JOURNAL OF BACTERIOLOGY, Oct. 1993, p. 6663–6670
0021-9193/93/206663-08$02.00/0
Copyright © 1993, American Society for Microbiology
Vol. 175, No. 20

Expression of the *Escherichia coli dnaX* Gene

KEN-SHIUNG CHEN, PRATIBHA SAXENA, AND JAMES R. WALKER*
Department of Microbiology, The University of Texas, Austin, Texas 78712-1095

Received 16 October 1991/Accepted 6 August 1993

The *Escherichia coli dnaX* gene encodes both the τ and γ subunits of DNA polymerase III. This gene is located immediately downstream of the adenine salvage gene *apt* and upstream of *orf12-recR*, *htpG*, and *adk*. The last three are involved in recombination, heat shock, and nucleotide biosynthesis, respectively. *apt*, *dnaX*, and *orf12-recR* all have separate promoters, and the first two are expressed predominantly from those separate promoters. However, use of an RNase E temperature-sensitive mutant allowed the detection of lesser amounts of polycistronic messengers extending from both the *apt* and *dnaX* promoters through *htpG*. Interestingly, transcription of the weak *dnaX* promoter is stimulated 4- to 10-fold by a sequence contained entirely within the *dnaX* reading frame. This region has been localized; at least a portion of the sequence (and perhaps the entire sequence) is located within a 31-bp region downstream of the *dnaX* promoter.

The *Escherichia coli dnaX* gene, required for DNA chain elongation, is located in a group of six genes, all or most of which are involved in nucleic acid metabolism (Fig. 1). These include *apt*, *dnaX*, *orf12-recR*, *htpG*, and *adk*. *apt* (encoding adenine phosphoribosyl transferase) is an adenine salvage enzyme which catalyzes synthesis of AMP from 5′-phosphoribosyl-1′-pyrophosphate and adenine (20). *dnaX* encodes both the τ and γ subunits of DNA polymerase III holoenzyme (5, 15, 23, 32, 39). The function of *orf12* is unknown, but *recR* is involved in DNA repair and plasmid recombination (26). HtpG is a heat shock protein (4). Although the function of HtpG is not known, at least three other heat shock proteins are involved in nucleic acid metabolism (2, 38), and it is conceivable that HtpG is also. Adenylate kinase, the *adk* product, catalyzes the phosphorylation of AMP to ADP (9). In addition to these six genes that are known to be expressed, three long reading frames are oriented from right to left within the *apt-adk* region (Fig. 1). Although they all start with ATG codons, it is not known if they are expressed.

All six genes known to be transcribed are oriented in the same direction, the distances between them are short, and an *orf12-recR* promoter is located within the upstream, *dnaX* reading frame (43). The *dnaX* promoter (designated *Xp*) has been localized within the region between *apt* and *dnaX*. Although most *apt* and *dnaX* transcripts are monocistronic in wild-type cells, small amounts of transcripts which extend from *apt* or *dnaX* through *htpG* were observed in a temperature-sensitive RNase E mutant. In addition, a sequence internal to the *dnaX* reading frame stimulates *dnaX* expression.

MATERIALS AND METHODS

Bacterial strain, plasmids, and bacteriophages. Strain HB101 (27) was the bacterial host for all experiments unless otherwise indicated. The RNase E wild-type and temperature-sensitive mutant strains, N3433 and N3431 (18), respectively, were obtained from B. Bachmann. Plasmid pKK232-8 (7) was the promoter-cloning vector. pCM7 (12) is a derivative of pBR327 (40) and contains the Tn*9* chloramphenicol acetyltransferase (CAT) gene flanked by *Hin*dIII sites; it was purchased from Pharmacia. pGEM-7Z and pGEM-3Z (Promega) both contain Sp6 and T7 promoters flanking the multiple cloning site. ptac1 consists of pKK232-8 into which the tac promoter has been inserted to drive CAT expression (43). pUC7 has been described previously (30).

pBJ1 is the 6.2-kb *Eco*RI fragment which contains *apt* through a portion of *adk* cloned into pBR322 (43). In order to insert the 3,080-bp *Eco*RI-*Pst*I *apt-dnaX* region into the promoter-cloning vector, a 3.1-kb *Cla*I-*Pst*I fragment, which consists of 25 bp of vector pBR322 DNA from the *Cla*I site to the vector *Eco*RI site plus the 3,080-bp *Eco*RI-*Pst*I *apt-dnaX* region from pJH16 (32), was inserted into *Acc*I- and *Pst*I-cut M13mp18, producing M13kcx1 (Fig. 1). M13kcx1 replicative-form DNA was cut at the *Bam*HI and *Hin*dIII sites which bracket the insert (partial digest with *Hin*dIII) and the *apt-dnaX* region inserted into pKK232-8, generating pKKC50 (Fig. 1).

pKKC31, pKKC32, and pKKC33 are the *Bam*HI-*Ssp*I, *Bam*HI-*Bst*EII, and *Bam*HI-*Nar*I fragments, respectively, with filled-in ends, of M13kcx1 cloned into the *Sma*I site of pKK232-8.

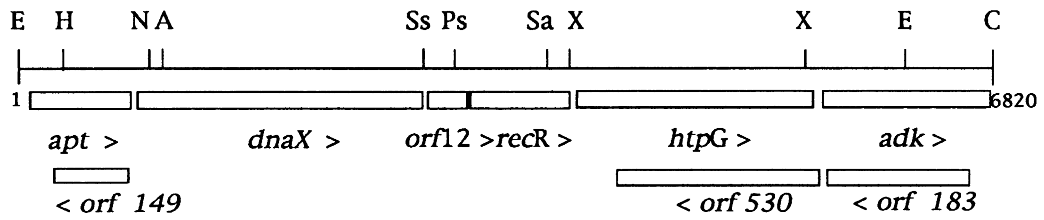
pKKC34 contains the fragment between the *Sma*I sites of the polylinker and the *apt* sequence of pKKC50 cloned into *Sma*I-cut pKK232-8. pKKC106 was made by deleting the *Hin*dIII fragment (from within the *apt* gene to the pKKC34 polylinker) from pKKC34.

pKKC22 consists of the pKKC34 *Hin*dIII fragment (cut within the *apt* gene and downstream of the insert within the polylinker region) cloned into *Hin*dIII-cut pKK232-8. pKKC2013 consists of the *Sma*I-*Nar*I region overlapping the 3′ end of *apt* and the 5′ end of *dnaX* cloned, after filling the *Nar*I end, into *Sma*I-cut pKK232-8. pKKC7 was constructed by cloning the *Nar*I-*Afl*II fragment of pKKC50, after filling the ends, into *Sma*I-digested pKK232-8. pKKC11 consists of the 514-bp *Bst*EII-*Ssp*I *recR* promoter fragment with filled ends inserted into *Sma*I-cut pKK232-8. pKKC123 is the *Sma*I-*Ssp*I fragment from pKKC50 cloned into *Sma*I-cut pKK232-8.

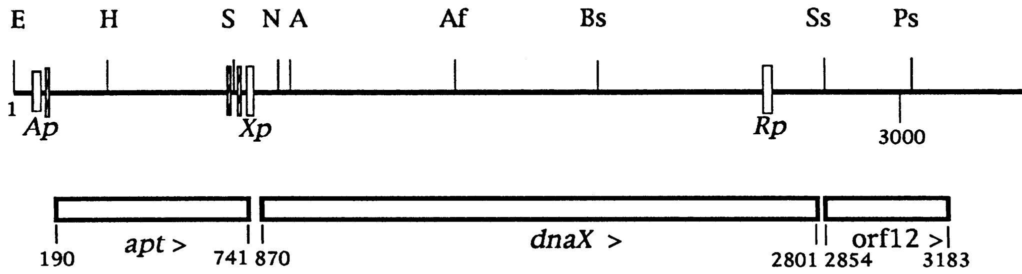
Plasmids pEU100, pEU200, pED100, and pED200 are derivatives of the *Xp*-containing pKKC2013 with the 621-bp *Nar*I-*Afl*II fragment inserted upstream in the normal (pEU100) or reversed (pEU200) orientation or downstream in the normal (pED100) or reversed (pED200) orientation. These constructs were made in several steps, the first of which was to insert *Bam*HI linkers up- or downstream of *Xp* in pKKC2013 in two different plasmids. pKKC2020 is pKKC2013 with a *Bam*HI site inserted upstream of *Xp* in the *Sma*I site. pKKC2040 has a *Bam*HI site inserted downstream of *Xp* in the

* Corresponding author.

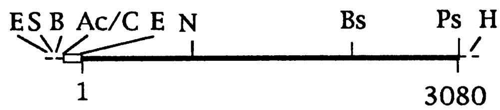
A.



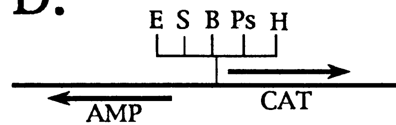
B.



C.



D.



E.

Plasmid	Promoter Strength
pKK232-8	11
pKKC50	1100
pKKC106	280
pKKC22	22
pKKC2013	95
pKKC7	20
pKKC11	180
pKKC123	610
pKKC34	260
pKKC33	140
pKKC32	580
pKKC31	500
pKKC54	400
pEU100	130
pEU200	150
pED100	600
pED200	68
ptac	35000

Ap Xp Rp

filled-in *Hin*dIII site located in the polylinker of pKKC2013. Second, the *Nar*I-*Afl*II fragment from pKKC50 had the ends filled in and was inserted into the *Hin*cII site of pUC7, i.e., between the *Bam*HI sites, creating pEB10. Third, the stimulatory sequence bracketed by *Bam*HI sites was cloned from pEB10 into the *Bam*HI sites, in both orientations, of pKKC2020 and pKKC2040.

Plasmids pED108, -109, -110, -120, -130, and -140 were made by polymerase chain reaction (PCR) amplification of fragments beginning with the *Sma*I site at position 732 and ending downstream of *Xp*. All six were amplified from linearized pJH16 DNA with the same forward primer, 5′ TTCCCG GGCCATTAATTAT 3′ (positions 727 to 745). Reverse primers and their positions were (i) 5′ CATTTTCGGGCTAA GACC (898 to 881) (pED110), (ii) 5′ CAGCAAAGCTTCCT GGCCGACGACGT (944 to 919) (pED108), (iii) 5′ AGATA AAGCTTATGAATACGCCCTAA (994 to 969) (pED109), (iv) 5′ TTTAGAAGCTTCGCCAGCAGTCGGGC (1057 to 1032) (pED120), (v) 5′ TTAACAAGCTTCGGCGGCTCTTC AAG (1321 to 1296) (pED130), and (vi) 5′ TGGCAAGCTTC TGGTCGGTCAGACTT (1548 to 1523) (pED140). The pED 110 insert extended from the *Sma*I site at position 732 to the blunt end of reverse primer i. This piece was cloned into *Sma*I-cut pKK232-8. Reverse primers ii to vi all introduced a *Hin*dIII site. Amplified pieces synthesized with these reverse primers were cut with *Sma*I and *Hin*dIII and cloned into *Sma*I- and *Hin*dIII-cut pKK232-8.

Recombinant DNA technology. Standard techniques (27) were used for plasmid DNA isolation, restriction, filling sticky ends, 5′ labelling with [γ-^{32}P]ATP, ligation, transformation, and gel electrophoresis.

DNA sequencing. The chain termination method (35) with M13kcx1 DNA as the template was used for DNA sequencing.

Enzyme assays. CAT and β-lactamase were assayed in the same extracts by the procedures of Seed and Sheen (37) and Lupski et al. (25), respectively.

Primer extension and Northern (RNA) blot analysis. The method of Inouye et al. (22) was used for primer extension. Avian myeloblastosis virus reverse transcriptase was from Promega. The primer was the deoxyribonucleotide 5′ CATTT TCGGGCTAAGACC 3′, which corresponds to nucleotides 898 to 881. The procedures for RNA isolation and Northern blots were described previously (24).

Preparation of RNA probes. Radiolabelled RNA probes were prepared as described previously (28). For the CAT probe, the 780-bp *Hin*dIII fragment containing the CAT gene from pCM7 (12) was cloned into pGEM-7Z to generate pKCX77, which was linearized with *Bam*HI and transcribed by T7 RNA polymerase. To prepare the probe for the β-lactamase transcript, the 237-bp *Sca*I-*Pst*I fragment containing part of the β-lactamase gene from pBR322 was cloned into pGEM-3Z to generate pKCX80, which was linearized with *Eco*RI and transcribed by using Sp6 RNA polymerase.

In vitro transcription. The method of Bracco et al. (6) and *E. coli* RNA polymerase (Pharmacia) were used for in vitro transcription.

PCR. PCR was carried out with *Eco*RI-linearized pJH16 DNA as the template. The reactions were performed in a programmable heating block with 30 rounds of temperature cycling (94°C for 1 min, 55°C for 2 min, and 72°C for 3 min); 100 pmol of each primer, 3×10^{-5} pmol of template, and 2.5 U of *Taq* polymerase (Perkin Elmer Cetus) were used in a final volume of 100 μl. The reaction buffer was that recommended by the manufacturer. On completion of the reaction, the PCR product was subjected to phenol-chloroform and chloroform extractions followed by ethanol precipitation.

RESULTS

Localization of *Xp* and quantitative assays of promoter strength. Promoters for *apt* (*Ap*) and *orf*12-*recR* (*Rp*) have been identified previously (Fig. 1). Hershey and Taylor (20) identified *Ap* (nucleotides 54 to 82) by S1 mapping of the *apt* transcript. Yeung et al. (43) identified *Rp* (nucleotides 2620 to 2648) within the *dnaX* reading frame by promoter cloning, S1 mapping, and primer extension. Potential promoters for *dnaX* include nucleotides 629 to 656 within the *apt* sequence (GTG AAG-16 bp-CATTAT) (16) and the sequence TCGCCG-17 bp-TAGCAT, which extends from position 798 to 826 within the intergenic region between *apt* and *dnaX* (16, 44). To identify *Xp* and to measure its strength, various fragments of the *dnaX* region were cloned into the promoter-cloning vector pKK232-8 (7), which uses the CAT gene as the reporter gene. The presence of *Ap* upstream of *apt* was confirmed by the construction of pKKC106 (Fig. 1), which contains the 5′ end of *apt* on an *Eco*RI-*Hin*dIII fragment. The presence of *Rp* was confirmed by cloning the *Bst*EII-*Ssp*I fragment upstream of *orf*12 in pKKC11 (Fig. 1).

Xp was localized on the insert of pKKC2013, which carries the *Sma*I-*Nar*I fragment from position 729 to 897. All three promoters, *Ap*, *Xp*, and *Rp*, are extremely weak, directing the synthesis of only 0.8, 0.3, and 0.5%, respectively, as much CAT as directed by the induced tac promoter (ptac1). Promoter strength is indicated as the ratio of CAT to β-lactamase to normalize on the basis of copy number. No promoter was found upstream of the *Sma*I site (at position 729) on the *Hin*dIII-*Sma*I insert (position 305 to 734) of pKKC22 or within the 5′ end of the *dnaX* reading frame on the *Nar*I-*Afl*II fragment (position 897 to 1525) of pKKC7.

To locate the *dnaX* transcription start point, primer exten-

FIG. 1. (A) The 6,820-bp *dnaX* region of the chromosome. Open bars represent open reading frames, the directions of which are indicated by the arrowheads. E, *Eco*RI; H, *Hin*dIII; N, *Nar*I; A, *Aat*II; Ss, *Ssp*I; Ps, *Pst*I; Sa, *Sal*I; X, *Xmn*I; C, *Cla*I. This structure was adapted from that published by Yeung et al. (43) and is based on data from Hershey and Taylor (20), Yin et al. (44), Flower and McHenry (16), Mahdi and Lloyd (26), Yeung et al. (43), and Brune et al. (9). *orf*149, -530, and -183 are designated to reflect the number of codons they contain. They are oriented from right to left; it is not known if they are expressed. (B) Expanded *apt-dnaX-orf*12 region. The promoters for *apt* (*Ap*), *dnaX* (*Xp*), and *orf*12-*recR* (*Rp*) are indicated by vertical open bars. Hatched vertical bars are potential DnaA boxes. S, *Sma*I; Af, *Afl*II; Bs, *Bst*EII; other abbreviations are as in panel A. (C) M13kcx1 polylinker region plus insert. The heavy bar is the *Eco*RI-*Pst*I *apt-dnaX* fragment. The open box is pBR322 DNA from the pJH16 (32) *Cla*I site to the *Eco*RI site. The dashed line is M13mp18 polylinker. B, *Bam*HI; Ac/C, junction of half of the *Acc*I site of M13mp18 to half of the *Cla*I site of the insert; other abbreviations are as in panels A and B. (D) Promoter-cloning vector pKK232-8 (7). AMP, β-lactamase gene; CAT, CAT gene; other abbreviations are as in panels A and C. Arrows represent transcripts. (E) *apt-dnaX* fragments cloned into pKK232-8 for promoter activity assay. Promoter strength refers to the ratio of CAT to β-lactamase expressed as counts per minute over absorbance change. The values shown are the averages of three experiments; the standard deviations ranged from 10 to 25%, except for background values of 11 to 22, for which the standard deviation was 50%. Boxes containing arrows indicate stimulatory sequences cloned in specific orientations as described in the text.

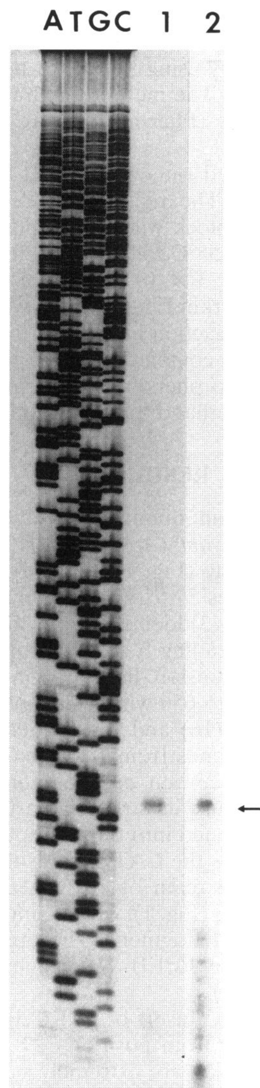


FIG. 2. *dnaX* transcription start point. RNA extracted from a strain harboring pED100 (in vivo) and RNA transcribed in vitro from pED100 by using *E. coli* RNA polymerase were primed for reverse transcription with a 5′-end-labelled synthetic oligodeoxyribonucleotide, 5′ CATTTTCGGGCTAAGACC 3′ (complementary to nucleotides 898 to 881). The products were denatured, electrophoresed, and located by autoradiography. Size markers were provided by chain-terminating sequencing reactions with the same primer and M13kcx1 DNA as the template. The sequencing reaction products are labelled A, T, G, and C. Lane 1, RNA synthesized in vivo; lane 2, RNA synthesized in vitro. The arrow indicates primer extension products.

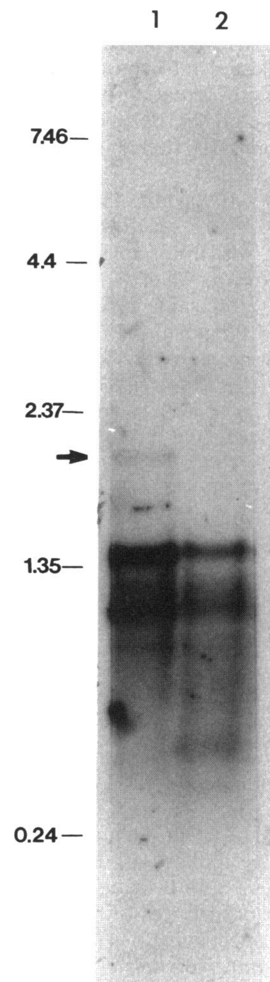


FIG. 3. Analysis of transcripts initiated from *Ap* and *Xp* by Northern blot hybridization. Total RNA was extracted from strain HB101 carrying pKKC33 (*ApXp*-CAT) (lane 1) or pKKC2013 (*Xp*-CAT) (lane 2). Twenty micrograms of RNA was electrophoresed, transferred to GeneScreen, and hybridized to 10^6 cpm of strand-specific CAT probe per ml in a total volume of 10 ml. The RNA ladder from Bethesda Research Laboratories was used for size markers (shown on the left, in kilobases). The arrow indicates a 2.1-kb transcript.

sion analysis was used. A synthetic oligodeoxyribonucleotide complementary to nucleotides 898 to 881 was used as a primer for extension of mRNA isolated from cells harboring pED100, which contains the sequences from nucleotide 729 to 1520 (Fig. 1). The product was denatured, and its size was measured by electrophoresis and autoradiography. Transcription initiated with a U at position 833, 7 nucleotides from the 3′ end of the *Xp* −10 region (Fig. 2). Transcription also began with nucleotide 833 in vitro. Transcripts synthesized by RNA polymerase from pED100 DNA in vitro also were used for primer extension, with the same result as that found in vivo (Fig. 2).

Some transcripts initiated at *apt* and *dnaX* run through *htpG*. To determine if transcripts initiated from *Ap* run into the *dnaX* region, the promoter activity of a fragment containing both *Ap* and *Xp* was compared with the activities of fragments carrying *Ap* or *Xp*. *Ap* (on pKKC106) directed the synthesis of about 280 U of CAT, whereas *Xp* (on pKKC2013) directed the synthesis of about 95 U (Fig. 1). The presence of both promoters (on pKKC33) directed the synthesis of about 140 U. This intermediate value suggests that some of the *apt* transcripts extend into the *dnaX* reading frame.

A Northern blot analysis confirmed that conclusion. RNAs extracted from cells carrying *ApXp*-CAT (pKKC33) and from cells carrying *Xp*-CAT (pKKC2013) fusions were electrophoresed, transferred to a filter, and probed with a transcript complementary to the full-length CAT fragment (Fig. 3). The *Xp*-CAT fusion (pKKC2013, Fig. 3, lane 2) produced a principal transcript of 1.4 kb, consistent with initiation from *Xp* and termination at the CAT terminator. This fusion transcript would consist of 84 nucleotides of *dnaX* mRNA and about 1.3

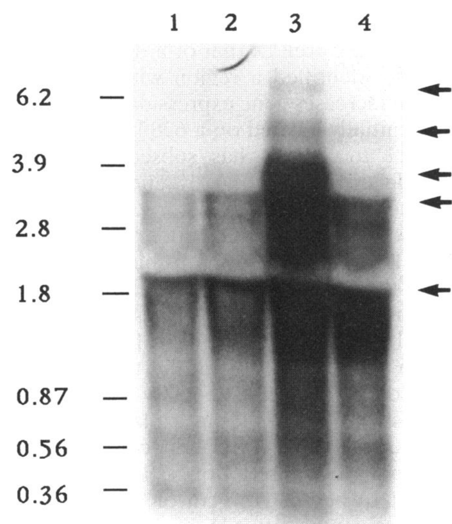


FIG. 4. Analysis of transcripts complementary to a *dnaX* probe. Wild-type (lanes 1 and 2) and RNase E mutant (lanes 3 and 4) strains were transformed with pBJ1. Cultures were grown at 30°C (lanes 2 and 4), portions were shifted to 43°C (lanes 1 and 3), incubation was continued at both temperatures for 30 min, and total RNA was extracted. Twenty micrograms of each preparation was analyzed as described for Fig. 3 except that the probe was a 5′-end-labelled oligodeoxyribonucleotide complementary to nucleotides 1 to 60 of the *dnaX* transcript. Arrows mark transcripts of 6.5, 4.9, 3.7, 3.1, and 1.8 kb. To demonstrate the 6.5-kb transcript, the film was exposed so long that the 3.7- and 3.1-kb transcripts appear as one broad band. The RNA size markers (shown on the left, in kilobases) were from Promega.

kb of CAT gene sequences. (Also produced were a 1.1-kb transcript and lesser amounts of smaller transcripts, all of which might have been degradation products.)

When the *ApXp*-CAT fusion was the template, the principal transcript was again 1.4 kb, consistent with initiation at *Xp*. However, small amounts of a 2.1-kb transcript also were produced, consistent with initiation at *Ap*; synthesis through the 740-nucleotide *apt* region, 84 nucleotides of *dnaX*, and about 1.3 kb of CAT; and termination downstream of the CAT gene.

Polycistronic *dnaX* messengers were sought also by Northern blot analysis of RNA extracted from a temperature-sensitive RNase E mutant. This enzyme is known to be involved in mRNA processing (31). Plasmid pBJ1, the 6.2-kb *Eco*RI insert of which extends from *apt* through half of the *adk* gene (Fig. 1), was transformed into the wild-type and RNase E temperature-sensitive isogenic pair of strains, N3433 and N3431 (18). The strains were grown at 30°C, a portion of each culture was shifted to 43°C for 30 min, and RNA was extracted from both 30- and 43°C-grown cultures. Northern blots were probed with a 5′-end-labelled oligodeoxyribonucleotide complementary to the first 60 nucleotides of the *dnaX* mRNA. RNA extracted from the wild-type cells at 30 and 43°C and from the RNase E mutant at 30°C had two principal transcripts, 3.1 and 1.8 kb (Fig. 4). When the RNase E mutant was incubated at 43°C, longer transcripts of 6.5, 4.9, and 3.7 kb were found along with the 3.1- and 1.8-kb transcripts.

Probing of RNA from wild-type and RNase E mutant cells which were not overproducing *dnaX* messenger (i.e., plasmidless cells) with a *apt* probe revealed that the wild-type cells growing at 30 and 43°C and the RNase E mutant growing at 30°C contained only one 750-nucleotide transcript, which corresponds to *apt* messenger. However, the RNase E mutant growing at 43°C contained, in addition to the 750-nucleotide *apt* transcript, small amounts of 6.0-, 3.7-, 2.3-, and 2.0-kb transcripts (data not shown).

In summary, *apt* and *dnaX* are expressed predominantly from *Ap* and *Xp*, respectively, and *orf*12-*recR* has a separate promoter also. However, small amounts of polycistronic messengers are produced. Of the two polycistronic messengers which initiate at *apt*, one (3.7 kb) extends through *recR*, and the other (6 to 6.5 kb) extends at least through *htpG*. Messengers which initiate at *Xp* extend through *dnaX* (1.8 kb), *recR* (3.1 kb), or *htpG* (4.9 kb). From the results with the RNase E mutant, we conclude that this enzyme participates in processing the longer transcripts. Whether some short transcripts are generated also by termination is not known.

A sequence within the *dnaX* reading frame increases expression. A sequence which increased expression was identified in the *dnaX* coding region between the *Nar*I and *Afl*II sites (Fig. 1). This fragment had no promoter activity when fused in either orientation to the CAT gene. For example, pKKC7 did not confer chloramphenicol resistance or produce detectable CAT (Fig. 1). However, expression from an *ApXp* fragment which extended from nucleotide 1 to 897 (pKKC33) was increased fourfold by extension of the 3′ end to the *Bst*EII site at nucleotide 2225 (pKKC32) (Fig. 1). The stimulation affected *dnaX* as shown by the results with two plasmids, one carrying *Xp* and the other carrying *Xp* plus the stimulatory sequence. A plasmid carrying *Xp* on a fragment which extended from the *Sma*I site at nucleotide 729 to the *Nar*I site at nucleotide 897 directed the synthesis of 95 U of CAT (pKKC2013), but the plasmid expressed 600 U when the fragment extended to the *Afl*II site at nucleotide 1520 (pED100) (Fig. 1). (The *Sma*I-*Nar*I and *Nar*I-*Afl*II fragments in pED100 are joined by a *Bam*HI linker [see Materials and Methods].)

This internal sequence had little or no effect when inserted upstream of *Xp* in its normal (pKKC2013 versus pEU100) or reverse (pEU200) orientation. When the sequence was located downstream in its normal position, the stimulation was sixfold, but in reverse orientation (pED200), the *Nar*I-*Afl*II fragment reduced expression.

The internal stimulatory sequence overlaps nucleotides 904 to 935. To localize further the sequence responsible for increased expression, a set of overlapping fragments carrying *Xp* and various lengths of downstream sequence were fused to the CAT gene. All fragments started at the *Sma*I site at position 732. The control plasmid was pKKC2013, in which the cloned fragment terminated at the 3′ end with a filled-in *Nar*I site (ending at position 903) joined to the blunt *Sma*I site upstream of the CAT gene (Fig. 5). Six additional constructs were made by PCR. pED110 joined *dnaX* position 898 to the *Sma*I site upstream of the CAT gene. The remaining five constructs had *Hin*dIII sites added to the 3′ ends by use of mismatch PCR primers; all were joined to the *Hin*dIII site upstream of the CAT gene. The *dnaX* sequences in pED108, -109, -120, -130, and -140 ended at positions 935, 983, 1049, 1310, and 1538, respectively.

The two shortest fragments, pKKC2013 and pED110, had strengths of 95 to 100; each of the longer fragments had five- to eightfold-greater activity. Thus, at least a portion of the stimulatory region and perhaps the entire region is present on the sequence from 904 to 935, that is, between the 3′ ends of pKKC2013 and pED108. From the start point of transcription, these positions are at +72 to +103 nucleotides.

Plasmid	Xp Insert 3' end		Promoter-Cloning Vector Sequence	Promoter Strength
	Last Nucleotide NO.	Sequence		
pKKC2013	903	GCC	GGG-19bp-AAGCTT-SD-ATG	95
pED110	898	ATG	GGG-19bp-AAGCTT-SD-ATG	100
pED108	935	GAA	GCTT-SD-ATG	630
pED109	983	CAT	AAGCTT-SD-ATG	500
pED120	1049	AAG	CTT-SD-ATG	470
pED130	1310	CCG	AAGCTT-SD-ATG	670
pED140	1538	CAG	AAGCTT-SD-ATG	860

FIG. 5. Localization of the *dnaX* internal stimulatory sequence. Fragments starting at position 732 and terminating at various positions downstream of *Xp* were fused to the *Sma*I site (dotted underline) or *Hin*dIII site (solid underline) of the promoter-cloning vector pKK232-8. SD, Shine-Dalgarno. The CAT initiation codon is in boldface. Four stop codons in three reading frames are located between the vector *Hin*dIII site and the initiation codon (8). Promoter strength is the CAT/β-lactamase ratio.

DISCUSSION

Transcription from the weak *dnaX* promoter, *Xp*, begins with the U seven nucleotides downstream of the −10 sequence. Among 263 *E. coli* transcription start points screened, only 31, or 12%, were U (19). Most *E. coli* transcripts start with a purine at the seventh nucleotide position (downstream of −10). If the seventh base is not a purine, transcription usually starts with a purine at position six or eight. In the case of *dnaX*, the nearest purines are 2 or 10 bases from the −10 sequence, which is outside the usual 4- to 8-nucleotide window (3). Interestingly, *dnaX* transcription initiates at nucleotide 833, which also is the last nucleotide of some upstream transcripts initiated from *apt* (20).

In addition to transcripts which initiate at position 833, some of the *apt* transcripts also run into *dnaX* as previously suggested by Hershey and Taylor (20). This suggestion was based on the observation that a probe which extended downstream through the *Xp* promoter, used to identify the 3′ end of *apt* mRNA, was fully protected in S1 nuclease protection experiments. Our analysis and the recent study by Flower and McHenry (17) provide direct evidence that some *apt* transcripts extend into *dnaX*, although *dnaX* is expressed principally from its own promoter. We report here the detection of monocistronic *dnaX* transcripts; on the other hand, Flower and McHenry (17) did not detect 3′ ends of *dnaX* transcripts between *dnaX* and *orf*12-*recR* and concluded that these three genes constitute a *dnaX* operon.

Additionally, polycistronic messengers from both *Ap* and *Xp* extending through *htpG* were detected in small amounts but only in a temperature-sensitive RNase E mutant grown at a nonpermissive temperature. We conclude, therefore, that some monocistronic messengers are generated by RNase E processing of longer transcripts.

Inasmuch as most *apt* transcripts are monocistronic, the question then arises as to the mechanism by which they are terminated. Although the intergenic region between *apt* and *dnaX* contains two regions of dyad symmetry, neither stem-loop resembles a rho-independent terminator (20). An interesting question is whether the portions of *apt* transcripts which run into *dnaX* are regulated, perhaps by DnaA protein. Between *Ap* and *Xp* are three potential DnaA boxes (each with seven of nine matches to the consensus sequence); two of these were pointed out by Flower and McHenry (16). All three potential DnaA boxes are oriented in the direction of transcription (on the noncoding strand). It has been demonstrated that a DnaA box in the direction of transcription can attenuate *dnaA* transcription as well as that of a downstream gene (36).

We have also identified a region within the *dnaX* coding sequence which increases gene expression by 4- to 10-fold. This sequence was initially located on a 628-bp region that extends from codon 11 to 219; it has subsequently been further localized by deleting from the 3′ end. When the 3′ end extended to nucleotide 904, stimulatory activity was abolished; when the 3′ end extended to nucleotide 935, the sequence was fully functional. Perhaps the responsible sequence is located entirely within these 31 bp, which extend over the region from +72 to +103 nucleotides from the transcription start.

This internal sequence is thought to boost gene expression by an effect on transcription or on the transcript because the constructs used to detect its activity were transcriptional fusions. At least three models can explain the activity of this sequence. First, an activator protein could bind within this region and stimulate transcription. Stimulation could result from induced DNA bending directly or from activator protein-RNA polymerase contact after looping. Second, an antitermination or antipausing mechanism could extend nascent messengers beyond a termination or pause site within the 5′ end of *dnaX*. Third, an effect on mRNA processing or degradation could result in increased expression levels.

With regard to the possibility that the internal sequence activates transcription initiation, it is interesting that this region contains 16 bp which have extensive similarity to the purine regulon operator. Genes involved in purine and pyrimidine nucleotide biosynthesis are negatively regulated by the purine repressor binding, in the presence of corepressor guanine or hypoxanthine, to operators (10, 29, 34). The consensus purine regulon operator and the similar sequence from the *dnaX* internal region are

purO	aCGCAAAC GTTTtCnT
dnaX	CCACAAAC CTTTGCTG

Among the 13 most conserved positions in *purO*, 10 in the *dnaX* region are identical. Perhaps the purine repressor protein, which normally represses at the purine operators, acts as an activator at the *dnaX* site. Alternatively, a similar protein, but not the purine repressor itself, could be the activator.

The possibility that a repressor, in this case the purine repressor, can alternately repress or activate is not without precedence. The *tyrP* gene, which encodes the tyrosine transport system (41), is alternately repressed or stimulated from one promoter by the TyrR protein, depending on the identity of the cofactor. Tyrosine-mediated repression requires two TYR R boxes, the downstream one of which overlaps the *tyrP* promoter −35 region. Phenylalanine-mediated activation requires only the upstream TYR R box (1). In λ lysogens, the *c*I repressor simultaneously represses transcription from the λ rightward promoter (Pr) and enhances expression from the leftward *c*I promoter (Prm) (21). A natural repressor, the RepA protein of plasmid pLS1, can repress or activate transcription from a particular promoter of this plasmid, depending on the helix phasing of the RepA target relative to the promoter (33). In eucaryotes, the glucocorticoid receptor protein, when bound to hormone, stimulates or represses promoters near positive or negative glucocorticoid response elements. Moreover, a composite glucocorticoid response element of only 25 bp can be either positively or negatively regulated, depending on the physiological context (14).

The internal sequence does not function as a typical enhancer because it is position and orientation specific. There are several examples of downstream activator sequences in eucaryotic systems which do not have properties typical of enhancers. The bovine leukemia virus long terminal repeat functions

as a transcriptional promoter and contains an activator sequence downstream of the transcription start site. The activator was effective only when located immediately downstream of the RNA start (13). The murine c-*myc* proto-oncogene first exon contains a positive control element which is active only in its sense orientation, 3′ of a nearby promoter (42). The mouse ribosomal protein gene *rpL32* first intron contains an element which stimulates transcription by 5- to 10-fold. This element did not function when translocated upstream of the transcription start site or downstream of its normal location (11).

ACKNOWLEDGMENTS

This work was supported by American Cancer Society grant MV-429N, National Science Foundation grant DMB-9105555 and, in part, by Public Health Service grant GM34471 from the National Institutes of Health and by Welch Foundation grant F-949.

ADDENDUM

After this paper was submitted for publication, the paper by Flower and McHenry (17) reported the identification of *dnaX* and *orf12-recR* promoters and the conclusion that *dnaX* and *orf*12-*recR* constitute an operon.

REFERENCES

1. **Andrews, A. E., B. Lawler, and A. J. Pittard.** 1991. Mutational analysis of repression and activation of the *tyrP* gene in *Escherichia coli*. J. Bacteriol. **173:**5068–5078.
2. **Ang, D., G. N. Chandrasekhar, M. Zylicz, and C. Georgopoulos.** 1986. *Escherichia coli grpE* gene codes for heat shock protein and host growth at high temperature. J. Bacteriol. **167:**25–29.
3. **Aoyama, T., and M. Takanami.** 1985. Essential structure of *E. coli* promoter. II. Effect of the sequences around the RNA start point on promoter function. Nucleic Acids Res. **13:**4085–4096.
4. **Bardwell, J. C. A., and E. A. Craig.** 1988. Ancient heat shock gene is dispensable. J. Bacteriol. **170:**2977–2983.
5. **Blinkowa, A., and J. R. Walker.** 1990. Programmed ribosomal frameshifting generates the *E. coli* DNA polymerase III γ subunit from within the τ subunit reading frame. Nucleic Acids Res. **18:**1725–1729.
6. **Bracco, L., D. Kotlarz, A. Kolb, S. Diekmann, and H. Buc.** 1989. Synthetic curved DNA sequences can act as transcriptional activators in *Escherichia coli*. EMBO J. **8:**4289–4296.
7. **Brosius, J.** 1984. Plasmid vectors for the selection of promoters. Gene **27:**151–161.
8. **Brosius, J., and J. R. Lupski.** 1987. Plasmids for the selection and analysis of prokaryotic promoters. Methods Enzymol. **153:**54–68.
9. **Brune, M., T. Schumann, and F. Wittinghofer.** 1985. Cloning and sequencing of the adenylate kinase gene (*adk*) of *E. coli*. Nucleic Acids Res. **13:**7139–7151.
10. **Choi, K. Y., and H. Zalkin.** 1990. Regulation of *Escherichia coli pyrC* by the purine regulon repressor protein. J. Bacteriol. **172:**3201–3207.
11. **Chung, S., and R. P. Perry.** 1989. Importance of introns for expression of mouse ribosomal protein gene *rpL32*. Mol. Cell. Biol. **9:**2075–2082.
12. **Close, T. J., and R. Rodriguez.** 1982. Construction and characterization of the chloramphenicol-resistance gene cartridge: a new approach to the transcriptional mapping of extrachromosomal elements. Gene **20:**305–316.
13. **Derse, D., and J. W. Casey.** 1986. Two elements in the bovine leukemia virus long terminal repeat that regulate gene expression. Science **231:**1437–1440.
14. **Diamond, M. I., J. N. Miner, S. K. Yoshinaga, and K. R. Yamamoto.** 1990. Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. Science **249:**1266–1272.
15. **Flower, A., and C. S. McHenry.** 1990. The γ subunit of DNA polymerase III holoenzyme of *Escherichia coli* is produced by ribosomal frameshifting. Proc. Natl. Acad. Sci. USA **87:**3713–3717.
16. **Flower, A. M., and C. S. McHenry.** 1986. The adjacent *dnaZ* and *dnaX* genes of *Escherichia coli* are contained within one continuous open reading frame. Nucleic Acids Res. **14:**8091–8101.
17. **Flower, A. M., and C. S. McHenry.** 1991. Transcriptional organization of the *Escherichia coli dnaX* gene. J. Mol. Biol. **220:**649–658.
18. **Goldblum, K., and D. Apirion.** 1981. Inactivation of the ribonucleic acid-processing enzyme ribonuclease E blocks cell division. J. Bacteriol. **146:**128–132.
19. **Harley, C. B., and R. P. Reynolds.** 1987. Analysis of *E. coli* promoter sequences. Nucleic Acids Res. **15:**2343–2361.
20. **Hershey, H. V., and M. W. Taylor.** 1986. Nucleotide sequence and deduced amino acid sequence of *Escherichia coli* adenine phosphoribosyl transferase and comparison with other analogous enzymes. Gene **43:**287–293.
21. **Hochschild, A., N. Irwin, and M. Ptashne.** 1983. Repressor structure and the mechanism of positive control. Cell **32:**319–325.
22. **Inouye, S., A. Nakazawa, and T. Nakazawa.** 1987. Expression of the regulatory gene *xylS* on the TOL plasmid is positively controlled by the *xylR* gene product. Proc. Natl. Acad. Sci. USA **84:**5182–5186.
23. **Kodaira, M., S. B. Biswas, and A. Kornberg.** 1983. The *dnaX* gene encodes the DNA polymerase III holoenzyme τ subunit, precursor of the γ subunit, the *dnaZ* gene product. Mol. Gen. Genet. **192:**80–86.
24. **Lindsey, D. F., D. A. Mullin, and J. R. Walker.** 1989. Characterization of the cryptic lambdoid prophage DLP12 of *Escherichia coli* and overlap of the DLP12 integrase gene with the tRNA gene *argU*. J. Bacteriol. **171:**6197–6205.
25. **Lupski, J. R., A. A. Ruiz, and G. N. Godson.** 1984. Promotion, termination, and antitermination in the *rpsU-dnaG-rpoD* macromolecular synthesis operon of *E. coli* K-12. Mol. Gen. Genet. **195:**391–401.
26. **Mahdi, A. A., and R. G. Lloyd.** 1989. Identification of *recR* locus of *Escherichia coli* K-12 and analysis of its role in recombination and DNA repair. Mol. Gen. Genet. **216:**503–510.
27. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
28. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient *in vitro* synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12:**7035–7056.
29. **Meng, L. M., M. Kilstrup, and P. Nygaard.** 1990. Autoregulation of *purR* repressor synthesis and involvement of *purR* in the regulation of *purB*, *purC*, *purL*, *purMN*, and *guaBA* expression in *Escherichia coli*. Eur. J. Biochem. **187:**373–379.
30. **Messing, J., and J. Vieira.** 1982. A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene **19:**269–276.
31. **Mudd, E. A., P. Prentki, D. Belin, and H. M. Krisch.** 1988. Processing of unstable bacteriophage T4 gene 32 mRNAs into a stable species requires *Escherichia coli* ribonuclease E. EMBO J. **7:**3601–3607.
32. **Mullin, D. A., C. L. Woldringh, J. M. Henson, and J. R. Walker.** 1983. Cloning of the *Escherichia coli dnaZX* region and identification of its products. Mol. Gen. Genet. **192:**73–79.
33. **Pérez-Martín, J., and M. Espinosa.** 1991. The *repA* repressor can act as a transcriptional activator by inducing DNA bends. EMBO J. **10:**1375–1382.
34. **Rolfes, R. J., and H. Zalkin.** 1990. Autoregulation of *Escherichia coli purR* requires two control sites downstream of the promoter. J. Bacteriol. **172:**5758–5766.
35. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5469.
36. **Schaefer, C., and W. Messer.** 1989. Directionality of DnaA protein/DNA interaction. Active orientation of the DnaA protein/*dnaA* box complex in transcription termination. EMBO J. **8:**1609–1613.
37. **Seed, B., and J. Y. Sheen.** 1988. A simple phase-extraction assay

for chloramphenicol acetyltransferase activity. Gene **67:**271–277.
38. **Tilly, K., N. McKittrick, M. Zylicz, and C. Georgopoulos.** 1983. The *dnaK* protein modulates the heat-shock response of *Escherichia coli*. Cell **34:**641–646.
39. **Tsuchihashi, Z., and A. Kornberg.** 1990. Translational frameshifting generates the γ subunit of DNA polymerase III holoenzyme. Proc. Natl. Acad. Sci. USA **87:**2516–2520.
40. **West, R. W., and R. L. Rodriguez.** 1982. Construction and characterization of *E. coli* promoter probe plasmid vectors. III. pBR322 derivatives with deletions in the tetracycline resistance promoter region. Gene **20:**291–304.
41. **Wookey, P. J., J. Pittard, S. M. Forrest, and B. E. Davidson.** 1984. Cloning of the *tyrP* gene and further characterization of the tyrosine-specific transport system in *Escherichia coli* K-12. J. Bacteriol. **160:**169–174.
42. **Yang, J.-Q., E. F. Remmers, and K. B. Marcu.** 1986. The first exon of the c-myc proto-oncogene contains a novel positive control element. EMBO J. **5:**3553–3562.
43. **Yeung, T., D. A. Mullin, K.-S. Chen, E. A. Craig, J. C. A. Bardwell, and J. R. Walker.** 1990. Sequence and expression of the *Escherichia coli recR* locus. J. Bacteriol. **172:**6042–6047.
44. **Yin, K.-C., A. Blinkowa, and J. R. Walker.** 1986. Nucleotide sequence of the *Escherichia coli* replication gene *dnaZX*. Nucleic Acids Res. **14:**6541–6549.