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Cloning of the *Escherichia coli* gene for primosomal protein i: The relationship to *dnaT*, essential for chromosomal DNA replication

(prepriming/stable DNA replication/SOS signals/lagging-strand synthesis/oligonucleotide screening)

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ABSTRACT **The *Escherichia coli* gene encoding one of the primosomal proteins, protein i, was cloned by the use of synthetic oligonucleotide probes. Nucleotide sequence analysis revealed a coding region for protein i of 537 base pairs preceded by a possible promoter sequence. The gene is located adjacent to the *dnaC* locus, probably both being in a single operon. The protein i gene was shown to be closely related to the *dnaT* locus based on the following observations. (*i*) A multicopy plasmid carrying only the protein i gene suppresses the temperature-sensitive phenotype of a *dnaT* strain and restores the ability of the strain to carry out stable DNA replication in the absence of protein synthesis. (*ii*) An extract from a *dnaT* strain does not support replication of the plasmid pBR322 *in vitro*; addition of purified protein i restores its activity. These results indicate that protein i is encoded by *dnaT* and that it is essential for chromosomal DNA replication and is involved in the induction of stable DNA replication during the SOS response.**

Replication of double-stranded DNA takes place in two successive stages (1). The first stage may involve recognition of the replication origin by a replicon-specific initiation protein followed by the initiation of leading-strand synthesis. The second stage is characterized by the initiation of lagging-strand synthesis coupled with the movement of the replication fork. Intensive study of resolution and reconstitution of conversion of single-stranded DNA (SS) to replicative form DNA (RF) of phage ϕX174 *in vitro* presented an attractive model for the initiation of lagging-strand DNA synthesis (2–4). The model includes (*i*) the recognition of a specific nucleotide sequence on a SS binding protein (SSB)-coated SS by protein n′ (2, 5); (*ii*) assembly of prepriming proteins n, n′, n″, i, *dnaB*, and *dnaC* to form a prepriming protein complex; (*iii*) formation of a primosome with the participation of primase (*dnaG* product); (*iv*) movement of the primosome along the SS in the 5′ → 3′ direction (toward the fork movement) and synthesis of multiple RNA primers of a lagging strand.

In spite of its essential role in the prepriming stage of ϕX174 DNA replication *in vitro*, the function of the primosome in chromosomal DNA replication is not clear, mainly because of the lack of genetic analysis of prepriming proteins. Therefore, we decided to isolate the gene for one of the prepriming proteins, protein i. Our strategy included the determination of the partial amino acid sequence of protein i and synthesis of mixed oligonucleotide probes to screen an *Escherichia coli* genomic library. A positive clone was sequenced and was found to code for a protein of the predicted amino acid sequence. The gene for protein i was found to be located immediately upstream of the *dnaC*. Furthermore, evidence is presented that the previously isolated *dnaT* (6) carries a defect in the structural gene for protein i, suggesting that protein i is essential for normal chromosomal DNA replication and stable DNA replication.

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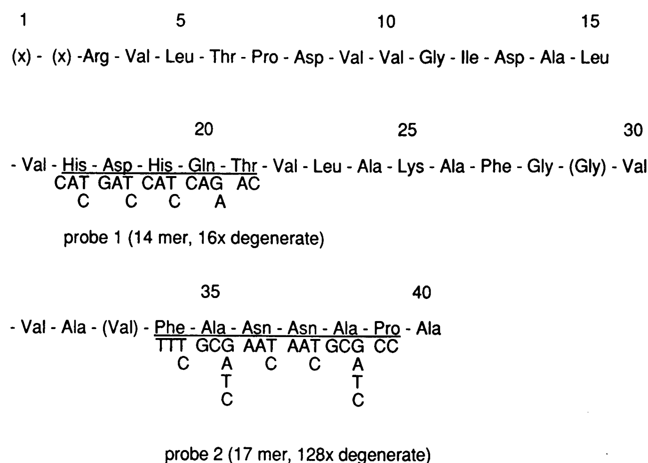

FIG. 1. N-terminal amino acid sequence of protein i and oligonucleotide probes synthesized. Protein i was purified to near homogeneity from *E. coli* K-12 strain HMS83 as described (11). The N-terminal sequence of protein i was determined by an automated gas-phase protein sequencer (Applied Biosystems, Foster City, CA) as described (12). Amino acids with parentheses indicate the presence of ambiguity. Two mixed oligonucleotides with all possible codon choices as indicated were synthesized on a DNA synthesizer (Applied Biosystems) and were used to screen the *E. coli* genomic library. x, Unknown.

MATERIALS AND METHODS

Strains, Enzymes, and Reagents. *E. coli* 15 T^- strains UT205 (*thyA, deoB, arg, trp, metE, rpsL, dnaT1*) and UT3062 (*thyA, arg, trp, metE, rpsL*) (6) were obtained from K. Lark. All enzymes were purchased either from Bethesda Research Laboratories or New England Biolabs. Enzyme reactions were undertaken as described (7).

Screening of the Library with Mixed Oligonucleotide Probes. The *E. coli* genomic library was constructed by inserting *Sam*3A1 partial digests of high molecular weight DNA from *E. coli* K-12 strain C600 at the *Bam*HI site of pUC8. Approximately 2000 transformants were grown in microtiter plates. Plasmid DNA was isolated from pools containing 48 individual clones and analyzed by Southern hybridization (7, 8). The DNA from one subgroup gave a positive band that hybridized to both probes. This subgroup was further divided into eight second subgroups, each of which contained 6 individual clones. The DNAs from each of the eight groups were analyzed in the same way and one positive second subgroup was picked up. Six individual clones from the group

Abbreviations: SS, single-stranded DNA; bp, base pair(s); RF, replicative form DNA; kb, kilobase pair(s); SSB, SS binding protein.

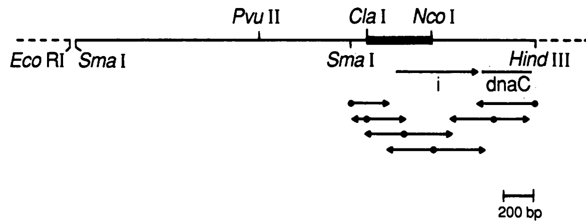

FIG. 2. Restriction map of the pHMI100 clone that was positive on the screening and the strategy for nucleotide sequencing. Both mixed oligonucleotide probes specifically hybridized to the 0.4-kb *Cla* I–*Nco* I fragment (thick bar). Dashed lines indicate the DNA derived from the pUC8 vector. The arrows indicate the direction and an approximate extent of nucleotide sequencing. The dots indicate the 5′ end of the fragments cloned into M13 vector from which each sequencing was performed into the direction of the arrows. bp, Base pairs.

were analyzed, and a single clone pHMI100 that hybridizes with both probes was obtained.

Preparation of Extract and *in Vitro* DNA Replication of pBR322. Fraction II was prepared by the method of Fuller *et al.* (9), except that 0.47 g of ammonium sulfate was added per ml of fraction I. The standard reaction mixture for the *in vitro* DNA replication (25 μl) contained 40 mM Hepes/KOH (pH 8.0), 40 mM KCl, 5% (wt/vol) polyethylene glycol 8000, 24 mM creatine phosphate, 240 ng of creatine kinase per ml, 2 mM ATP, 0.5 mM (each) CTP, GTP, and UTP, 0.1 mM (each) deoxyribonucleotide with [^{3}H]- or [^{32}P]dTTP (200–400 cpm/pmol of dNTP), 50 μM cAMP, 0.3 μg of template DNA, and 200–500 μg of protein in the extract. The reaction mixture was incubated at 30°C for 60 min.

Other Method. Nucleotide sequence was determined by the M13 dideoxy chain-termination method (10).

RESULTS

Cloning of the Gene Encoding Protein i. We have determined the N-terminal amino acid sequence of purified protein i (Fig. 1). Two mixed oligodeoxynucleotides synthesized according to the amino acid sequence as shown in Fig. 1 were used as probes to screen the *E. coli* genomic library. A clone, pHMI100, carrying a 3-kilobase-pair (kb) insert that hybridized with both probes was isolated (data not shown). Restriction digestion and Southern analysis (7) localized the hybridizing region to a 0.4-kb *Cla* I–*Nco* I fragment (Fig. 2). By nucleotide sequence analysis of the fragment and the surrounding region, an open reading frame was found whose predicted amino acid sequence matched that determined for protein i except for one position (the 27th amino acid) (Fig. 3).

Nucleotide Sequence of the Protein i Gene. The nucleotide sequence of the protein i gene (Fig. 3) shows the coding region of the gene is preceded by a Pribnow box-like sequence TAAAAT (13) (position 181–186) and a −35 sequence TTGTAC (position 158–163). At 7 bp upstream of

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        10         20         30         40         50         60         70
CCCGGGCATA TCGGCTTATA CCGCAATGAT TTCGGCGGTA AAAATCAGCC AGTTAGGTTA CAGCGAACCG

        80         90        100        110        120        130        140
TTGATGATTA CCCTGTTAAC CAACTTTCTT ACAGCTTCAT CGATTGTTGG TGCGTTATCC ATCGGTCTTT

       150        160        170        180        190   ↓    200        210
CCATTCCTGG ATTATGGTTG TACCGCAAGC CCCTCGCGTA TAAAATTGCC TCGCTTAAGC ACACGGATGA

       220        230        240        250        260        270        280
GAGACAGCCT CCTCTCCTCC GTGTGTTACT ATAAAAGTTA TCTCCCTTCT CGTTCATCGT TCCATATTTG

                            304            319            334
AGAAACAGT ATG TCT TCC AGA GTT TTG ACC CCG GAC GTC GTT GGT ATT GAC GCC
          MET Ser Ser Arg Val Leu Thr Pro Asp Val Val Gly Ile Asp Ala

              349            364            379
CTG GTA CAC GAT CAC CAA ACC GTT CTG GCA AAA GCT GAA GGC GGT GTG GTT GCC
Leu Val His Asp His Gln Thr Val Leu Ala Lys Ala Glu Gly Gly Val Val Ala

    394            409            424            439
GTA TTT GCT AAC AAT GCC CCG GCG TTT TAT GCC GTC ACG CCT GCA CGC CTG GCT
Val Phe Ala Asn Asn Ala Pro Ala Phe Tyr Ala Val Thr Pro Ala Arg Leu Ala

              454            469            484
GAA CTG CTG GCG CTG GAA GAA AAG CTG GCG CGT CCG GGA AGC GAT GTC GCT CTG
Glu Leu Leu Ala Leu Glu Glu Lys Leu Ala Arg Pro Gly Ser Asp Val Ala Leu

499            514            529            544
GAC GAT CAA CTC TAT CAG GAA CCG CAA GCC GCT CCC GTT GCT GTA CCC ATG GGG
Asp Asp Gln Leu Tyr Gln Glu Pro Gln Ala Ala Pro Val Ala Val Pro Met Gly

    559            574            589            604
AAA TTC GCC ATG TAT CCG GAC TGG CAA CCC GAT GCC GAT TTT ATC CGC CTG GCG
Lys Phe Ala Met Tyr Pro Asp Trp Gln Pro Asp Ala Asp Phe Ile Arg Leu Ala

              619            634            649
GCG CTA TGG GGC GTG GCG CTA AGA GAG CCG GTG ACC ACC GAA GAA CTG GCC TCA
Ala Leu Trp Gly Val Ala Leu Arg Glu Pro Val Thr Thr Glu Glu Leu Ala Ser

    664            679            694            709
TTC ATT GCC TAC TGG CAG GCG GAA GGT AAA CTC TTT CAC CAT GTG CAG TGG CAA
Phe Ile Ala Tyr Trp Gln Ala Glu Gly Lys Val Phe His His Val Gln Trp Gln

              724            739            754
CAA AAA CTG GCG CGC AGC CTG CAA ATC GGT CGT GCC AGC AAC GGC GGA CTG CCG
Gln Lys Leu Ala Arg Ser Leu Gln Ile Gly Arg Ala Ser Asn Gly Gly Leu Pro

769            784            799            814
AAA CGA GAT GTG AAT ACG GTC AGC GAA CCT GAC AGC CAA ATT CCA CCA GGA TTC
Lys Arg Asp Val Asn Thr Val Ser Glu Pro Asp Ser Gln Ile Pro Pro Gly Phe

829                       846            861
AGA GGG TAA CG ATG AAA AAC GTT GGC GCC CTG ATG CAA CGC CTG CAA AAA ATG
Arg Gly  .     MET Lys Asn Val Gly Ala Leu Met Gln Arg Leu Gln Lys Met
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FIG. 3. Nucleotide sequence of the gene for protein i. The nucleotide sequence of the part of the insert of pHMI100 was determined. The first cytosine of the *Sma* I site within the insert is taken as position 1. Open reading frames, corresponding to the coding region of protein i and the N-terminal part of the *dnaC*, are indicated by the amino acids under the nucleotide sequences. The first methionine of protein i is cleaved off. Underlines indicate a putative Pribnow box and a −35 region and the vertical arrow at position 193 indicates a putative transcription initiation site. An inverted repeat is indicated by two arrows written under the nucleotide sequences (position 190–247). Hyphenated lines indicate possible ribosome binding sites for the protein i gene (position 280–283) and the *dnaC* gene (position 821–825). Initiation codons for the protein i gene and the *dnaC* gene are marked by boxes at positions 290 and 832, respectively.

Table 1. Overproduction of protein i activity measured by ϕX174 SS-to-RF reconstitution assay and by *dnaT* complementation assay

			Activity, units* × 10^{-3}/mg of protein	
Plasmid	Vector	Insert	ϕX174	pBR322
None	—	—	0.5 (1)	0.6 (1)
pHMI106†	pBR322	6.8-kb *Bam*HI	1.0 (2)	1.1 (2)
pHM4059‡	pBR322	3-kb *Eco*RI–*Hin*dIII	6.0 (12)	8.0 (13)
pHM3089§	pUC8	1.2-kb *Sma* I–*Hin*dIII	11.5 (23)	27.8 (46)
pHMI100	pUC8	3-kb *Eco*RI–*Hin*dIII	9.0 (18)	17.2 (29)

Fraction II from *E. coli* K-12 strain HB101 harboring the plasmid indicated was prepared as described (11) except that 0.25 g of ammonium sulfate was added per ml of crude lysate and the backwash step was omitted. The protein i activity was measured by *in vitro* reconstitution assay of ϕX174 SS-to-RF conversion ("ϕX174") (15) and *dnaT* complementation assay of pBR322 DNA replication *in vitro* ("pBR322") (Fig. 6*B*). The replication of ϕX174 SS in the reconstitution system was strictly dependent on addition of protein i. The values in parentheses indicate the extent of overproduction.

*One unit is defined as the incorporation of 1 pmol of total nucleotide per min.

†pHMI106 carries a 6.8-kb *Bam*HI *E. coli* chromosomal DNA fragment containing the protein i and *dnaC* gene at the *Bam*HI site of pBR322.

‡pHM4059 carries the 3-kb *Eco*RI–*Hin*dIII fragment from the initial clone pHMI100.

§pHM3089 carries a 1.2-kb *Sma* I–*Hin*dIII fragment of pHMI100 in pUC8 with the protein i gene in the right orientation with the *lac* promoter in the vector.

ATG, a possible ribosome binding site GAGA (position 280–283) is also found (14). The coding region of the protein i gene consists of 537 bp—i.e., 179 amino acid residues with a calculated M_r of 19,300, close to the observed value of 22,000 (11). The amino acid composition predicted from DNA sequence analysis is also consistent with the observed one (data not shown). The coding region of the protein i gene is characterized by frequent use of rare codons, such as three AGA for arginine, eight CAA for glutamine, and two GGA and two GGG for glycine.

Overproduction of Protein i Activity. Definitive evidence that the cloned DNA fragment carries the structural gene for protein i was obtained by the demonstration that an extract prepared from the cells harboring the plasmid carrying the cloned gene overproduces the protein i activity. As shown in Table 1, fraction II from HB101 cells harboring these plasmids overproduced protein i activity. The initial clone pHMI100 as well as the clone pHM3089 that carries a 1.2-kb *Sma* I–*Hin*dIII fragment in the pUC8 vector overproduced the activity by >20-fold.

The Protein i Gene Is Located Immediately Upstream of *dnaC*. An open reading frame was found immediately downstream of the protein i gene (Fig. 3). Its predicted amino acid sequence completely matched that determined for the N-terminal region of the *dnaC* protein (unpublished results). The nucleotide sequence of *dnaC* was determined for an independently isolated clone, and an open reading frame that is identical to the protein i gene was found immediately upstream of the *dnaC* gene. These results established that the genes for protein i and *dnaC* are located next to each other at 99 min on the *E. coli* genetic map. Southern hybridization analysis of *E. coli* genomic DNA with the protein i gene probe revealed that there is only one copy of the gene in the genome of *E. coli* K-12 strain (data not shown).

Overproduction of Wild-Type Protein i Suppresses the Phenotypes of a *dnaT* Strain. Lark *et al.* has isolated a *dnaT* mutant that is defective in stable DNA replication and has mapped the mutation in the vicinity of *dnaC* (6). The *dnaT* mutant strain stops DNA replication at 42.5°C (6). To determine if the cloned protein i gene is related to *dnaT*, a

Insert	Vector	Gene i	Gene dnaC	Growth at 30°C	Growth at 42°C	Ratio 42°C / 30°C
NONE	pUC8	-	-	+	-	$<10^{-5}$
NONE	pBR322	-	-	+	-	NT
	pUC8	+	-	+	+	1
	pBR322	+	-	+	+	1
	pBR322	+	-	+	+	1
Km	pBR322	-	-	+	-	$<10^{-4}$
Km	pUC8	+	-	+	+	NT
	pBR322	-	+	+	-	NT

FIG. 4. Suppression of the temperature-sensitive phenotype of *dnaT* by a multicopy plasmid carrying the wild-type protein i gene. The bars represent the DNA region inserted in the vector. The triangles indicate that a 1.4-kb DNA fragment containing the kanamycin-resistance marker gene (Km) is inserted at that position (*Cla* I or *Nco* I). + or − in the column of "Gene" indicates the presence or absence, respectively, of the functional gene indicated. A plasmid in the bottom line represents pJK137 (16) that carries only the *dnaC*. Cells picked from a plate incubated at 30°C were restreaked on two M9 plates containing 50 μg of ampicillin per ml. After incubation at 30°C or 42°C for 12 hr, the growth was checked. To compare the plating efficiency at 30°C and 42°C, the cultures were grown in M9 medium containing ampicillin (50 μg/ml) at 30°C overnight. Equal amounts of cells were plated on two M9 plates with ampicillin after appropriate dilution and were incubated at 30°C and 42°C. The number of growing colonies was counted and the ratio was calculated. NT, not tested.

multicopy plasmid carrying the protein i gene was introduced into the *dnaT* strain. As shown in Fig. 4, the mutant strain was able to grow at a nonpermissive temperature when the protein i gene was introduced on a multicopy plasmid. A plasmid carrying only *dnaC* could not complement the temperature-sensitive growth of the cells. The insertion of a DNA fragment carrying a kanamycin-resistance marker from transposon *Tn903* into the coding region of the protein i gene abolished the suppression. The *dnaT* strain harboring the plasmid carrying the protein i gene could respond to thymine starvation and supported the DNA synthesis in the absence of protein synthesis (Fig. 5). The vector alone or the plasmid carrying only *dnaC* could not restore the ability to undergo stable DNA replication (Fig. 5). These results indicate that the temperature-sensitive phenotype and the defect in stable DNA replication of the *dnaT* strain can be suppressed by the overproduction of wild-type protein i.

Extract from *dnaT* Does Not Support Replication of pBR322 and Is Complemented by Addition of Purified Protein i. The results shown above still cannot rule out the possibility that the protein i gene is an extragenic suppressor for *dnaT*. Since protein i is involved in pBR322 DNA replication *in vitro* (17, 18), we examined DNA replication of the plasmid in an extract from the *dnaT* strain. As shown in Fig. 6*A*, incorporation was low in fraction II from *dnaT*, whereas a high level of replication was observed in fraction II from the parent strain UT3062. Addition of purified protein i to the *dnaT* extract restored the replication close to a level that is observed in the parent extract (Fig. 6*B*). Similar results were also obtained with fraction I (data not shown). The extent of overproduction of protein i activity determined by the *dnaT* complementation assay agrees with that determined by the

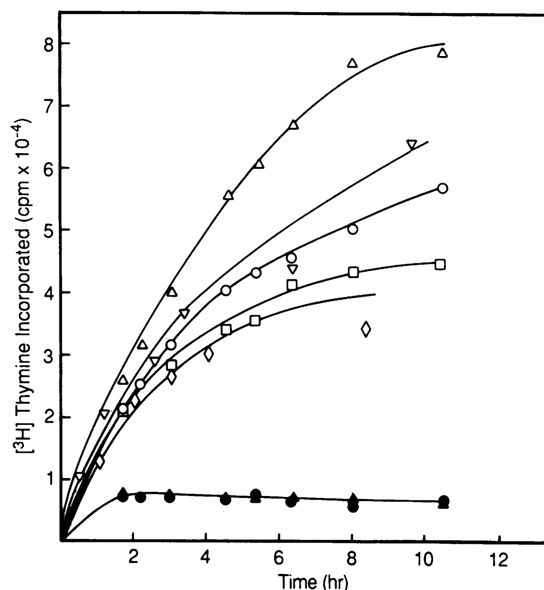
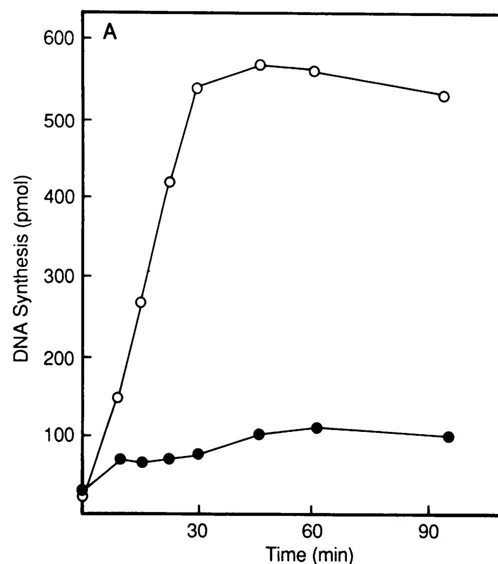


FIG. 5. Assay of stable DNA replication of a *dnaT* strain carrying various plasmids. Cultures were grown at 37°C in M9 medium containing 50 μg of ampicillin per ml and 4 μg of thymine per ml. At $OD_{600} \approx 0.2$–0.3, cells were harvested, washed once with M9 buffer, and resuspended in M9 medium lacking thymine. Incubation was continued at 37°C for 1 hr. Cells were harvested and resuspended in M9 medium lacking required amino acids. Chloramphenicol (150 μg/ml) and [³H]thymine (10 μCi/4 μg/ml; 1 Ci = 37 GBq; Amersham) were added and the incubation was continued at 37°C. At the times indicated, 100-μl samples were withdrawn and the acid-insoluble radioactivity was measured. ○, UT205 (pHMI100); □, UT205 (pHM3089); △, UT205 (pHM4059); ◇, UT205 (pHMI106); ▲, UT205 (pJK137); ●, UT205 (pUC8); ▽, UT3062. pJK137 is a derivative of pBR322 (16) that carries the intact coding region of *dnaC* but only a part of the protein i gene. Other plasmids are described in Table 1.



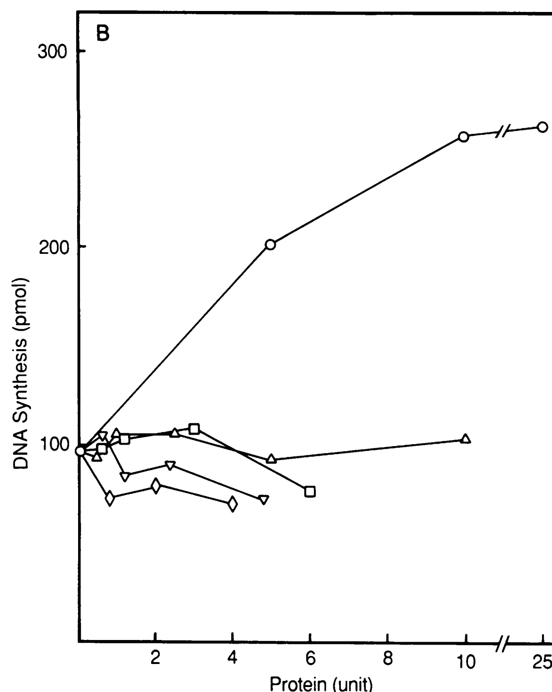


FIG. 6. (*A*) Replication of pBR322 in the extract from the *dnaT* strain. UT205 (*dnaT1*) (●) and UT3062 (*dnaT*⁺) (○) were grown in L broth at 30°C. At $OD_{600} = 0.8$, the culture was transferred to 42°C and the growth was continued for 1 hr. Fraction II was prepared and assayed for pBR322 DNA replication at 30°C. A 10× reaction mix (250 μl) was assembled and 25-μl samples were taken at the times indicated to measure the acid-insoluble radioactivity. One thousand picomole (as nucleotide) of template DNA per assay was used. (*B*) Complementation of pBR322 replication in an extract from *dnaT1* by purified replication proteins. Four microliters of fraction II (250 μg of protein) from UT205 and 1000 pmol (as nucleotide) of template DNA were used. ○, Protein i; □, *dnaB* protein; △, *dnaC* protein; ▽, primase; ◇, protein n'. One unit is defined as incorporation of 1 pmol of total nucleotide per min in the φX174 SS-to-RF reconstitution assay.

φX174 SS-to-RF reconstitution assay in each strain tested (Table 1). These results suggest that functional protein i is lacking in the extract from the *dnaT* strain.

DISCUSSION

We have cloned the *E. coli* chromosomal gene for prepriming protein i by screening an *E. coli* genomic library with mixed

oligonucleotide probes chosen on the basis of the amino acid sequence of the protein i (Fig. 1). Several lines of evidence demonstrated that the cloned DNA fragment actually contains the structural gene for protein i. (*i*) Nucleotide sequence analysis confirmed the presence of an open reading frame that codes for a protein of M_r 19,300 and the predicted amino acid sequence was in agreement with that determined for protein i (Fig. 3). (*ii*) The cloned DNA directed the synthesis of a protein of M_r 20,000 in an *in vitro* coupled transcription/translation system (data not shown). (*iii*) An extract from *E. coli* cells harboring the multicopy plasmid carrying the cloned gene had an elevated level of protein i activity (Table 1). The protein i gene was found to be located immediately upstream of *dnaC* on the *E. coli* chromosome. The termination codon of the protein i gene and ATG initiation codon of the *dnaC* are separated only by 2 bp (Fig. 3). This, in conjunction with the absence of obvious RNA polymerase recognition sequences immediately upstream of *dnaC*, lead us to speculate that genes for protein i and *dnaC* are cotranscribed. Unpublished results indicate that this is actually the case. Use of rare codons in the coding region of the protein i gene may partly account for the low level expression of the gene.

The relationship between the protein i gene and *dnaT*, which was isolated as a mutant defective in stable DNA replication (6), was investigated. The strain also exhibited a temperature-sensitive phenotype in chromosomal DNA replication, probably due to the defect in termination stage (6). We have shown that the cloned protein i gene on a multicopy plasmid can complement the temperature-sensitive phenotype (Fig. 4) and the defect in stable DNA replication of the *dnaT* strain (Fig. 5). Furthermore, an extract prepared from the *dnaT* strain is deficient in supporting pBR322 DNA replication *in vitro* (Fig. 6*A*) and this defect is overcome by adding purified protein i (Fig. 6*B*). These results strongly indicate that the *dnaT* encodes protein i, which is essential for normal chromosomal replication. The mutant protein i may be defective in its ability to reorganize the replication complex at the termination stage prior to the new round of DNA replication.

The *dnaT* extract supports the ϕX174 SS-to-RF to about half the extent of that shown with the parent extract. This may be due to the presence of an alternative pathway to replicate ϕX174 SS in the absence of protein i function or may indicate that the mutant protein i from *dnaT* lacks the ability that is essential for duplex DNA replication while retaining the activity to initiate primosome-dependent replication of ϕX174 SS.

The exact role of the primosome in double-stranded DNA replication is not clear at this moment. Although there are no protein n′ recognition sequences in the vicinity of *oriC* (19), such sequences were found near the origin of ColE1-related plasmids (20, 21) and the F plasmid (22). We found that *in vitro* DNA replication of ColE1-type plasmids requires the protein n′ recognition sequence and the function of protein i for extensive replication (Fig. 6; refs. 17 and 18), very likely for synthesis of the lagging strand (unpublished results), which strongly indicates that the primosome is involved in the initiation of lagging-strand synthesis of ColE1-type plasmid replication *in vitro*. A recent report by Minden and Marians (23) also indicates that the function of primosomal proteins is required for the pBR322 DNA replication *in vitro* composed of purified proteins.

At present, we do not know how protein i is involved in stable DNA replication. It is tempting to speculate that the primosome is involved in the primer synthesis during stable DNA replication and undergoes multiple rounds of replication in the absence of protein synthesis (24). The conservation of the primosome during the successive stages of DNA replication (25) is in keeping with this idea. Multiple protein n′ recognition sites on the *E. coli* chromosome (19) may function as origins of stable DNA replication to trigger primosome-dependent DNA replication. The mutant protein i may be unable to stabilize the primosome and may result in termination of stable DNA replication. Isolation of more conditional lethal mutants within the protein i gene and isolation of the genes for other prepriming proteins will shed more light on the role of the primosome in chromosomal DNA replication.

We thank Doug Villaret for excellent technical assistance in the screening of the *E. coli* genomic library, Karl Pope and Dan Gorman for synthesizing oligonucleotide probes, and Karl Lark for providing the *dnaT* strain. We thank Naoki Nakayama for his original recognition during the DNA sequencing of *dnaC* that the protein i gene is located upstream of *dnaC* and for helpful discussions. We also thank Arthur Kornberg and Yoshito Kaziro for their encouragement throughout the course of this work.

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