathway (20). Although the recR mutation had little effect on recombination after conjugation or transduction in an otherwise wild-type strain, it did cause deficiency in plasmid recombination (20).

The recR gene was mapped near min 11 (20), clockwise of and near the DNA replication gene dnaX (16, 22). We report here that the region between dnaX and the nearby htpG gene (3) contains two overlapping reading frames which encode proteins of 12 and 22 kilodaltons (kDa). The first reading frame is designated orf12 in accordance with the proposal of Mahdi and Lloyd (21), who also sequenced this region. (Their report appeared while this manuscript was in preparation.) The second is the recR gene, as shown also by Mahdi and Lloyd (21).

The orf12 and recR frames overlap by one nucleotide pair, suggesting translational coupling, and the promoter which expresses both is located upstream of orf12 within the dnaX coding sequence.

MATERIAL AND METHODS

Bacterial strains, plasmids, and bacteriophages. The E. coli K-12 strains listed in Table 1. pBJ1 is a 6.2-kilobase-pair (kb) EcoRI fragment carrying apt-dnaX-orf12-recR-htpG and a portion of the adk region cloned into pBR322 (Fig. 1). pBJ10 is a pBJ1 derivative with a 1.3-kb kanamycin resistance (Kanr) cassette from pUC4K (31, 33) inserted into the recR Sall site. pTTG20 is a NarI-XmnI fragment carrying most of the dnaX gene, the entire orf12 and recR genes, and a portion of htpG cloned into the pUC18 (36) NarI and SmaI sites. pTTG30 and pTTG40 are pTTG20 derivatives deleted for a portion of recR and portions of orf12 and recR, respectively. pTTG30 was constructed by opening pTTG20 with SalI, blunt-ending the Sall site, digestion with HpaI, and religating. pTTG40 was constructed by opening pTTG20 with SalI and BstEII, blunt-ending both sites, and ligating. pKKC11 consists of the 514-base-pair (bp) BstEII-SspI (positions 2225 to 2738) recR promoter fragment cloned into pKK223-8 (6). pTTG1 is a 664-bp PstI-Sall fragment cloned into pUC19 (36) restricted by the same enzymes. TTG2 is an M13mp19 (36) derivative carrying the 2,303-bp NarI-HpaI fragment cloned into its Acel-SmaI sites. TTG3 is an M13mp19 derivative with the 747-bp PstI-XmnI fragment in its PstI and SmaI sites.

A 311-bp BamH1 tac fragment was cloned into pKK223-8 to generate pTG1, in which tac is fused to the chloramphenicol acetyltransferase (CAT) gene. The tac fragment was cut from pMB211 (M. Bröker, personal communication) after first deleting an EcoRI lacZ fragment.

Recombinant DNA technology. Standard techniques (2) were used for plasmid DNA isolation, restriction, generation of blunt ends by use of T4 DNA polymerase, Bal31 exonuclease digestion, ligation, transformation, gel electrophoresis, and Southern blotting.

DNA sequencing. The chain termination method (26) was used to sequence a set of overlapping fragments from pTTG1 cloned into M13mp18 or mp19 (36). The PstI site (position 3074) and the Sall site (position 3732) were sequenced across by using TTG2 and TTG3, respectively, as templates.

Plasmid recombination. The method of Kolodner et al. (18) was used to measure recombination between two mutant tetracycline resistance alleles carried on one plasmid to generate a tetracycline-resistant wild-type allele.

Transduction. P1 virus was used for transduction by the method of Willetts et al. (34).
TABLE 1. E. coli strains used

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Radiolabeling of proteins in vivo. Proteins were labeled with <sup>35</sup>S]methionine in the maxicell (29) system with strain CSR603 as the host for the plasmids and with d-cycloserine (100 µg/ml) instead of ampicillin.

Electrophoresis of radiolabeled proteins. For electrophoresis, 15% denaturing, discontinuous polyacrylamide-sodium dodecyl sulfate gels and 5% stacking gels were used (2). Labeled proteins were visualized by exposing gels to Kodak X-Omat XAR-1 film. Molecular weight standards were from Bio-Rad Laboratories.

Enzyme assays. CAT and β-lactamase were assayed in the same extract by the procedures of Seed and Sheen (27) and Lupski et al. (19), respectively. Extracts were prepared as described before (19).

S1 nuclease protection assay. The procedure of Ohta et al. (23) was used for S1 assays. The 650-bp BstEII fragment (positions 2225 to 2875) (Fig. 1) was purified by electroelution and 5'-end labeled. This fragment was denatured and used as probe, or it was cut at the HaeII site (position 2354) and the resulting 526-bp, 3'-HaeII-BstEII-5' subfragment (labeled only at the 5' end) was purified and used. Probe sufficient to provide 200,000 cpmp was used.

Primer extension. The method of Inouye et al. (14) was used for primer extension, except that the crude RNA preparation was purified by sedimentation through a 5.7 M CsCl solution (8). Avian myeloblastosis virus reverse transcriptase was from Promega. The primer was the deoxyribonucleotide 5'-CGACGCGAGGTCGGAA-3'.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper have been submitted to GenBank under accession no. M37084.

RESULTS

Nucleotide sequence of the orf12-recR region. The region between the dnaX and htpG genes (Fig. 1A) was sequenced by the chain termination method (26). The sequence downstream of dnaX to the PstI site beginning at nucleotide 3074 was reported by Flower and McHenry (9); the sequence upstream of htpG to the Sall site at position 3732 was reported by Bardwell and Craig (3). We determined the sequence between the PstI and Sall sites on both strands and sequenced across both sites on one strand. While this manuscript was in preparation, Mahdi and Lloyd (21) reported the sequence between the BstEII (position 2875) and KpnI (position 4198) sites. The sequences are identical except that we read as CCG the nucleotides beginning at

A.

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FIG. 1. Structure of the apt-dnaX-orf12-recR-htpG-adk region. (A) A 6,820-bp fragment with reading frames indicated by open bars and derivative plasmids and phages. The nucleotides are numbered beginning with the first nucleotide of the EcoRI site upstream of apt. Where differences in sequence were reported in the interval between htpG and adk (3, 7), we used the data from the recent work. The overlap between the orf12 and recR frames is indicated by the darkened area of the bar. E, EcoRI; H, HindIII; N, NalI; Bs, BstEII; Ha, HaeII; Ps, PstI; HpaI; V, EcoRV; Sa, SalI; X, XmaI; C, ClaI. (B) Plasmids constructed for use in identifying the Orf12 and RecR proteins. These plasmids contain the dnaX gene repressed at the NalI site. Deletions are indicated by dashed lines. The promoter and transcription direction are indicated by the P and the horizontal arrow, respectively.
3274, whereas Mahdi and Lloyd published CGC for that region.

The interval between dnaX and hipG contains two open reading frames which would be transcribed from left to right (Fig. 1B). Assuming that translation begins at the ATG, the first starts at 2854 and extends for 109 codons to the stop codon at 3183 to 3185, encoding a protein deduced to have a molecular weight of 12,015. This reading frame is designated orf12 (21); its 5' end was first reported by Flower and McHenry (9). The second reading frame, assuming that ATG initiates its translation also, would begin at position 3185 and extend to 3788. This is designated recR, based on the observation that disruption of this frame by a kanamycin resistance cassette (below) generated a mutant similar in phenotype to the original recR strain of Mahdi and Lloyd (20), the mapping of recR to the dnaX region of the chromosome (20), and the demonstration (21) that a clone of this region complemented the original recR strain. The recR product is deduced to be a 201-amino-acid protein of $M_r$ 21,951. The two reading frames overlap by one nucleotide, the A of the orf12 terminator serving as the first nucleotide of the recR initiator. Both proteins are predicted to be strongly acidic, with isoelectric points of 4.9 and 5.1 for Orf12 and RecR, respectively.

Codon usage indicates that Orf12 should be efficiently expressed, whereas the codon distribution of RecR is more typical of a rarely expressed gene. Strongly expressed genes favor NNC over NNU for phenylalanine, tyrosine, asparagine, and isoleucine, whereas weakly expressed genes favor NNC over NNU for proline, arginine, glycine, and alanine (11). The orf12 sequence fits the strongly expressed pattern in five of the eight pairs, two have pairs members used equally, and one pair is typical of weakly expressed genes. On the other hand, the recR sequence is more likely to be weakly expressed because four of the pairs are typical of rare proteins, one pair does not discriminate, and three pairs favor the strongly expressed pattern.

Strongly expressed genes also discriminate against codons corresponding to minor isoacceptor tRNAs, whereas weakly expressed genes do not (13, 28). The orf12 sequence contained only one rare codon (GGA, Gly) and no rare CUA (Leu), AUA (Ile), UCG (Ser), CCC (Pro), CGA, CGG, AGA, AGG (Arg), or GGG (Gly) codons, consistent with its assignment as a highly expressed gene. RecR, however, depends on translation of three UCG, one CCC, one each CGC and CGG, and five GGG rare codons.

Genes of the recT region. Availability of the orf12-recR sequence allowed completion of a physical map of a 6,820-bp EcoRI-ClaI fragment which carries the recT region and adjacent genes (Fig. 1A). These are apt (12), dnaX (9, 37), orf12-recR, hipG (3), and ack (7). All these genes are transcribed left to right, as presented in Fig. 1. Based on a comparison of restriction sites, this segment of the chromosome is located, in clockwise orientation, between kb 502 and 509 on the Kohara physical map (17) of the whole chromosome.

Insertion mutagenesis of the chromosomal recR gene. Site-directed insertion mutagenesis (15, 35) was used to disrupt the chromosomal recR allele. The 1.3-kb kanamycin resistance fragment from pUC4K (31, 33) was introduced into the SalI site within the recR reading frame on pBJ1, producing pBJ10 (Fig. 1A). pBJ10 was linearized by Fstl and used to transform the recR recB recC sbeC strain JC7623 to kanamycin resistance. One of the recombinants with a recR::Kan' allele (strain JCB325) was chosen for further study. Southern analysis confirmed the recR disruption (data not shown).

Strain JCB325 (recR::Kan' recBC sbeBC) grew slowly at all temperatures and could not form colonies at 17°C. This cold sensitivity resulted from the combination of recR::Kan' with recBC sbeBC rather than from the recR mutation directly. The recR::Kan' allele, moved from the recBC sbeBC background to generate strain TY108, caused UV sensitivity and plasmid DNA recombination deficiency. Measured by the method of Kolodner et al. (18), plasmid recombination frequency decreased 50-fold in strain TY108 compared with that in the wild-type strain AB1157 (data not shown). This confirms the identity of the reading frame containing the SalI site, rather than orf12 (Fig. 1B), as the recR gene, because these are the properties associated with recR mutants (20).

Protein products of orf12 and recR. The translation products of orf12 and recR were labeled by $[^35]$S)methionine in vivo in maxicells and in vitro in a coupled transcription-translation system (22, 25) by using plasmids which carried both orf12 recR, only orf12, or neither orf12 nor recR. Plasmid pTTG20, the insert of which contains orf12, recR, and their promoter (Fig. 1B) (see below), was deleted for most of recR (i.e., between the HpaI and SalI sites) to form pTTG30 and for both orf12 and recR (i.e., between the BstEII site at position 2225 and the SalI site) to form pTTG40. In vivo, both the 12-kDa Orf12 protein and the RecR protein product of 26 kDa apparent size were encoded by pTTG20 (Fig. 2). Identification of the apparent 26-kDa protein as the recR product was based on the fact that it was not produced from the deleted pTTG30 or pTTG40. The RecR protein had a molecular weight of about 22,000 determined by sequence analysis; the reason for the discrepancy is unknown. In vitro, the orf12 product was detectable as a 12-kDa protein encoded by both pTTG20 and pTTG30 (data not shown). Synthesis in vitro of the RecR protein was not observed. Mahdi and Lloyd (21) also identified the orf12 and recR products as 12- and 22-kDa proteins.

Translation of recR was much less efficient than that of orf12. Its level was about 5 to 10% of the level of Orf12 (the different content of methionines, nine in orf12 and seven in recR, did not contribute significantly to the observed difference in intensity of the autoradiogram). The failure to observe RecR synthesis in vitro could be related to inefficient translational coupling in extracts.

orf12-recR promoter. Analysis of the nucleotide sequence upstream of orf12 identified a potential promoter, TTG AAC-N$_{17}$-GATAAT, within the 3' end of the dnaX reading frame.
(9). To determine whether this region could act as a promoter, the 514-bp \(Bst\)EII-XbaI fragment (positions 2225 to 2738) (Fig. 1B) containing the predicted promoter was cloned into the promoter-cloning vector pKK232-8 so that it would direct transcription of CAT (6). This plasmid, pKKC11 (Fig. 1B), directed the synthesis of CAT and conferred chloramphenicol resistance on the host strain HB101, whereas the vector pKK232-8 did not. This confirmed the identity of the promoter, which directed the synthesis of 170 CAT units per unit of plasmid-directed \(\beta\)-lactamase activity. This was about 0.3% of the activity of the induced tac promoter. When the tac promoter was cloned into the same vector, generating pTC1, and induced with isopropyl-\(\beta\)-D-thiogalactoside, it directed the synthesis of 47,000 CAT units per unit of \(\beta\)-lactamase.

Located 6 bp upstream of the orf12 was the sequence AGAGAG, which should serve as a ribosome-binding site (30), as predicted by Flower and McHenry (9).

On the other hand, the recR reading frame appears not to have a separate promoter or ribosome-binding site, as judged by sequence analysis. Its translation is probably coupled to that of orf12, as proposed also by Mahdi and Lloyd (21), although the presence of a weak promoter specific for recR cannot be excluded.

**orf12-recR** transcript. S1 nuclease protection experiments were used to localize, approximately, the 5' end of the messenger. RNA was extracted from cultures of strains JM103, JM103 carrying the vector pUC18, and JM103 carrying the orf12* recR* plasmid pTTG20 (Fig. 1B). A probe complementary to the messenger was prepared by purifying the orf12-recR promoter-containing 650-bp BstEII fragment (Fig. 1A). This fragment was 5'-end labeled, denatured, and hybridized to cellular RNA extracts. S1 nuclease-protected fragments were identified by electrophoresis and autoradiography (Fig. 3). The principal protected fragment from the strain carrying orf12 and recR on a multicolor plasmid was approximately 225 nucleotides in length (Fig. 3, lane E), which confirms the recR promoter location predicted from the sequence and indicates that transcription starts at about position 2655, about 10 nucleotides downstream of the -10 sequence (Fig. 4). The same conclusion was reached when the probe was the 526-nucleotide 3'-HaeII-BstEII-5' fragment (Fig. 1A) labeled at the 5' end (data not shown), which also confirmed that the 650-bp BstEII probe, which was labeled on both ends, was protected by orf12-recR messenger and not an antisense RNA. Similarly sized orf12-recR transcripts were present in cells carrying only a haploid copy of the recR region (Fig. 3, lanes C and D) but at very low levels. These transcripts were clearly visible when the autoradiogram was overexposed.

Minor transcripts of about 650 nucleotides, which protected the full-length probe, were detected in all extracts and could represent dnaX transcripts which extend into the orf12-recR region or renatured probe. Minor amounts of fragments of approximately 195, 185, and 145 nucleotides were detected; their identities are unclear.

**Transcription start point.** The S1 nuclease protection experiment indicated that transcription initiated about 10 nucleotides downstream from the center of the -10 sequence (Fig. 4). To locate the exact start point, primer extension analysis was used. The same RNA preparation used for S1 mapping was further purified by sedimentation through a 5.7 M CsCl solution (8) and used as a substrate for reverse transcription after priming with a 5'-end-labeled synthetic oligonucleotide complementary to nucleotides 2740 to 2756 (Fig. 4). The product was denatured and its size was measured by electrophoresis and autoradiography. The standards were provided by chain-terminating sequencing reaction products provided by the same primer hybridized to TTG2 DNA, an M13mp19 derivative containing the 2,303-bp NarI-HpaI fragment (Materials and Methods).

The longest RNA detected (number 1 in lane A, Fig. 5) initiated with the G at nucleotide 2655 (Fig. 4). Based on the similarity of the sequence over nucleotides 2620 to 2648 to the consensus of \(E.\ coli\) promoter, the S1 mapping, and the primer extension, it is proposed that orf12-recR transcription begins at nucleotide 2655 and that the messenger has a long, untranslated region of 199 nucleotides. Minor amounts of transcripts beginning at positions 2656 and 2657 were also observed.

A transcript with the 5' end apparently at nucleotide 2675 (number 2 in lane A, Fig. 5) was more abundant than that beginning at position 2655. It is possible that two promoters initiate transcription at positions 2655 and 2675 or that the majority of the transcript initiated at 2655 is processed between 2674 and 2675. However, we favor the interpretation that the reverse transcriptase terminated primer extension synthesis prematurely at position 2675 because the S1 nuclease protection analysis did not detect a major transcript beginning at 2675 and because there is no sequence analysis evidence to indicate the presence of a second promoter.

The significance, if any, of the transcript with a 5' end at position 2700 (number 3, lane A, Fig. 5) is unknown.

**DISCUSSION.**

The \(E.\ coli\) orf12 and recR reading frames encode proteins of 12 and 22 kDa, respectively, assuming that they initiate with the ATG codons at positions 2834 and 3183, respectively. These products have been identified after synthesis in vivo in maxicells as proteins which migrate on sodium dodecyl sulfate-polyacrylamide gels at 12 and 26 kDa. We conclude that these reading frames are transcribed from one promoter, located 206bp upstream of orf12, which initiates
transcription starting at nucleotide 2655. The function of the long (199-nucleotide) untranslated leader is, at this time, unknown. The apparent absence of a separate promoter for recR and the one-nucleotide overlap between the orf12 termination codon and the recR initiation codon suggest that RecR translation is coupled to that of Orf12. Of the two models proposed for translational coupling (i.e., translation of the downstream reading frame is facilitated by translating ribosomes opening the secondary structure of the messenger and the “hand-over” model for coupled genes separated by short intercistronic regions [24]), the hand-over model seems more nearly appropriate for Orf12 and RecR. Whatever the mechanism, RecR translation is less efficient by a factor of about 10 to 20 than that of Orf12, at least when labeled in maxicells.

The specific function of the RecR protein is unknown. It is required, however, for plasmid recombination and UV repair. This suggests that it participates in a repair process which depends on recBC-independent recombination (21).

It is interesting that orf12 and recR are located within a group of genes (Fig. 1), most (and possibly all) of which are involved in nucleic acid metabolism. apt encodes adenine phosphoribosyltransferase, which catalyzes the synthesis of AMP from adenine and phosphoribosylpyrophosphate (12). dnaX encodes the r and r subunits of DNA polymerase III (9, 37). htpG encodes a heat shock protein which is dispensable but which is required for normal growth rate (3, 4); some heat shock proteins are involved in nucleic acid metabolism (1, 32). Adenylate kinase, an enzyme essential for growth, is the product of adk (7). All are transcribed in the same direction, there are short intervals between the open reading frames, and there are overlapping termination (or processing) sites and promoters. Each gene except recR seems to have a unique promoter, but in the case of orf12 and recR, this promoter is located within the dnaX reading frame. This location raises the question of whether dnaX transcripts also extend through orf12-recR. Other interesting questions include the function of the untranslated orf12-recR leader and the significance, if any, of the gene organization in this region.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

Alonso, Shirahige, and Ogasaوارa (personal communication) have found that Bacillus subtilis contains two adjacent open reading frames, orf107 and recM, which are homolo-
gous to orf12 and recR of E. coli. orf107 encodes a 107-amino-acid protein that is 39.8% identical to orf12 protein; recM encodes a 217-amino-acid protein that is 43.2% identical to RecR. Moreover, orf107 and recM are adjacent to, and transcribed in the same direction as, dnaX. There are, however, major differences between these regions in the two organisms. First, the B. subtilis dnaX gene has 562 codons, compared with 643 in E. coli. Second, the B. subtilis dnaX-orf107-recM genes seem to form one operon.

LITERATURE CITED


