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The *Escherichia coli polB* Gene, Which Encodes DNA Polymerase II, Is Regulated by the SOS System

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The *dinA* (damage inducible) gene was previously identified as one of the SOS genes with no known function; it was mapped near the *leuB* gene, where the *polB* gene encoding DNA polymerase II was also mapped. We cloned the chromosomal fragment carrying the *dinA* region from the ordered *Escherichia coli* genomic library and mapped the *dinA* promoter precisely on the physical map of the chromosome. The cells that harbored multicopy plasmids with the *dinA* region expressed very high levels of DNA polymerase activity, which was sensitive to *N*-ethylmaleimide, an inhibitor of DNA polymerase II. Expression of the polymerase activity encoded by the *dinA* locus was regulated by SOS system, and the *dinA* promoter was the promoter of the gene encoding the DNA polymerase. From these data we conclude that the *polB* gene is identical to the *dinA* gene and is regulated by the SOS system. The product of the *polB* (*dinA*) gene was identified as an 80-kDa protein by the maxicell method.

In *Escherichia coli*, three DNA polymerases have been identified (15). DNA polymerase III has been shown to be essential for chromosomal DNA replication and to consist of multiple species of subunits. The nucleotide polymerizing activity resides in the α subunit encoded by the *dnaE* (*polC*) gene. DNA polymerase I, encoded by *polA*, has been shown to be involved in DNA repair and to be required for DNA replication when the cell is growing fast on rich medium (11). However, no biological role has been assigned to DNA polymerase II, encoded by *polB*. The *polB* mutants that have been isolated exhibit no defect in DNA replication and DNA repair (5, 9).

We suspected that the existing *polB* mutants retained substantial levels of DNA polymerase II activity in vivo and that this might have prevented us from revealing the specific phenotype of the mutants so far. To elucidate the role of DNA polymerase II in DNA replication, repair, recombination, and mutagenesis, we thought it necessary to construct a *polB* deletion mutant by using a cloned gene (25). Biochemical studies on DNA polymerase II have been hampered by the relative scarcity of the enzyme in the cell. The overproduction of the enzyme with the cloned gene would facilitate these studies. Therefore, we have been trying to clone the *polB* gene. Bonnar et al. (3) presented evidence that synthesis of DNA polymerase X, presumably DNA polymerase II, is regulated by LexA repressor. The *dinA* gene (13) was identified as an SOS-regulated gene with no known function; it was mapped near *leuB*, where *polB* was also mapped (4, 9). It occurred to us that *dinA* might be identical to *polB*. In this work, we examined this possibility and found that it is indeed so. The *polB* gene was recently cloned by Chen et al. (6).

MATERIALS AND METHODS

Bacterial strains, plasmids, phages, and media. The *E. coli* strains used in this study are listed in Table 1. Strain HRS5200 was constructed by P1 transduction of *leu*$^+$ and *polB100* of HMS83 (5) into ME6268. Plasmids pUC18 (26), pSY343 (27), pSCH18 (10), and pRS528 (22) were used for cloning. An *E. coli* gene library constructed with lambda vectors was kindly provided by Y. Kohara (14). LB medium was described by Miller (18).

Materials. [α-^{32}P]TTP, [^{35}S]methionine, [^{35}S]cysteine, and ^{14}C-labeled proteins for molecular weight standards were purchased from Amersham Japan (Tokyo, Japan). Mitomycin C (MMC) and *N*-ethylmaleimide were from Kyowa Hakko (Tokyo, Japan) and Sigma (St. Louis, Mo.), respectively. The enzymes used for recombinant DNA techniques were from Takara Shuzo (Kyoto, Japan).

Assay of β-galactosidase activity. β-Galactosidase activity was assayed in cells harboring pSCH18 derivatives encoding *dinA'-'lacZ* or other *lacZ* fusion genes grown in the presence or absence of MMC (2 μg/ml) for 2 h. The procedures for the enzyme assay and calculation of the activity were as described by Miller (18).

Preparation of cell extracts. To measure the activity of DNA polymerase II in the overproducing strains, cells of *E. coli* HRS5200 harboring pSY343 derivatives carrying *polB* were grown to an optical density at 600 nm of 0.1 in 10 ml of LB medium containing kanamycin (50 μg/ml) at 28°C. After the incubation temperature was shifted to 37°C for 8 h, the cells were collected by centrifugation.

To assay SOS-induced DNA polymerase II activity, the cells harboring pSCH18 derivatives were grown to an optical density at 600 nm of 0.3 in LB medium containing ampicillin (30 μg/ml) at 37°C, and MMC was added to 2 μg/ml. The cells were cultured for 2 h and then collected by centrifugation. The wet cell paste was washed with 5 ml of buffer containing 50 mM Tris hydrochloride (pH 8.0)–25% sucrose and then suspended in 50 μl of the same buffer. After an equal volume of lysis buffer (50 mM Tris hydrochloride [pH 7.5], 25% sucrose, 60 mM spermidine hydrochloride, 20 mM NaCl, 12 mM dithiothreitol, 0.5 mg of lysozyme per ml) was added, the suspension was chilled at 4°C for 45 min. To the suspension, 20 μl of 5% (wt/vol) Brij 58 was added, and the mixture was incubated at 37°C for 5 min. After the lysate was chilled on ice, a clear supernatant was obtained by centrifugation (30,000 × *g*) for 30 min at 4°C.

Assay of DNA polymerase activity. The standard reaction

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TABLE 1. *E. coli* strains used

Strain	Relevant markers	Source or reference
ME6268	*polA1 leu*	A. Nishimura
HRS5200	*polA1 polB100 leu*$^+$	This study
DM2558	*recA*$^+$ *lexA*$^+$ (*lac-pro*)*XIII*	7
DM844	*lexA*(Ind$^-$) *lacY1*	D. W. Mount
DM2570	*lexA*(Def) (*lac-pro*)*XIII*	7
DE241	*recA1* (*lac-pro*)*XIII*	7
DM2571	Δ*recA lexA*(Def) (*lac-pro*)*XIII*	7
CSR603	*recA1 uvrA6 phr-1*	21

mixture (40 μl) contained 30 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)–KOH (pH 7.6), 8 mM magnesium acetate, 100 mM potassium glutamate, 5 mM dithiothreitol, 33 μM (each) dATP, dGTP, and dCTP, 3.3 μM [α-^{32}P]TTP (ca. 5,000 to 10,000 cpm/pmol), 25 mM activated salmon DNA (1) for the template, and 5 μl of the cell lysate (usually the original lysates described above were diluted 25-fold with buffer containing 30 mM HEPES-KOH [pH 7.6] and 10% glycerol). After incubation at 37°C for 30 min, the reaction was stopped by adding EDTA to 20 mM. DNA synthesis was assayed by the radioactivity adsorbed to DE81 filter disks (Whatman), which was measured by liquid scintillation counting, and the activity was expressed in picomoles of dTTP incorporated into DNA during a 30-min reaction (20). The specific activity of the extract was calculated by dividing the activity by the quantity of protein measured with a protein assay kit (Bio-Rad).

Other methods. We used standard recombinant DNA techniques (17). The proteins encoded by plasmids were analyzed after the cells were labeled with [^{35}S]methionine and [^{35}S]cysteine by the maxicell procedure of Sancar et al. (21).

RESULTS

Location of the *dinA* promoter on the physical map of the chromosome. The *dinA* locus was originally identified as a DNA-damage-inducible locus by taking advantage of Mu d1 (Ap *lac*) operon fusion and was mapped near 2 min on the *E. coli* chromosome (13). One of the *dinA*::*lacZ* fusion genes was cloned into pBR322, and the resultant plasmid was termed pGW510 (12). The 2.8-kb *Bam*HI-*Hin*dIII fragment of the plasmid contained the *dinA* promoter. Kohara et al. (14) constructed an ordered *E. coli* genome library with phage vectors. We examined which clone of the Kohara et al. library contained the *dinA* promoter by dot blot hybridization with the 2.8-kb *Bam*HI-*Hin*dIII fragment of pGW510 as a probe. Among the 13 Kohara clones mapped around 2 min, clone 5H5 strongly hybridized with the probe, clone 8D2 weakly hybridized, and the rest did not hybridize (data not shown). This result suggested that clone 5H5 contained a larger part of the probe than clone 8D2. From the clone 5H5 we recloned the 5.3-kb chromosomal *Bam*HI-*Eco*RI fragment, which hybridized with the same probe (data not shown), into the *Bam*HI-*Eco*RI site of pUC18, giving pTH100.

We constructed restriction maps of the 5.3-kb *Bam*HI-*Eco*RI fragment carried on pTH100 (Fig. 1A) and several plasmids carrying various fragments of this region (Fig. 1C). To locate the *dinA* promoter on this region more precisely, *lacZ* operon fusions with the various fragments of the *Bam*HI-*Eco*RI region were constructed with these plasmids, and we assayed β-galactosidase activity in the cells carrying these operon fusions grown in the presence or the absence of MMC, an SOS-inducing agent (Table 2). Plasmid pTH1528, which carried the promoterless *lacZ* gene, was employed as the negative control, and plasmid pTH1510, which carried the *dinA-lacZ* fusion gene obtained from pGW510 (12), was employed as the positive control for the induction of the SOS-regulated promoter. Strain DM2558 (*recA*$^+$ *lexA*$^+$) carrying either of plasmid pTH1116 or pTH1118 showed about fivefold-higher β-galactosidase activity in the presence of MMC than in its absence. Strains DM2570 [*lexA*(Def)] and DM2571 [Δ*recA lexA*(Def)] carrying pTH1116 or pTH1118 showed constitutive expression of β-galactosidase; the levels of activity observed were similar to that in MMC-treated DM2558 carrying pTH1116 or pTH1118. Strains DM844 [*lexA*(Ind$^-$)] and DE241 (*recA1*) carrying these plasmids showed low levels of expression that were similar to those of uninduced DM2558 carrying the same plasmids. These results suggest that promoter carried on pTH1116 and pTH1118 is repressed by LexA repressor and regulated by the SOS system. Any strain carrying pTH1115 expressed little β-galactosidase. Therefore, we conclude that the 1.2-kb *Bam*HI-*Cla*I-1 fragment does not contain an SOS-inducible promoter or any other strong promoter oriented from the left to the right of the restriction maps in Fig. 1A. All strains carrying pTH1100, pTH1102, pTH1104, or pTH1117 showed high levels of β-galactosidase activity, and always the levels of expression observed were higher in the MMC-induced cells than in the uninduced cells. However, the induction was independent of the *recA* and *lexA* gene functions. Therefore, another promoter that is not under SOS control should be located between the *Cla*I-2 and *Mlu*I-2 sites. From these results, we concluded that the SOS-regulated *dinA* promoter is located in the *Cla*I-1–*Acc*I region in Fig. 1C.

Some new information obtained by the present work was added to the local chromosomal map around the *dinA* region constructed by Kohara et al. (14) (Fig. 1A). The *leuB6* mutation (*E. coli* AB1157 [2]) was complemented by the *Bam*HI fragment shown in Fig. 1A as a shaded area. The location of the *araBAD* operon and the *araC* gene was identified by comparing the restriction map constructed by Kohara et al. (14) with the published DNA sequence of the *ara* region (16).

The *dinA* region contains the *polB* gene. Since both the *dinA* gene and the *polB* gene (which encodes DNA polymerase II) are located near *leuB* (4, 9, 13; this work), we examined whether the *polB* gene was encoded on the cloned 5.3-kb *Bam*HI-*Eco*RI fragment. Cells of MM386 (*polA12 polB*$^+$), a temperature-sensitive *polA* mutant strain (19), carrying pTH100 grown at 28°C gave about 20-fold higher DNA polymerase activity than did cells carrying pUC18 when assayed at 37°C (data not shown). Since pUC18 and the derivatives cannot replicate in the *polA1 polB100* strain, which we wished to use for DNA polymerase II assay, we recloned the DNA fragments to be assayed into the *Bam*HI-*Eco*RI site on a runaway plasmid, pSY343, whose replication is independent of *polA* function. HRS5200 (*polA1 polB100*) harboring a plasmid carrying the 5.3-kb *Bam*HI-*Eco*RI region (pTH200) and the 4.8-kb *Mlu*I-1–*Mlu*I-2 region (pTH202) gave several hundred times the DNA polymerase activity of the control strain carrying the vector plasmid (Table 3). The addition of *N*-ethylmaleimide, an inhibitor of DNA polymerase II, to the reaction mixture drastically reduced the activity. Since the 4.8-kb *Mlu*I chromosomal fragment mapped near the *leuB* locus expressed *N*-ethylmaleimide-sensitive DNA polymerase activity in a copy number-dependent fashion, we conclude that this fragment con-

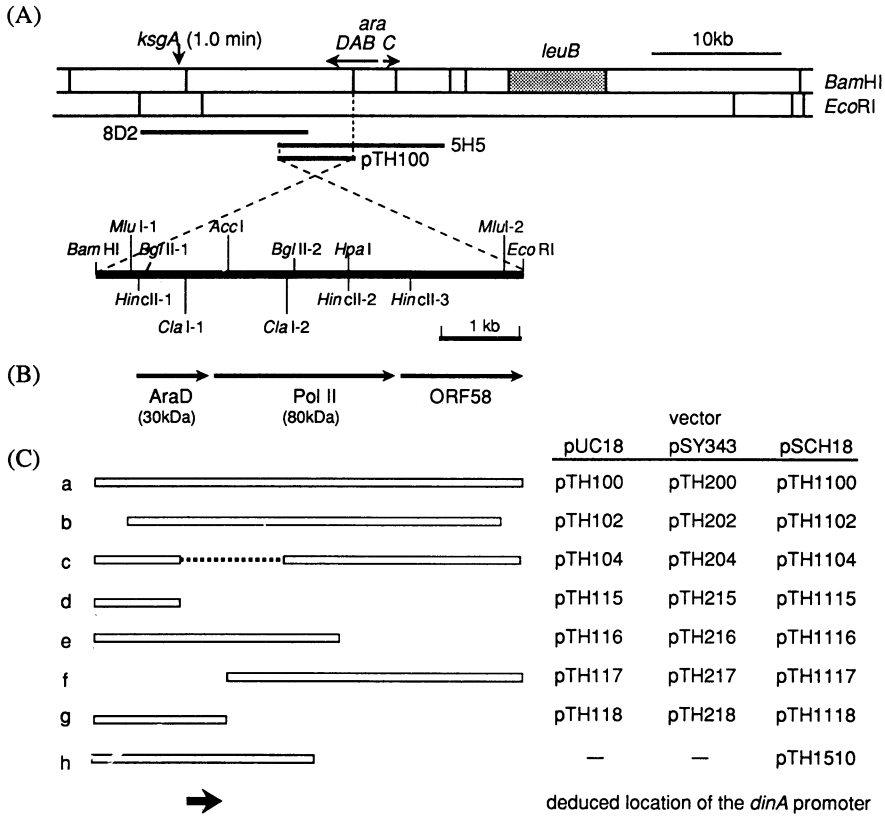

FIG. 1. (A) Physical map of the *dinA* region. The restriction patterns by *Bam*HI and *Eco*RI digestion and the chromosomal DNA regions carried by the λ clones 5H5 and 8D2 were determined by Kohara et al. (14). The shaded area represents the region that complemented the *leuB6* mutation. The locations of the *araBAD* operon and *araC* on the physical map were determined by comparing the nucleotide sequences (16) with the restriction map (14). The directions of the arrows show the directions of transcription. Plasmid pTH100 is a pUC18 derivative carrying the 5.3-kb *Bam*HI-*Eco*RI fragment of clone 5H5, which hybridized with the *dinA* probe. The *Eco*RI site of clone 5H5 was one of the cloning sites on EMBL4 vector. (B) Coding frames of the proteins deduced from the maxicell experiments (Fig. 2) and the *araD* sequence (16). (C) Structure of the plasmids carrying various fragments of the *dinA* region. A series of deletion plasmids (pTH1XX) derived from pTH100 was constructed. Fragment a was the 5.3-kb *Bam*HI-*Eco*RI fragment of clone 5H5. For fragment b, the protruding ends of the 4.8-kb *Mlu*I fragment were converted to blunt ends and cloned into the *Sma*I site of pUC18. The DNA fragment in pTH102 was inserted in the same orientation with the fragment in pTH100 relative to the *Bam*HI and *Eco*RI sites of pUC18. Fragment c was the 4.3-kb DNA fragment that deleted the *Cla*I-1–*Cla*I-2 region of fragment a. For fragment d, the protruding end of the *Cla*I-1 site was converted to a blunt end, and an *Eco*RI linker (5′-GGAATTCC) was attached to it. For fragment e, an *Eco*RI site was created at the *Hpa*I site by inserting the *Eco*RI linker. For fragment f, the protruding end of the *Acc*I site was converted to a blunt end, and a *Bam*HI site was created by attaching a *Bam*HI linker (5′-CGGATCCG) to the blunt site. For fragment g, the protruding end of the *Acc*I site was converted to a blunt end, and an *Eco*RI site was created by attaching the linker. The *Bam*HI-*Eco*RI fragments of pTH1XX plasmids were recloned into the *Bam*HI-*Eco*RI site of pSY343 (a *polA*-independent runaway plasmid) and pRS528 (a pBR322-derived vector for construction of *lacZ* operon fusion); the resultant plasmids were named pTH2XX and pTH10XX, respectively. The *Pst*I-*Sal*I fragments carrying the fusions between the fragments of the *dinA* region and *lacZ* in pTH10XX were recloned into the *Pst*I-*Sal*I site of pSCH18 (a *polA*-independent low-copy-number vector), giving pTH11XX. All of the plasmids with the same numbers (XX) carry the same chromosomal fragments as the inserts. For fragment h, the chromosomal region of the *dinA′-′lacZ* fusion gene of pGW510 was used as the *dinA* probe for hybridization. pTH1510 carries the 12-kb *Bam*HI fragment containing the fusion gene that was recloned from pGW510.

tains the *polB* gene. Furthermore, the failure to detect an increase in DNA polymerase activity in strains carrying pTH204, pTH215, pTH216, pTH217, and pTH218 is consistent with the coding region for DNA polymerase II being to the right of the *Cla*I-1 site, covering the *Acc*I site, and extending at least to the *Hpa*I site.

Activity of DNA polymerase II is SOS inducible. If the expression of the *polB* gene is regulated by the *dinA* promoter, DNA polymerase II encoded by *dinA* should also be induced by the treatments that damage DNA. The DNA polymerase activity of the *polA1 polB*$^+$ cells was induced 2.5-fold by MMC treatment, whereas the activity of *polA1 polB100* cells was not (Table 4). The activity in the *polA1 polB100* cell lysate should be that of DNA polymerase III, and the activity in the *polA1 polB*$^+$ cell lysate should be the combined activity of DNA polymerases II and III. No increase in the activity attributable to DNA polymerase III was observed in the *polA1 polB100* cells treated with MMC. Therefore, the induced DNA polymerase activity should reflect the activity of DNA polymerase II. The MMC-treated *polA1 polB100* cells harboring a low-copy-number *polB*$^+$ plasmid also showed higher polymerase activity than did untreated cells (Table 4). These results support the conclusions that the *polB* gene is identical to the *dinA* gene and that the expression of *polB* is mostly regulated at the transcriptional level by the SOS system. A 16-bp sequence very

TABLE 2. Regulation of β-galactosidase synthesis encoded by the fusion genes

Plasmid[a]	MMC	β-Galactosidase synthesis[b]				
		DM2558 (wild type)	DM844 [*lexA*(Ind⁻)]	DM2570 [*lexA*(Def)]	DE241 (*recA1*)	DM2571 [Δ*recA lexA*(Def)]
pTH1528	−	2.0	1.7	1.1	1.4	1.0
	+	2.6	1.0	1.3	1.0	1.0
pTH1510	−	199	94	1,040	91	1,205
	+	1,091	101	1,270	79	1,015
pTH1100	−	2,360	2,075	2,010	2,125	2,700
	+	3,915	2,880	2,625	2,700	3,350
pTH1102	−	1,827	1,830	1,875	1,810	2,435
	+	4,015	2,645	2,865	2,505	3,060
pTH1104	−	2,097	2,130	1,680	2,100	2,105
	+	3,905	2,570	2,715	2,925	3,385
pTH1115	−	8.4	7.4	3.9	8.1	4.7
	+	9.6	6.6	5.0	4.3	4.6
pTH1116	−	380	355	1,785	271	1,715
	+	2,165	399	2,255	260	1,535
pTH1117	−	2,215	1,630	1,414	2,065	1,770
	+	3,660	1,915	2,095	2,940	2,700
pTH1118	−	380	358	2,000	291	2,265
	+	1,993	415	2,975	303	2,215

[a] pTH1528 and pTH1510 are pSCH18 derivatives carrying the 7.8-kb *PstI-SalI* fragment encoding the promoterless *lacZ* gene of pRS528 and the 12-kb *Bam*HI fragment encoding the *dinA'-'lacZ* fusion gene of pGW510, respectively. For the structure of the operon fusions carried on the other plasmids, see Fig. 1C.
[b] β-Galactosidase units per unit of optical density at 600 nm (18).

similar to the SOS box (LexA-binding site), CTG-TATAaAaccACAG (23), was found just downstream of the *araD* gene (16), which might be the SOS box of the *polB* gene.

Identification of the *dinA* (*polB*) gene product. To identify the products of the genes encoded by the 5.3-kb *Bam*HI-*Eco*RI DNA fragment, we labeled the plasmid-coded proteins by the maxicell method with [^{35}S]methionine and [^{35}S]cysteine (Fig. 2). Plasmid pTH100 encoded three proteins (80, 58, and 30 kDa) in addition to the β-lactamase (28 kDa) and pre-β-lactamase (30 kDa) encoded by the vector (lane 2). We assigned coding frames for these proteins (Fig. 1B). An 80-kDa protein was expressed in the cells harboring plasmids that contained the 4.8-kb *Mlu*I region; the *polA1 polB100* cells harboring the plasmids that contained this region showed high levels of DNA polymerase II activity, whereas the plasmids that did not express the 80-kDa protein showed no DNA polymerase II activity in the *polA1 polB100* cells (Fig. 1 and 2, Table 3). For the synthesis of the 80-kDa protein and DNA polymerase II activity, the region covering the *Acc*I and *Hpa*I sites was required. A strong 58-kDa band expressed by pTH100, pTH104, and pTH117 (Fig. 2, lanes 2, 4, and 6) was replaced by a 45-kDa band, expressed by pTH102 (lane 3), that might be a C-terminal truncated product of the 58-kDa protein. Therefore, the 58-kDa protein should be encoded by the region covering the *Mlu*I-2 site, and the orientation of the gene should be from the left to the right as shown in Fig. 1B. Since 46- and 11-kDa proteins were always detected together with the 58-kDa protein and the combined molecular mass of the two proteins is approximately equal to 58 kDa (Fig. 2B), they are likely to be the degradation products of the 58-kDa protein. The coding region for the *araD* gene was assigned by nucleotide sequence data (16) and physical mapping studies (14; this work), and the *araD* product may be the 30-kDa protein identified as the upper band of the doublet (Fig. 2, lanes 2, 3, 5, and 7). The 65-kDa band found in lane 4 of Fig. 2 might be a fusion protein of the N-terminal part of AraD and the C-terminal part of DNA polymerase II. The 60-kDa protein in lane 5 may be a C-terminal truncated product of the 80-kDa protein. The above data are all consistent with the assignment of the coding regions of these proteins shown in Fig. 1B. However, it is quite possible that the gene corresponding to open reading frame 58 extends beyond the

TABLE 3. DNA polymerase activity of the *polA1 polB1* strain (HRS5200) harboring the plasmids with the DNA fragments of the *dinA* region

Plasmid[a]	DNA polymerase activity[b] (pmol of dTTP/mg of protein/30 min	
	−NEM[c]	+NEM
pTH200	6,110	32.2
pTH202	8,980	46.7
pTH204	9.2	3.4
pTH215	11.3	7.5
pTH216	11.3	6.3
pTH217	14.3	5.3
pTH218	13.7	1.0
pSY343	13.9	3.6

[a] See Fig. 1C for the structure of the plasmids.
[b] DNA polymerase activity was assayed as described in Materials and Methods.
[c] NEM, *N*-Ethylmaleimide.

TABLE 4. Induction of DNA polymerase II by MMC

Strain/plasmid[a]	MMC	DNA polymerase activity (pmol of dTTP/mg of protein/30 min)
ME6268/pTH1528 (*polA1 polB⁺/polB⁻*)	−	44
	+	110
HRS5200/pTH1528 (*polA1 polB100/polB⁻*)	−	17
	+	18
HRS5200/pTH1102 (*polA1 polB100/polB⁺*)	−	110
	+	434

[a] See Fig. 1C for the structure of pTH1102. pTH1528 was described in Table 2.

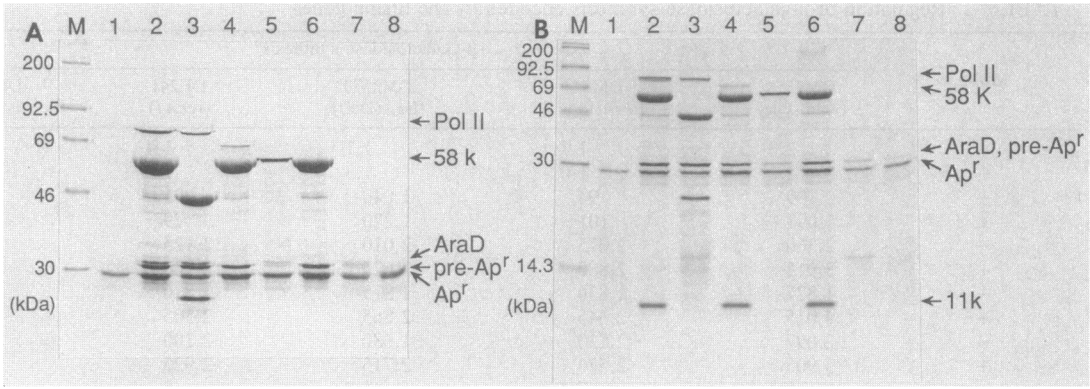

FIG. 2. Identification of the *dinA* (*polB*) gene product by the maxicell method (21). Plasmid-encoded proteins were labeled with [^{35}S]methionine and [^{35}S]cysteine in CSR603 strains carrying various plasmids, separated by (A) 10% and (B) 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and visualized by autoradiography. Lanes: 1, pUC18; 2, pTH100; 3, pTH102; 4, pTH104; 5, pTH116; 6, pTH117; 7, pTH118; 8, pTH115. The arrows on the right indicate the positions of the plasmid-encoded proteins. Ap^r and pre-Ap^r indicate β-lactamase and its precursor with signal peptide, respectively, which are encoded by the *bla* gene on the vector.

*Eco*RI site, and the 58-kDa protein, which was unstable, may be a product of the truncated gene. Expression of the *araD* product and the 58-kDa protein was not regulated by the SOS system (Table 2). Only the *dinA* coding region was regulated by the SOS system, and this region encoded only the 80-kDa protein. Therefore, the maxicell data also strongly support that the *dinA* gene is identical to the *polB* gene.

DISCUSSION

Recently, Chen et al. (6) also cloned the *polB* gene by using an in vivo mini-Mu cloning system (8). The restriction map of the *polB* region that they cloned was very similar to that of the *dinA* region that we analyzed here, except that the *Mlu*I-2 site (Fig. 1) was absent in their clone and that one of *Bam*HI sites in their restriction map was absent in ours. These minor differences in the restriction maps might be attributable to the difference in *E. coli* strains used for cloning the *polB* region. The strain they used carried the *ara* region transferred from *E. coli* B (8), and the *polB* gene was tightly linked to the region (Fig. 1A). The *polB* region analyzed here was cloned from W3110, a derivative of *E. coli* K-12 (14). However, the restriction maps within the *polB* (*dinA*) gene were the same.

Bonner et al. (3) reported that synthesis of a DNA polymerase was induced by nalidixic acid and regulated by LexA repressor and that the enzyme was very likely to be DNA polymerase II. The present work confirmed their results and supplied stronger evidence that the *polB* gene, encoding DNA polymerase II, is regulated by the SOS system. The molecular mass of the enzyme was estimated to be 84 kDa by them, which agrees well with the molecular mass of the *dinA* (*polB*) gene product (80 kDa) identified by us. They have also shown that the inducible DNA polymerase that was partially purified was capable of insertion and bypass at abasic lesion in DNA. The mode of the *polB* regulation and the properties of DNA polymerase II (3, 24) indicate that the enzyme has some role in DNA repair and mutagenesis. However, the *dinA* mutant was as UV mutable and UV resistant as the wild type (G. C. Walker, unpublished data). There seem to be two possible explanations. The first is that DNA polymerase II is not required for UV mutagenesis or UV repair. The second is that a truncated or fusion protein is made that has some DNA polymerase II activity. The in vivo role of the *polB* gene will be elucidated by studying the properties of the deletion mutants, whose construction will be facilitated by the cloned *polB* gene (25).

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ADDENDUM IN PROOF

We recently determined the nucleotide sequence of the *polB* gene, which revealed that *E. coli* DNA polymerase II is highly homologous to the α-like DNA polymerases, including human DNA polymerase α, yeast DNA polymerases I and II, and T4 DNA polymerase.

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