Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 4907–4911, August 1982
Biochemistry

Regulation of expression of the *Escherichia coli dnaG* gene and amplification of the dnaG primase

(thermoinduction of primase/RNA polymerase σ gene/primase gene–σ gene operon/transcription attenuation/regulation of DNA and RNA synthesis)

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Communicated by Hamilton O. Smith, May 10, 1982

ABSTRACT **We have isolated λ transducing phages carrying the *Escherichia coli* primase gene (*dnaG*) and mapped restriction sites in the cloned bacterial DNA segments. Several different DNA fragments containing the *dnaG* gene were inserted into multicopy plasmids. An analysis of the primase levels in cells harboring such plasmids indicates that sequences far upstream from the *dnaG* gene are required for optimal primase expression. Using this knowledge, we constructed a plasmid with a thermoinducible copy-number, pRLM61, which was employed to amplify intracellular primase levels approximately 100-fold. The *dnaG* gene is transcribed clockwise with respect to the *E. coli* genetic map, and a *Hin*dIII site located 180 base pairs upstream from the *dnaG* gene separates the gene from its primary promoter. An apparent transcription termination signal is positioned 30–70 base pairs in front of the primase gene. Transcription proceeds past this strong terminator only when RNA polymerase has first transcribed the bacterial DNA segment proximal to the *Hin*dIII site. We suggest that primase expression in *E. coli* is positively regulated by a mechanism of transcription antitermination mediated by a bacterial factor. We propose, furthermore, that the neighboring structural genes for primase and for the σ subunit of RNA polymerase are coordinately regulated as part of an operon. This arrangement may enable the bacterial cell to readily control the level of initiation of DNA and RNA synthesis and thus to respond quickly and efficiently to changing conditions.**

Initiation of DNA synthesis by the DNA polymerase III holoenzyme during DNA replication in *Escherichia coli* is strictly dependent on the prior action of primase to synthesize the prerequisite primer fragments (1–7). Detailed physical and functional studies of this prototype primase, the product of the *dnaG* gene, have been hampered by the difficulty in obtaining significant quantities of the purified enzyme. To remedy this situation, we sought to amplify the intracellular level of primase by inserting the *dnaG* gene into multicopy plasmids. To this end we have constructed a thermoinducible plasmid carrying the *dnaG* gene, pRLM61, which enables intracellular primase levels to be amplified approximately 100-fold. Difficulties encountered in this construction stimulated an investigation into the nature of the regulation of *dnaG* gene expression. We report here that primase expression appears to be regulated by a mechanism of transcription attenuation. Moreover, we suggest that the neighboring *rpoD* gene, coding for the σ subunit of RNA polymerase, is situated in an operon with the *dnaG* gene. The possibility that this gene arrangement reflects the capacity of the bacterial cell to coordinately regulate the rate of initiation of both DNA and RNA synthesis is discussed.

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MATERIALS AND METHODS

Bacterial and Phage Strains. The *E. coli* K-12 strains used and their relevant genetic characteristics were as follows: Q1 (highly transformable) (8); SK2267 (*recA*, *endA*, highly transformable) (obtained from Sidney Kushner); N100 (*recA*) (9); N100(λ*c*I$^+$); RLM774, which is N99(λ*bio*10*c*I857*Pam*3) (temperature-inducible, nonkilling lysogen obtained from Martin Rosenberg); BT308 (*dnaG*ts) (10); RLM658 (*dnaG*ts, *recA*); RLM596 (*dnaG*ts, λ lysogen); and RLM605 (*dnaG*ts, λ*imm*21 lysogen) (ts, temperature-sensitive).

Pools of λKR2 phage containing *Hin*dIII or *Xho* I fragments of *E. coli* DNA and a pool of λKR4 phage containing *Bam*HI fragments of *E. coli* DNA were generously provided by Stephen Rogers. These phages were screened for the ability to transduce *dnaG* by using them to infect RLM596 or RLM605 and selecting colonies able to grow at 42°C. Such colonies were used as the source of the desired phage.

DNA Procedures. Plasmid DNAs were prepared either by the method of Clewell and Helinski (11) or by an alkaline extraction procedure (12). Restriction endonucleases and T4 DNA ligase were purchased from either New England BioLabs or Bethesda Research Laboratories and used according to the directions of the supplier. The 5′ extensions of fragments with cohesive ends were filled in with *E. coli* DNA polymerase I (13) prior to insertion of the fragments into the *Hpa* I site of pKC30. Plasmid transformation of *E. coli* was accomplished by a $CaCl_2$ treatment procedure (14). Chimeric plasmids carrying the *dnaG* gene were isolated from colonies growing at 42°C after transformation of *dnaG*ts *E. coli* strains BT308, RLM658, or RLM596 with plasmid DNA. Plasmid and restriction fragment DNA samples were analyzed by electrophoresis through 0.8% agarose gels containing 0.05 M Tris, 0.38 M glycine, and 2 mM EDTA, pH 8.3. DNA sequence determination was by the chemical degradation method (15).

Cell Growth, Preparation of Cell Extracts, and Primase Assays. Cells to be assayed for intracellular primase levels were grown in AZ broth (16) at 30°C to 3×10^8 to 1×10^9 cells per ml and harvested and frozen as described (1). Where noted, media contained either ampicillin at 100 μg/ml or kanamycin at 20 μg/ml. After preparation of soluble extracts (16), primase was partially purified by ammonium sulfate fractionation as described (1), except that the protein precipitate was resuspended in a minimal volume of buffer A (1) containing 0.1 M NaCl. Primase was assayed by a complementation assay (1) that measures the conversion of phage G4 single-stranded DNA to the duplex replicative form in the presence of a heat-inactivated

Abbreviations: kb, kilobase(s); bp, base pair(s); ts, temperature-sensitive; Ap^R, ampicillin resistant (resistance); Km^R, kanamycin resistant (resistance).

ammonium sulfate fraction from *dnaG*ts strain BT308. A unit of primase catalyzes the incorporation of 1 pmol of dNMP into DNA per min at 30°C.

RESULTS

Pools of λ phages carrying either *Hin*dIII or *Xho* I fragments of *E. coli* DNA were screened for their ability to transduce temperature-sensitive *E. coli dnaG* mutants to temperature resistance. Transducing phages were isolated that carried a 9.2-kilobase (kb) *Hin*dIII fragment or a 3.3-kb *Xho* I fragment of *E. coli* DNA, denoted fragments A and G, respectively (Fig. 1*a*). In *recA*⁻ *E. coli*, however, only phage carrying *Hin*dIII fragment A complemented *dnaG*ts mutations. This suggests that only a portion of the *dnaG* gene is carried on *Xho* I fragment G. Reports published while this work was in progress (19–22) demonstrated that the coding region for the σ subunit of RNA polymerase was situated between positions 7.1 and 8.9 kb on the map in Fig. 1*a*. We conclude that the *dnaG* gene maps between positions 5.0 and 7.1 kb.

The *Hin*dIII/*Bam*HI fragment carrying the *dnaG* gene (fragment C, Fig. 1*a*) was inserted into pKC7 (23), a kanamycin-resistant (Km^R) derivative of pBR322, to create plasmid pRLM40. *E. coli* cells carrying this multicopy plasmid were lysed and assayed for primase levels. Surprisingly, primase levels in strains harboring pRLM40 were not increased above the levels found in plasmid-free *E. coli* (Table 1, lines 1 and 2). It seemed likely that fragment C did not contain the entire *dnaG* gene or was lacking sequences necessary for optimal expression of primase. It was also possible that the *dnaG* gene was autogenously regulated.

To distinguish between these possibilities, we isolated and characterized the properties of the overlapping *E. coli Bam*HI fragment that carried the *dnaG* gene. From pools of λ phage carrying *Bam*HI fragments of *E. coli* DNA, we isolated phage that could complement *dnaG*ts, *recA* mutants of *E. coli*. These phage carried an 8.4-kb *Bam*HI fragment of *E. coli* DNA (fragment B, Fig. 1*a*). Insertion of *Bam*HI fragment B into plasmid pKC7 yielded pRLM47. Primase levels in cells containing pRLM47 were determined and found to be approximately 10-fold higher than the levels in cells without plasmid (Table 1, lines 1 and 3). Optimal expression of the *dnaG* gene product is thus dependent on sequences present between positions 0 and 5.0 kb. A plasmid carrying *Bgl* II fragment E (Fig. 1*a*), pRLM57, yielded intermediate levels of primase (Table 1, line 4). This suggests that sequences that stimulate primase expression map on both sides of the *Bgl* II site at position 2.1 kb.

Table 1. Primase levels yielded by cloned *dnaG* gene fragments

Plasmid	*dnaG* fragment	Host bacteria	Primase specific activity, units/mg protein
pKC7; none	None	N100, SK2267, Q1	640
pRLM40	C	N100, SK2267	580
pRLM47	B	N100, SK2267	8,000
pRLM57	E	Q1	1,700

Fragments containing the *dnaG* gene (described in Fig. 1*a*) were inserted into plasmid pKC7. Cells carrying the chimeric plasmids were grown to 0.8–1.0 × 10^9 cells per ml in the presence of ampicillin (pRLM40 and pRLM57) or kanamycin (pKC7 and pRLM47).

Location of the *dnaG* Gene. The large difference in primase levels yielded by pRLM40 and pRLM47 led us to consider the possibility that the *Hin*dIII site at position 5.0 kb was located within the *dnaG* gene or between the primase structural gene and its promoter or ribosome-binding site. To clarify the situ-

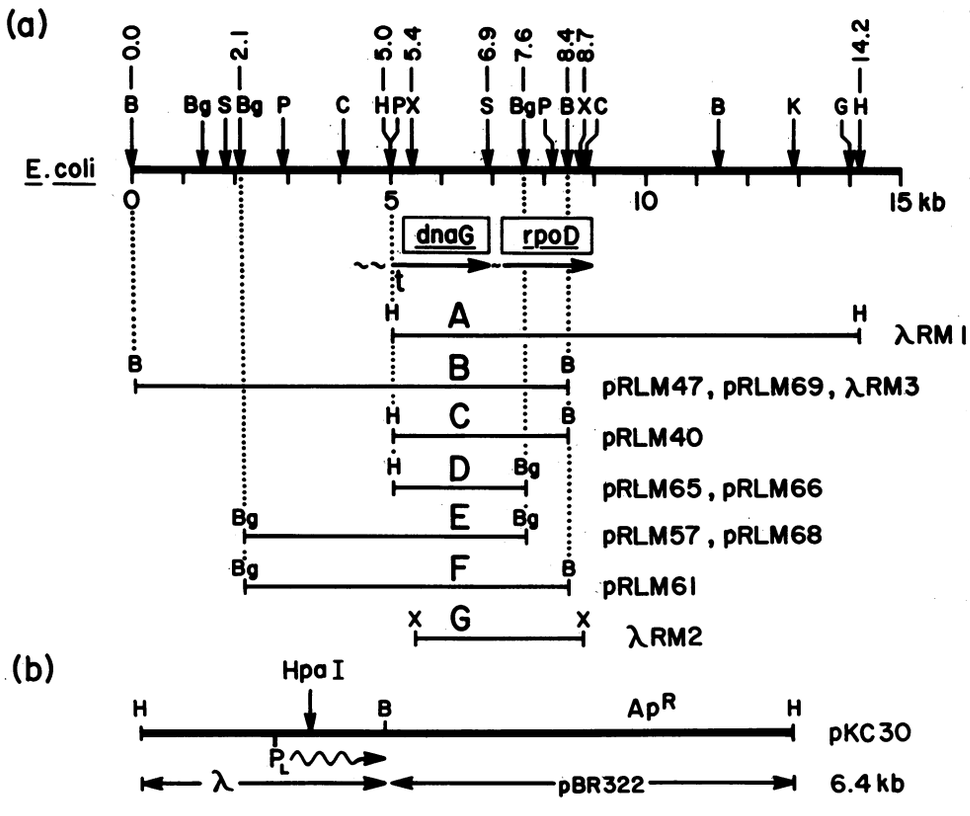

FIG. 1. (*a*) Restriction map of *E. coli* DNA in the region surrounding the *dnaG* gene and diagrams of fragments from this region present in chimeric plasmids and λ phage. The positions of restriction sites were determined from analysis of single and double enzyme digests of λRM1 and pRLM47. *Cla* I sites were taken from Lupski *et al.* (17). The region between 8.4 and 14.2 kb was not tested for the presence of *Bgl* II or *Pst* I sites. Positions of the *dnaG* (18) and *rpoD* (19) genes are indicated by blocks. The arrows depict the direction of transcription of these genes, whereas the wavy lines indicate other regions believed to be transcribed as part of the presumptive *dnaG–rpoD* operon. *t* represents the transcription termination signal located just proximal to the *dnaG* gene. The map is oriented such that transcription from left to right is equivalent to transcription in the clockwise direction on the *E. coli* map. DNA fragments A–G represent segments containing all or part of the *dnaG* gene that were inserted into λ phage or plasmids, whose names are listed adjacent to the fragment they carry. Restriction enzymes used: B, *Bam*HI; Bg, *Bgl* II; H, *Hin*dIII; X, *Xho* I; S, *Sac* I; P, *Pst* I; C, *Cla* I; K, *Kpn* I; G, *Sal*GI. (*b*) Diagram of plasmid pKC30. The location of the λ P_L promoter, the direction of transcription initiated at P_L, and the position of the *Hpa* I cloning site are indicated. Ap^R, ampicillin resistance.

ation, we determined the nucleotide sequence surrounding the *Hin*dIII site (data not shown). Within the first 170 base pairs (bp) to the right of the *Hin*dIII site (as oriented in Fig. 1*a*) termination codons are located in each of the six possible reading frames. The first possible initiation codon is located 182 bp to the right of the *Hin*dIII site. This eliminates the possibility that *dnaG* coding sequences extend to the left of this *Hin*dIII site and confirms the presence of the entire *dnaG* structural gene and its associated ribosome-binding site on the *E. coli Hin*dIII/*Bam*HI fragment C carried on pRLM40. Therefore, the enhanced expression of primase from fragment B on pRLM47 suggests that the *dnaG* gene is transcribed primarily from a promoter located to the left of the *Hin*dIII site and thus is transcribed in the direction from left to right (Fig. 1*a*).

Insertion of the *dnaG* Gene Downstream from λ Promoter P_L. Plasmid pKC30 (Fig. 1*b*) (24) carries the strong, but controllable, P_L promoter from phage λ and contains a single *Hpa* I site located 321 bp downstream from the start site of P_L transcription. DNA inserted at the *Hpa* I site of pKC30 can be transcribed in a highly efficient manner from the P_L promoter. The activity of this promoter can be negatively regulated by the λ cI repressor present in lysogenic cells. Alternatively, the P_L promoter can be activated by placing lysogens, carrying a temperature-sensitive mutation in the phage repressor gene, at a restrictive temperature. Three different fragments that carried the *dnaG* structural gene (fragments B, D, and E, Fig. 1*a*) were inserted into the *Hpa* I site of pKC30 after conversion of protruding termini to blunt ends. Recombinant plasmids carrying the *dnaG* gene were selected by their capacity to transform BT308(λ$^+$) to temperature resistance. The desired plasmids were isolated, characterized by restriction mapping, and subsequently used at permissive temperatures to transform *dnaG*$^+$ lysogens containing either wild-type or thermosensitive λ cI repressor.

Primase levels yielded in the presence of active λ cI repressor by these P_L-containing plasmids, pRLM65, -66, -68, and -69 (Fig. 1), are listed in Table 2 (lines 1–4). Again, the stimulatory effect of sequences far upstream from the *dnaG* gene was observed. Furthermore, those plasmids carrying the *Bam*HI B and *Bgl* II E fragments inserted in the proper orientation downstream from the repressed P_L promoter generated primase levels approximately 3-fold higher than those given by similar plasmids lacking the P_L promoter (compare Tables 1 and 2). This increase presumably reflects the inability of the repressor made from the single-copy prophage to completely repress the 20–30 copies of plasmid-based P_L promoter. Unexpectedly, however, complete derepression of the powerful P_L promoter on these plasmids by thermal inactivation of the temperature-sensitive cI repressor present in strain RLM774 elicited little or no amplification of intracellular primase concentrations (Table 2, lines 5–10). Unfortunately, plasmid pRLM69 could not be included in this analysis because repeated attempts to introduce it into this inducible strain by transformation failed. This was presumably caused by the inability of the mutant repressor to prevent lethal expression of gene products coded by the cloned *Bam*HI fragment.

Table 2. Primase levels yielded by *dnaG* gene fragments cloned downstream from the λ P_L promoter

Plasmid	*dnaG* fragment*	Host bacteria	Primase specific activity, units/mg protein
1. pRLM65	D (n)	N100(λ)	700
2. pRLM66	D (u)	N100(λ)	690
3. pRLM68	E (n)	N100(λ)	5,700
4. pRLM69	B (n)	N100(λ)	21,000
5. pRLM65	D (n)	RLM774	750
6. pRLM65	D (n)	RLM774	1,600†
7. pRLM66	D (u)	RLM774	560
8. pRLM66	D (u)	RLM774	460†
9. pRLM68	E (n)	RLM774	8,300
10. pRLM68	E (n)	RLM774	9,000†

Fragments containing the *dnaG* gene, denoted as in Fig. 1*a*, were inserted into the *Hpa* I site of plasmid pKC30 (Fig. 1*b*) downstream from the P_L promoter. N100(λ) cells harboring hybrid plasmids were harvested at 8×10^8 cells per ml. RLM774 derivatives were grown at 30°C to 3×10^8 cells per ml, at which point a portion of the culture was heated to 42°C to activate transcription from the P_L promoter on the plasmid. All cells were grown in the presence of ampicillin.

* Fragments denoted by (n) were inserted into pKC30 such that P_L-directed transcription proceeded from left to right (in the sense of Fig. 1*a*) across the *dnaG* gene. Fragments denoted by (u) were inserted in the opposite orientation.

† Cells were grown at 42°C for 45 min prior to harvest.

Our failure to achieve a significant amplification of primase levels by P_L-directed transcription led us to consider the possibility that a transcription termination signal was positioned upstream from the *dnaG* gene. Indeed, examination of the DNA sequence in the region of the *Hin*dIII site at position 5.0 kb revealed the presence of a probable ρ-independent transcription termination site. The sequence of this site, situated between 114 and 152 nucleotides downstream from the *Hin*dIII site, is depicted in Fig. 2. The G+C-rich region of dyad symmetry followed by a run of uridine residues found in this sequence is a typical feature of ρ-independent transcription terminators (27). Apparently, transcription initiated at promoters positioned upstream from the *Hin*dIII site, as in pRLM47, -57, -68, and -69, can proceed through the presumptive termination signal and transcribe the *dnaG* gene. On the other hand, our data suggest that P_L-directed transcription most likely terminated at this signal if the cloned fragment did not contain sequences proximal to the *Hin*dIII site. This occurred even though the λ *N* gene antitermination protein (synthesized from the induced prophage) and its required recognition sequence were present (24).

Amplification of Primase by Increased Gene Dosage. As an alternative approach, we sought to raise intracellular primase levels by increasing the dosage of the *dnaG* gene per cell. On the basis of the results reported here and those from additional experiments, we anticipated that prolonged high-level expression of primase and its neighboring gene products would be lethal to *E. coli*. For this reason, we decided to insert the *dnaG* gene into a bacteriophage λ–pBR322 chimeric plasmid whose

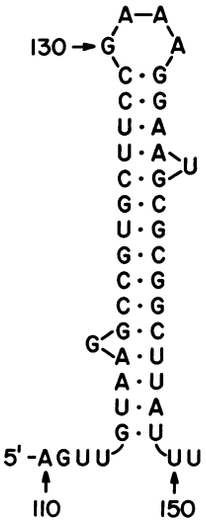

FIG. 2. Sequence and potential secondary structure of a probable transcription termination site just proximal to the *dnaG* gene. The sequence of the terminator signal, depicted here in its RNA form, is numbered from the 5′ base of the *Hin*dIII recognition site at position 5.0 kb (Fig. 1*a*). The potential free energy of formation of the stem and loop structure is approximately −26 kcal/mol (−109 kJ/mol), calculated by using the rules described in refs. 25 and 26.

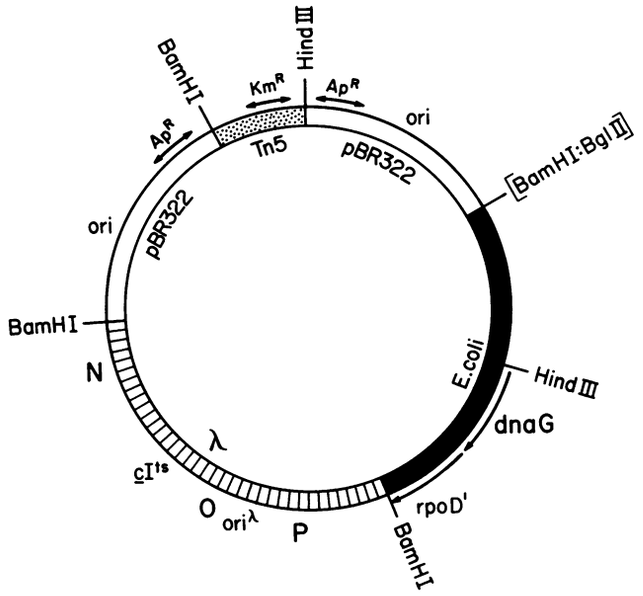

FIG. 3. Diagram of the structure of thermoinducible plasmid pRLM61 used for the amplification of primase. Details of the construction of pRLM61 will be presented elsewhere. Total length is 24 kb.

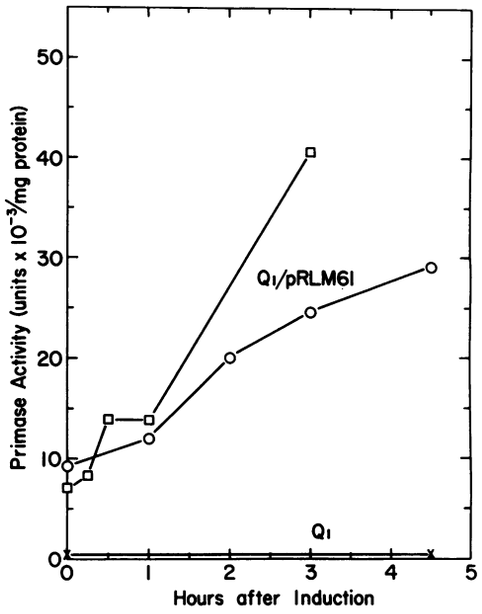

FIG. 4. Time course of primase amplification after thermoinduction of Q1/pRLM61. Cells were grown to 3×10^8 per ml at 30°C, at which point plasmid amplification was induced by shifting the growth temperature to 42°C. At the times indicated, portions of the culture were harvested. After preparation of soluble extracts and partial purification by ammonium sulfate fractionation, primase levels were determined. The time course for Q1/pRLM61 was determined twice (○, □). The primase specific activities yielded by the plasmid-free parent strain, Q1 (×), varied between 400 and 530 units/mg of protein.

copy number could be increased by thermal induction (28). The 7.3-kb *Bam*HI fragment of λ DNA contained in the parent plasmid (herein called the λ *NOP* fragment) carries the regulatory and replication region of the phage chromosome, including the viral replication origin and λ genes *N*, *O*, and *P*. Expression of these genes and initiation of plasmid replication at the λ origin is prevented at 30°C by a temperature-sensitive λ cI repressor coded by the λ *NOP* fragment. The repressor can be inactivated by cell growth at 42°C to induce plasmid amplification. Despite multiple attempts using several different approaches, we were unable to construct a viable plasmid chimera containing pBR322, the λ *NOP* fragment, and the *dnaG* gene in its most active form on the 8.4-kb *E. coli Bam*HI fragment B. At last, a viable plasmid containing this gene combination was constructed, but only after a 2.1-kb segment, containing sequences that stimulate primase expression, was deleted from the left end of the *E. coli Bam*HI fragment B (Fig. 1*a*). A map of this plasmid, pRLM61, is given in Fig. 3.

Thermal induction of strains harboring pRLM61 consistently resulted in 50- to 100-fold amplifications of the intracellular primase level as compared to the level found in control strains with no plasmid. The time course of primase induction in strain Q1/pRLM61 is depicted in Fig. 4. Maximal primase levels were obtained after 3–5 hr of induction, shortly after cell growth ceased. We recovered 425,000 units of primase per g of Q1/pRLM61 cell paste, which is more than 100-fold greater than the amount of primase recovered from wild-type *E. coli* (1, 5). From the specific activity of primase in a crude extract of induced Q1/pRLM61, we estimate that primase constitutes approximately 0.4% of the total soluble protein.

DISCUSSION

We have constructed a thermoinducible chimeric plasmid, pRLM61, carrying the *E. coli dnaG* gene that enables a 100-fold amplification of intracellular levels of *E. coli* primase. Using induced Q1/pRLM61 as a source, we can readily isolate 1 mg of nearly homogeneous primase from 10 g of cell paste (unpublished data). The availability of significant amounts of pure primase has made it possible to determine its NH_2-terminal amino acid sequence and, subsequently, to verify the nucleotide sequence of the *dnaG* gene (18).

Genetic complementation tests, supported by DNA sequence analysis, indicate that the complete *E. coli dnaG* gene is located on a 2.6-kb *Hin*dIII/*Bgl* II fragment (fragment D in Fig. 1*a*). Transcription directed across this fragment from the λ P_L promoter induced primase expression only when the *Hin*dIII terminus of the fragment was upstream from the *Bgl* II terminus (Table 2, lines 5–8). Thus, the *dnaG* gene is transcribed in the same direction, clockwise on the *E. coli* genetic map (29), as is the downstream neighboring *rpoD* gene (19–23). Our DNA sequence and genetic data delimit the beginning of the *dnaG* gene to a 240-bp segment, between a possible initiation codon 182 bp to the right of the *Hin*dIII site and the *Xho* I site at position 5.4 kb (Fig. 1*a*). Transposon mutagenesis of the *dnaG* gene (17), coupled with direct DNA sequence and protein sequence analysis (18), has recently been used to confirm the location of the primase initiation codon at 182 bp downstream from the *Hin*dIII site.

A single copy per cell of cloned *E. coli Hin*dIII fragment A directs expression of sufficient primase to allow survival of a *dnaG*ts *recA* host at a restrictive temperature. We conclude in this case that the cloned *dnaG* gene is transcribed from a promoter positioned in the 182-bp segment of *E. coli* DNA proximal to the start of the gene. Genetic experiments have also led other groups to conclude that the 9.2-kb *Hin*dIII fragment A carried a promoter for the *dnaG* gene (17, 21, 22). Nevertheless, the work reported here demonstrates that this promoter is very weak, because cellular primase levels were not increased when the promoter and accompanying *dnaG* gene were inserted into multicopy plasmids such as pRLM40 and pRLM65. The feebleness of this promoter is also evidenced by the absence of any sequence that even remotely resembles a canonical *E. coli* promoter sequence (30) in this region (data not shown).

Our studies on the expression of primase reveal that the *dnaG* gene is primarily transcribed from another promoter (or promoters) positioned further upstream than the weak promoter discussed above. At least one such promoter must reside in the 2.9-kb *Bgl* II/*Hin*dIII segment proximal to the *dnaG* gene. Primase levels yielded by plasmids carrying the *dnaG* gene were increased about 3-fold when the plasmid also carried this upstream region (Table 1). This amplification is considerably more than 3-fold, however, after correcting for the level of primase expression from the resident bacterial chromosome.

A strong ρ-independent transcription termination signal is located 30 bp upstream from the initiation codon for the *dnaG* gene. We surmise that transcription directed by the powerful λ P_L promoter present on pRLM65 usually terminates at this site (Table 2). It is surprising in this case that transcription ceases at the terminator, particularly because the λ N antitermination protein is present (supplied by the thermally induced prophage) and because the requisite transcription across a N protein recognition sequence (present on pRLM65 between P_L and the *E. coli* DNA insert) has occurred. Under such conditions, *E. coli* RNA polymerase would be expected to transcribe past most ρ-dependent and ρ-independent termination signals (27, 31). On the other hand, if RNA polymerase transcribes across *E. coli* DNA sequences present on the upstream *Bgl* II/*Hin*dIII fragment prior to reaching the terminator, then it is able to proceed past this signal more efficiently (Table 2). We suggest that the upstream *Bgl* II/*Hin*dIII DNA segment contains a recognition sequence for a bacterial factor that modifies the *E. coli* RNA polymerase to a form that can transcribe past the terminator signal proximal to the *dnaG* gene.

On the basis of the results reported here and genetic data that will be reported elsewhere, we propose that the *E. coli dnaG* and *rpoD* genes are situated in a common operon. We suggest, furthermore, that the syntheses of primase and the RNA polymerase σ polypeptide are closely coregulated by a mechanism of transcription attenuation at the terminator just proximal to the *dnaG* gene. Several lines of evidence are consistent with this hypothesis. The neighboring *dnaG* and *rpoD* genes are both transcribed in the same direction, clockwise on the *E. coli* genetic map (18–22). Moreover, the primary promoter employed for transcription of the σ structural gene maps at an unknown location upstream from the *Sac* I site at position 6.9 kb (20, 21). Because this *Sac* I site is located within the primase structural gene (18), this promoter probably is located proximal to the *dnaG* gene. Finally, as is reported here for primase, synthesis of σ appears to be regulated, at least in part, by a mechanism of transcription attenuation (32).

What benefit to the cell might be gained by its acquiring the capacity to coordinately regulate the synthesis of primase and σ? Both of these gene products are absolutely required for cell viability, primase to initiate DNA replication and σ to enable RNA polymerase to initiate RNA synthesis. Primase and σ are both found in limited quantities *in vivo* (1, 33) and may be rate-limiting components for these complex macromolecular processes. Thus, a simple coordinate regulation of the *dnaG* and *rpoD* genes might provide the means by which the overall rate of *initiation* of macromolecular synthesis (DNA and RNA) is controlled in *E. coli*. Moreover, given the inherent instability of *E. coli* mRNA, this control would presumably extend to the regulation of the initiation of protein synthesis. The cell, then, would have the capacity to respond rapidly to changing conditions, such as infection by temperate phage or alterations in its growth medium.

The 20-fold higher intracellular concentration of σ over that of primase (1, 33) is not in accord with the proposal made here that the *dnaG* and *rpoD* genes are transcribed from a common promoter. This wide discrepancy in protein levels, however, is apparently accounted for by the presence of a weak ribosome-binding site and an unusually large number of rare codons in the *dnaG* gene that hinder its translation (18).

We thank Stephen Rogers for supplying pools of λ phages carrying fragments of *E. coli* DNA. We also thank Marty Rosenberg, Nigel Godson and Jim Lupski, and Richard Burgess and Zachary Burton for communication of information prior to publication. We are grateful to Robert C. Benjamin and P. C. Huang for providing advice, assistance, and facilities for DNA sequence analysis. This work was supported by grants from the National Institutes of Health (GM-24282) and the American Cancer Society (NP-363).

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