Tandem Transcription Termination Sites in the dnaN Gene of Escherichia coli*

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The dnaN gene of Escherichia coli encodes the β-subunit of DNA polymerase III and maps between the dnaA and recF genes. We demonstrated previously that dnaN and recF constitute a transcriptional unit under control of the dnaN promoters. However, the recF gene has its own promoter region located in the middle of the dnaN structural gene. In this report, we use S1 mapping of mRNAs, transcriptional and translational fusions to the galK and lacZ genes, and in vitro mutagenesis to identify and characterize three tandem transcription termination sites responsible for transcriptional polarity in the dnaN-recF operon. These sites are located in the dnaN gene, downstream from the recF promoter region. Cumulatively, they terminate about 80% of the untranslated transcripts initiated at the recF promoters. As expected, they do not reduce transcription coming from the dnaN promoters unless dnaN translation was prematurely disrupted by the presence of a nonsense codon. The particular arrangement of regulatory elements (promoters and terminators) in the dnaN-recF region provides an exceptional in vitro system to confirm the latent termination site model of transcriptional polarity. In addition, our results contribute to the understanding of the complex regulation of the dnaA, dnaN, and recF genes. We propose that these three genes constitute an operon and that the terminators described in this work could be used to reduce expression of the distal genes of the operon under circumstances in which the dnaN translation happens to be slowed down.

Escherichia coli DNA polymerase III holoenzyme is a highly processive multisubunit complex responsible for chromosomal replication (1, 2). The β subunit of this enzyme plays an essential role in the formation of initiation complexes on primed templates as well as in the elongation reaction (3, 4). The structural gene for β, dnaN, maps between the dnaA and recF genes, at 83 min on the E. coli chromosome map. The dnaA gene product recognizes the E. coli chromosomal origin (oriC) and initiates replication (5, 6). Gene recF codes for a single-stranded DNA-binding protein of about 40 kDa whose precise function still remains unknown (7, 8). However, the phenotype of mutants indicates that recF is involved in DNA recombination and repair as well as in a certain type of stable DNA replication (see Refs. 7–11 and references cited therein).

The three adjacent genes, dnaA, dnaN, and recF, are all transcribed in the same direction (from dnaA toward recF) and show a very compact organization. Thus, the dnaN-recF junction has overlapping stop and start codons (7, and there are only four base pairs in the dnaA-dnaN intercistronic region (3, 12). This organization suggests that expression of these genes is interrelated. In fact, we showed recently that they constitute an operon under control of the dnaA promoters (13). Interestingly, both dnaN and recF have their own promoter regions (11, 14). Thus, there are at least three dnaN promoters located in the second half of the dnaA gene (see Fig. 1). These promoters provide sufficient dnaN and recF expression for complementation when both genes are present on a multicopy plasmid (11). The recF promoter region is located in the middle of the dnaN structural gene (14). This region contains three overlapping promoters, two of them (P1 and P2) initiating transcription toward recF and the other one (Px) in the opposite direction (see Fig. 1). Transcription from the two recF promoters is regulated negatively by sequences located downstream, in dnaN (14). It has been proposed that these sequences constitute a transcriptional terminator(s) responsible for polarity in the dnaN-recF operon and for the low expression of recF mediated by its own promoters (11, 13). The aim of this work is to localize such termination signals precisely and to study their effect on recF and dnaN expression.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Plasmids—The E. coli K-12 strains used were AB1157, N100, and MC1000, which have been described previously (15–16).

Plasmid pMLB1034 (16) contains the lacZ gene lacking a promoter, ribosome binding site, and the first eight codons for β-galactosidase. It also contains three unique restriction sites into which DNA fragments harboring promoter and translation initiation sites can be inserted to form gene fusions. The order of the three restriction sites before the lacZ gene is EcoRI, Smal, and BamHI. Plasmid pIC103 was constructed by cloning an EcoRI fragment (bp 3 to 3553, in Fig. 1) into the EcoRI site of pMLB1034 with the dnaN and recF genes oriented correctly with respect to lacZ. pIC340, a dnaN-lacZ translational fusion, was formed from pIC103 by deletion of the DNA fragment between the Smal site, on pMLB1034 DNA, and the XhoI site, on chromosomal DNA (bp 1490 in Fig. 1), followed by filling in of the XhoI protruding ends mediated by DNA polymerase I (Klenow fragment) and recircularizing in the presence of HindIII linkers (sequence CCAAGCTTGG). A set of dnaN-lacZ translational fusions was constructed by digestion of pIC103 with Bal-31 from the unique NcoI site (at bp 2586 in Fig. 1). Bal-31-cut DNA was treated with

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1 The abbreviation used is: bp, base pair(s).
DNA polymerase I (Klenow fragment), digested with Smal (to eliminate chromosomal DNA sequences between the dnaN and lacZ genes), and recircularized. Deletion end points were located by DNA sequencing in two selected plasmids, pIC368 and pIC371.

pIC494 is a transcription fusion vector derived from pMLB1034. In pIC449, the 660-bp EcoRI-ApaI fragment from pKO-1 (17), which contains 168 bp of the region normally preceding the galK gene and the NH2-terminal coding sequence of this gene, is inserted into the Smal site on pMLB1034 DNA in a way that disrupts this site and produces a galK-lacZ translational fusion. In this fragment, translational stop codons in all reading frames are located just upstream of the 168 bp of “leader” galT gene sequences preceding galK and downstream from the unique Smal cloning site. pIC449 contains the ρ fragment (18) inserted into the HindIII site located 15 bp upstream of the Smal site on pKO-1 DNA to prevent readthrough transcription from vector promoters. pIC449 has the advantage over pKO-1 that the β-galactosidase activity of the GalK-LacZ fusion protein can be used to quantify the length of regulatory elements in place of the radiocchemical method usually employed to assay galactokinase activity (17). In addition, pIC449 has the advantage over conventional lacZ transcription fusion vectors that a constant efficiency for lacZ translation is ensured by the galK ribosome binding site and the untranslatable leader galT gene sequences (17, 19).

pIC451 and pIC455 were obtained by inserting the 437-bp Sau3A1 fragment (bp 1486–1923, in Fig. 3) and 729-bp Hinfl fragment (bp 1429–2158, in Fig. 3) respectively, into the Smal site of pIC449. The chromosomal fragment carried by pIC468 and spanning nucleotides –3 to 2419 was obtained from pIC371 (see Table 1). Plasmids pIC475 and pIC476 were constructed by replacing the DNA fragment between the unique PstI site, on pIC449 DNA, and the unique XhoI site, on chromosomal DNA (bp 1490), with the unique XhoI site, on chromosomal DNA (bp 1490), in Fig. 4, of pIC451 and pIC468, respectively, by the PstI-XhoI fragment of pIC455. Plasmid pIC475 was formed by replacing the PstI-XhoI fragment of pIC451 by the PstI-Xhol fragment of pIC468. Plasmid pIC475 was constructed by cloning the EcoRI-Xhol fragment spanning nucleotides –3 to 1490 (see Fig. 4) into the Smal site of pIC449 after filling in the cohesive EcoRI and Xhol ends with DNA polymerase I (Klenow fragment). It was noted that the XhoI site is finally regenerated. This site was used subsequently to insert the 536-bp HaeIII fragment (bp 1866–2402, in Fig. 4) to construct pIC488.

To introduce nonsense codons in dnaN we used SMURFT linkers (Pharmacia LKB Biotechnology Inc.), which terminate translation by the insertion of a suppressible termination codon in all reading frames. pIC491 and pIC492 were obtained by inserting the SMURFT linker CTAGCTAGACTAG into the XhoI site (at bp 1490, in Fig. 4) of pIC468 and pIC475, respectively, after filling in the protruding XhoI ends with DNA polymerase I (Klenow fragment). Plasmids pIC498 and pIC500 were obtained by inserting the SMURFT linker CTAGCTAGACTAG into the RsrII site (at bp 1400, in Fig. 4) of pIC482 and pIC485, respectively, after filling in the cohesive RsrII ends with DNA polymerase I (Klenow fragment). DNA Manipulations—Standard methods were used for construction and cloning of plasmids, bacterial transformation, purification of plasmid DNA, and restriction enzyme analysis (20).

Enzyme Assays—For determination of β-galactosidase activity, cells harboring lacZ plasmids were grown in LBT (10) with 40 μg/ml ampicillin. Assays were performed, unless otherwise noted, on sodium dodecyl sulfate-chloroform-permeabilized cells as described by Miller (21). Galactokinase and β-lactamase activities were measured as described previously (22). S1 Mapping Assay and DNA Sequencing—Preparation of total in vivo RNA and S1 mapping experiments were performed as described previously (23).

The probe DNAs were labeled at the 3′ ends with terminal deoxynucleotidyl transferase and (α-32P)diodeoxy-ATP by using the DNA 3′ end labeling kit from Boehringer Mannheim. The Maxam and Gilbert chemical method (24) was used for sequencing fragments.

RESULTS AND DISCUSSION

S1 Mapping of 3′ Ends—Our previous work (14) indicated that sequences in dnaN negatively regulating expression from the recF promoters should be located either between the Hinfl and EcoRI sites at bp 2158 and 2212, respectively, or around the Hinfl site at bp 2158, in such a way that the cut at this site disrupts the sequences (see Fig. 1). We therefore decided to use the sense strand of the 536-bp HaeIII fragment, labeled at the 3′ end (Fig. 1, bp 1866–2402), to determine by S1 mapping the 3′ ends of transcripts initiated at the recF promoters.

One major and two minor bands of around 120, 250, and 330 nucleotides were obtained (see Fig. 2A), which could define three putative terminators (hereafter designated T1, T2, and T3, respectively). Although additional very faint bands were detected, we have focused all our attention on the three mentioned bands because they consistently appeared as the most prominent in all the experiments.

For more precise mapping of the 3′ ends at T1 we employed sequencing gels together with the chemically cleaved probe DNA. As shown in Fig. 2B, transcription appeared to stop predominantly at bp 1986 (T1, large arrowhead) with some termination near the T residue at bp 1984 (small arrowhead).

A 349-bp BstXI-HaeIII fragment (Fig. 1, nucleotides 2053–2402) was labeled with 32P at the 3′ ends, and the sense strand was used as a probe to localize precisely termination points at T2 and T3. As shown in Fig. 2C, transcriptional ends are near bp 2125 and 2193. The slight heterogeneity in the lengths of the protected fragments around bp 2193 may reflect actual heterogeneity in the 3′ ends of the RNAs terminating at this region. Alternatively, it may be caused by nipping of the 5′ ends in the hybrids because this region is A+T-rich.

Effect of the T1, T2, and T3 Terminators on Transcription—To determine the efficiencies of termination at T1, T2, and T3, DNA fragments carrying the recF promoters together with 3′-flanking sequences of variable length, including or not the termination points detected by S1 mapping, were inserted into the galK transcription fusion vector pKO-1 (17). Galactokinase activities for the resulting plasmids were assayed and normalized using the β-lactamase gene to exclude any effect of plasmid copy number upon the results. As can be seen in Fig. 3, the three tandem terminators show a gradient effect on transcription started at the recF promoters. Thus, termination is 44% effective for T1, 62% for T1+T2, and 80% for T1+T2+T3. Note that the presence of dnaN sequences extending down-
stream from the PvuII site at bp 2212 does not modify the percentage of termination contributed by T1, T2, and T3 (compare plasmids pIC165, pIC388, and pIC391). However, the recF promoters activity is abated further when the first half of the recF structural gene is included in a recombinant plasmid (see pIC124 in Fig. 3). This is in agreement with previous results indicating that sequences in recF negatively regulate expression of this gene (14).

We have not detected differences between the transcriptional activity of fragments 1429–1923, 1647–1923, 1486–1923, and 1539–1923 (Fig. 3 and data not shown). Moreover, fragments 1429–2419 and 1618–2419 show a transcriptional activity similar to that of fragment 1490–2419 (see Fig. 3). Therefore, it can be concluded that sequences between nucleotides 1429 and 1618 are neutral to downstream transcription, at least under standard growth conditions.

The results shown in Fig. 3 indicate that a real termination activity is associated with the DNA intervals 1923–2069, 2069–2158, and 2158–2212 and support the conclusion that the 3′ ends identified above are generated from termination events occurring in these intervals and not from degradation of transcripts terminated at a distal site.

DNA sequence analysis performed by using the Microgenie program (Beckman Instruments, Inc.) indicates that termination points T1, T2, and T3 are located in DNA regions that do not show the typical structure of simple terminators (25). Moreover, they do not work in a minimal system of in vitro transcription (14). Therefore, we propose that T1, T2, and T3 fall in the category of complex terminators (25). Preliminary in vivo analysis suggests that only T3 is dependent on the Rho protein (13). It is known that Rho mediates termination by binding to a specific region on the RNA called rut (26). Comparisons of known rut sequences have revealed a consensus motif: a cytosine-rich and guanosine-poor region located upstream of the transcription stop points (27). We have searched for regions of high C over G content using the Microgenie software according to Alifano et al. (27). We have identified a large C>G bubble upstream of T2 and T3, extending from bp 1985 to 2085 (data not shown). Curiously, the transcription stop signal for T3 is embedded in a C>G bubble spanning nucleotides 2170–2215. No C>C-rich bubble is observed in the dnaN region upstream of T1. Although more experiments are necessary to clarify the role of Rho on the activity of T1, T2, and T3, it must be pointed out that the tandem arrangement of Rho-dependent and Rho-independent termination sites in the second half of dnaN would not be unique since the simultaneous presence of both types of terminators has also been detected within the lacZ gene of E. coli (28).

Effect of the T1, T2, and T3 Terminators on Transcription started at the recF Promoters—The above results clearly

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**Fig. 3. Effect of the T1, T2, and T3 terminators on transcription started at the recF promoters.** The top of the figure shows part of the dnaN and recF genes. The numbering system is that of Fig. 1. Only relevant restriction sites are shown. Arrowheads indicate the location of terminators T1, T2, and T3, a, the bars below the map represent the DNA region inserted into the Smal site of pKO-1. Only recombinant plasmids carrying the dnaN-recF sequences correctly oriented with respect to the galK gene are shown. Plasmids pIC124, pIC165, pIC166, pIC179, and pIC185 have been described previously (14). Chromosomal fragments carried by pIC388 and pIC391 were obtained from pIC368 and pIC371 (see Table 1), respectively. b, colony phenotype represents the color of plasmid-bearing N100 colonies on Mac-gal-amp plates (11) after 17 h of incubation at 32°C. c, galactokinase units (GalK units) and β-lactamase units (Bla units) were determined in N100 cells as described under "Experimental Procedures." The results represent the averages of at least three independent experiments with the standard deviation usually not exceeding 10%. d, GalK/Bla = galactokinase units/β-lactamase units. e, the percentage of termination was calculated according to the formula

\[
100 - \frac{\text{GalK/Bla of terminator plasmid}}{\text{GalK/Bla of pKO1}} \times 100
\]

where terminator plasmid is any recombinant plasmid carrying the recF promoters and some or all the terminators.
show the presence of transcriptional terminators in the second half of the dnaN gene. Very few intracistronic terminators have been analyzed thus far. A molecular analysis of the sites located within four different cistrons of the his operon of Salmonella typhimurium (27) and a biochemical characterization of the sites within the rho, lacZ, and iboG-iboM genes of E. coli (28-31) have been performed recently. The analysis of many of these elements that are responsible for the phenomenon of transcriptional polarity appears crucial to obtain information on their structure as well as further insights into the molecular mechanisms leading to transcription termination within cistrons (26, 27).

To study the effect of T1, T2, and T3 on dnaN expression we constructed a series of translational fusions between dnaN and the lacZ reporter gene carried by plasmid pMLB1034. These fusions contain the dnaN transcription and translation initiation signals and a variable amount of dnaN-coded sequence at the NH2-terminal region, including or not the terminators. Fusion plasmids were transformed into MC1000 cells and the ability of T1, T2, and T3 to terminate transcripts initiated at the dnaN promoters determined from the production of β-galactosidase in the transformed cells. The activity of plasmid-encoded β-lactamase was used as an internal control to correct for differences in plasmid copy number. As can be seen in Table I, the presence of the terminators has a negligible effect, if any, on β-galactosidase production. Hence, it can be concluded that T1, T2, and T3 do not significantly affect expression of dnaN, at least when the gene is inserted in multicopy plasmids and the cells are growing under normal physiological conditions. This means that transcription started at the dnaN promoters is able to overcome the terminators in contrast to transcription started at the promoters of the recF gene, which is strongly affected (see Fig. 3).

It has been established that translation passing through simple or complex transcriptional terminators can reduce or abolish the effect of such transcription signals (28, 29, 31-34). This could explain the different behavior of transcription started at the recF or dnaN promoters. In the first case, transcripts reaching the terminators are not being translated because the recF ribosome binding site is located close to the recF structural gene, i.e. downstream from T1, T2, and T3 (9). In contrast, transcription started at the dnaN promoters is normally coupled to translation of the dnaN gene when reaching the terminators (see Fig. 1). This coupling apparently allows RNA polymerase to continue transcription through the terminators. If so, the uncoupling of transcription and translation of the dnaN gene should result in activation of T1, T2, and T3 to terminate transcripts started at the dnaN promoters. To test this we chose pIC449, a transcription fusion vector constructed recently in our laboratory from pMLB1034, and used lacZ expression as a quantitative assay of transcription reaching the lacZ gene (see "Experimental Procedures"). Our aim was to clone DNA fragments carrying the dnaN promoters and T1, T2, and T3 in pIC449 and analyze the ability of these terminators to stop the transcription started at the dnaN promoters when dnaN translation was disrupted prematurely by the presence of a nonsense codon.

First, we used pIC449 to analyze the transcriptional activity of some DNA fragments studied previously with the pKO-1 system (Fig. 3). By comparing transcriptional activity of fragments 1647–2069, 1429–2158, and 1618–2212 with that of fragment 1539–1923, we found that in MC1000 host, termination was 54% effective for T1, 82% for T1+T2, and 95% for T1+T2+T3 (data not shown). Although these percentages are higher than those seen with pKO-1 (Fig. 3), it seems clear that similar conclusions can be drawn from both cloning systems.

As shown in Fig. 4, the recF promoters are 4-fold stronger than the dnaN promoters (compare pIC473 and pIC466). Apparently, the two groups of promoters act independently because no functional interference is observed when they are operating simultaneously in pIC449 (compare pIC475 with pIC473 and pIC466). Note that transcription reaching the lacZ gene is greatly reduced when T1, T2, and T3 are cloned downstream from the dnaN and the recF promoters (compare the β-galactosidase/β-lactamase ratios of pIC475 and pIC468). According to the results shown in Fig. 3 and Table I, this reduction must be mainly a result of the action of terminators on transcription started at the recF promoters. This idea is supported by results from Fig. 4, which indicate that in the pIC449 system, T1, T2, and T3 reduce transcription started at the recF promoters by about 97% (compare pIC473 and pIC476) but have an insignificant effect on transcription started at the dnaN promoters. This can be inferred by comparing the β-galactosidase/β-lactamase ratios of pIC468 and pIC466 and assuming that the contribution of the recF promoters to the β-galactosidase/β-lactamase ratio of pIC468 is 3.5 (β-galactosidase/β-lactamase ratio of pIC476 – β-galactosidase/β-lactamase ratio of pIC449). The difference between the β-galactosidase/β-lactamase ratio of pIC468 corrected for the contribution of the recF promoters (28.7 – 3.5 = 25.2) and the β-galactosidase/β-lactamase ratio of pIC466 (31.7) appears irrelevant if we take into account the standard deviations.

To uncouple transcription and translation of the dnaN gene and analyze under these conditions the activity of terminators T1, T2, and T3 on transcription coming from the dnaN promoters, we inserted a nonsense codon into the XhoI site (at bp 1490 in Fig. 4) of pIC468. The β-galactosidase/β-lactamase ratio of the new plasmid (pIC491) was about half that of the original plasmid (compare pIC468 and pIC491 in Fig. 4). The contribution of the recF promoters must be subtracted from the β-galactosidase/β-lactamase ratio of

### Table I

| Plasmid | Fragment | Terminators | β-Gal units | Bla units | β-Gal/Bla
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<tr>
<td>pMLB1034</td>
<td>(-3)-1490</td>
<td>T1, T2, T3</td>
<td>0.08 ± 0.02</td>
<td>19.64 ± 1.41</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>pIC340</td>
<td>(-3)-2353</td>
<td>T1, T2, T3</td>
<td>64.82 ± 2.79</td>
<td>9.17 ± 0.53</td>
<td>7.07</td>
</tr>
<tr>
<td>pIC368</td>
<td>(-3)-2149</td>
<td>T1, T2, T3</td>
<td>87.80 ± 8.03</td>
<td>12.98 ± 0.81</td>
<td>6.77</td>
</tr>
<tr>
<td>pIC371</td>
<td>(-3)-2149</td>
<td>T1, T2, T3</td>
<td>96.72 ± 9.60</td>
<td>13.11 ± 0.96</td>
<td>7.30</td>
</tr>
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* See plasmid constructions in "Experimental Procedures."

* Chromosomal fragment fused to the lacZ gene. The numbering system is that of Fig. 1.

* β-Galactosidase units (β-Gal units) and β-lactamase units (Bla units) were determined in toluenized MC1000 cells harboring lacZ plasmids as described under "Experimental Procedures." All values are the mean of three or more independent experiments and are given with their standard deviations.

* β-Gal/Bla = β-galactosidase units/β-lactamase units.
pIC468 and pIC491 to evaluate the effect of the terminators on the transcription initiated at the dnaN promoters. By doing this, a termination efficiency of 73% is found from the equation: 100 - ((β-galactosidase/β-lactamase of pIC491 - β-galactosidase/β-lactamase of pIC476) × 100)/(β-galactosidase/β-lactamase of pIC468 - β-galactosidase/β-lactamase of pIC476). Therefore, T1, T2, and T3, in addition to regulating transcription from the recF promoters, are able to stop transcription started at the dnaN promoters when multiple regulatory elements are present. Therefore, we decided to clone the 536-bp HaeIII fragment (Fig. 4, bp 1866–2402), containing all three terminators, just downstream from the NH2-terminal coding sequence of the dnaN gene, thus excluding the recF promoters. pIC488 was constructed by inserting this HaeIII fragment into the XhoI site of pIC482 (bp 1490 in Fig. 4) in such a way that the dnaN reading frame was preserved in the fusion. As expected, no differences between the β-galactosidase/β-lactamase ratios of pIC482 and pIC488 were found (Fig. 4). However, the insertion of a nonsense codon into the RsrII site (bp 1400, Fig. 4) of pIC488 decreased transcription initiated at the dnaN promoters by about 90% (compare pIC500 with pIC488 or pIC498 in Fig. 4). These results clearly demonstrate that the uncoupling of transcription and translation of the dnaN gene allows T1, T2, and T3 to terminate transcripts coming from the dnaN promoters.

It is noticeable that the ability of T1, T2, and T3 to terminate untranslated transcripts started at the dnaN promoters appears greater when the terminators are inserted at the beginning of the dnaN structural gene (90% of termination efficiency) than when they remain in their normal configuration (73% of termination efficiency). Although it is possible that the lower efficiency calculated in the last case was caused, at least partially, by accumulation of statistical errors, it is tempting to speculate that the DNA region between nucleotides 1490 and 1866 (or its corresponding untranslated mRNA) confers ability to cause a partial read-through of some or all three terminators.

The particular arrangement of regulatory elements (promoters and terminators) in the dnaN-recF region provides an exceptional in vivo system to confirm the latent termination site model of transcriptional polarity (28, 27, 30, 32, 33). The terminators described here are latent for one gene (dnaN) but active for another (recF), and their activity is dependent on the concurrence of the transcription and translation processes.

Because of the different efficiency of T1, T2, and T3 to stop transcripts started at the dnaN and recF promoters, it can be proposed that although the recF promoters are four times stronger than the dnaN promoters, recF expression depends on the dnaN promoters to a greater extent than on its own promoters. This proposal is strongly supported by the higher rate of transcription overcoming T1, T2, and T3 that is obtained when the dnaN promoters are operating simultaneously with the recF promoters (compare pIC476 and pIC468 in Fig. 4). In fact, by using translational fusions between recF and the lacZ reporter gene we showed recently that the dnaN promoters increase severalfold the recF expression (13). More-
over, our results also indicated that recF is a distal gene of the dnaA operon and that dnaA, dnaN, and recF are predomin-
antly expressed from the same mRNA. However, there must be
transcriptional and/or post-transcriptional mechanisms
specifically involved in lowering expression of the recF gene
(13).

The fact that dnaA, dnaN, and recF are organized in an
operon suggests that under certain physiological conditions
there may be a necessity for coregulation of the three genes
and consequent synthesis coordination of their products.
However, the presence of multiple regulatory elements within
the dnaA operon (11, 13, 14, and this work) could be used to
accomplish discordant regulation as well as differential
expression of the operon genes. In fact, it has been reported
that some of the dnaN promoters are inducible in a dam
background under conditions of simultaneously reduced dnaA
transcription (35). Moreover, preliminary experiments sug-
gest that both dnaN and recF but not dnaA expression is
induced by DNA damage. Finally, the terminators described
in this work may contribute to reduce expression of the distal
genes of the operon under circumstances in which the dnaN
translation happens to be slowed down.

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