The Escherichia coli polB Locus Is Identical to dinA, the Structural Gene for DNA Polymerase II

CHARACTERIZATION OF Pol II PURIFIED FROM A polB MUTANT

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Pol II* was first isolated and purified in 1970 from an Escherichia coli strain deficient in Pol I (1). A Pol II mutant, polB100, was obtained by mutagenizing cells and assaying for the absence of Pol II activity in crude cell lysates (2). This "brute force" biochemical approach used to obtain Pol II mutants was similar to that taken by DeLucia and Cairns (3) to obtain Pol I mutants. Based on transduction mapping data, Pol II was located clockwise of leu, at about 2 min on the E. coli chromosomal map (4)(Fig. 1). The pol II mutant allele, designated as polB100, showed no phenotype (4). The mapping data for polB100 appeared consistent with the approximate location of an independently isolated polB1 mutant (5).

In 1988, we observed that Pol II was induced as part of the SOS-regulon under control of the Lex A repressor (6). The structural gene for Pol II was subsequently found to coincide with dinA (7, 8), a DNA damage-inducible gene under SOS control (9). In contrast to polB, dinA was located counterclockwise of leu, mapping at about 1 min, between the genetic markers thr and ara (9, 10). DNA sequence analysis of the region surrounding dinA demonstrated that dinA was situated immediately adjacent to araD (Fig. 1)(7). The distance between dinA and polB is about 1 min on the E. coli chromosome.

To resolve the uncertainty concerning the relationship between polB and dinA, we cloned and sequenced the structural gene for Pol II and surrounding sequences from a strain (E. coli HMS83) containing the polB100 gene and purified and characterized the Pol II gene product. We refer to this product as Pol B100. Past difficulties in measuring a phenotype for the polB100 strain will be discussed in light of the biochemical properties of Pol B100.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Plasmids, and Media—HMS83 (polA1, polB100, leu , cys , thy ) was obtained from Dr. R. Moses, Oregon Health Sciences Center, and MC1061 (hsdR, araD19, ΔaroABC-leu7679, galU, galK lacX74, rpsL, thi ) was obtained from Dr. K. McEntee, UCLA. XL1-Blue (supE44, hsdR17, recA1, endA1, gyrA46, thi, relA1, lacF* [proAB+, lacF], lacZDM15, Tn10(tet ) was purchased from Stratagene, San Diego, CA. SG22099 (same as MC4100, but clpA319::mini-kan) was provided by Dr. Michael Maurizi, National Cancer Institute, National Institutes of Health, Bethesda, MD. ZQ100 is similar to MC1061 but contains a kanamycin insertion at the clpA locus from S222909 and also carries F' from XLI-Blue. We have shown previously that Pol II is absent in MC1061 (11). Restriction enzymes were purchased from Promega Corp., Madison, WI. Anti-Pol I antibody was a generous gift from Dr. L. Loeb, University of Washington. E. coli Pol III β, γ complex and SSB were gifts from Dr. M. O'Donnell, Cornell University Medical College, NY. Antibiotic concentrations were: ampicillin, 100 µg/ml, and kanamycin, 30 µg/ml. Genetic and molecular procedures were standard (12, 13).

Cloning and Sequencing of polB100 from HMS83—Genomic DNA from HMS83 was prepared using phenol/chloroform extraction followed by ethanol precipitation (13). The genomic DNA was digested with BamHI and packaged using a Lambda EMBL4 vector, from Clontech, Palo Alto, CA., and plated on E. coli Y1088 (Stratagene). A 2.4-kilobase wild-type polB (dinA) fragment from phy400 (14) was used as the probe to isolate the polB100 gene from the HMS83 genomic library. Phage DNA from a positive clone was purified and digested with PvuI and PstI. A 5.0-kilobase fragment containing the polB100 gene, determined by Southern hybridization, was purified by agarose gel electrophoresis. The vector pT7T318U (Pharmacia Biotech Inc.) was digested with PvuI and Smal, and the 5.0-kilobase fragment was then subcloned into pUTT318U for sequencing. The entire polB gene and surrounding regions, spanning about 300 base pairs to each side, were sequenced using Sequenase (U. S. Biochemical Corp.).

Construction of polB100 Overexpression Plasmid—Site-directed mutagenesis (15) of the wild-type polB overexpression plasmid, pHV400,
was used to construct the polB100 overexpressing plasmid, pZQ83. A 25-mer oligonucleotide with a single base substitution in the middle was used. The primer sequence was 5'-CATCACGTAGCCGCTACGGG-CTGGCCG-3', where T, located 14 nucleotides from the 5' terminus, was incorporated in place of C to generate the mutant primer sequence. After this plasmid was constructed, the 2.4-kilobase polB fragment was resequenced to ensure that this was the only mutation in the gene.

**Purification of polB100 Mutant Pol II**—The pZQ83 plasmid (Amp+) was transformed into strain ZQ100 (KanR, TetR), a polB deletion strain, so that the mutant enzyme Pol B100 could be purified in the absence of wild-type Pol II. We succeeded in purifying Pol B100 using the same purification procedure used previously for wild-type Pol II (14, 16). Although we had no difficulty obtaining a high expression level of Pol B100 in the presence of wild-type Pol II, we were unable to overexpress Pol B100 substantially in a Pol II null mutant background. Because the specific activity for nucleotide incorporation of Pol B100 was also extremely low, about 5–10% of that of wild-type, Western gels were run with a high-titer anti-Pol II antibody (11) to identify Pol B100 after each purification step. The concentration of Pol B100 was determined by comparing its band intensity in a Western blot with that of a known amount of wild-type Pol II. Total protein concentrations were determined using the Bradford assay kit from Bio-Rad, Hercules, CA.

**DNA Polymerase and 3' to 5' Exonuclease Activity Assays—Extension of a 5'-32P-labeled primer was used as an assay for DNA polymerase activity. Two primer-template DNA constructs were used: the first contained a 15-mer annealed over an unlabeled 15-mer downstream from the 32P-labeled primer to generate a 50-mer gap. The assays were carried out as described previously (14, 16). Following a 5-min reaction, the elongated primer extension product was resolved by polyacrylamide gel electrophoresis. The integrated intensities of the primer extension bands were measured using a PhosphoImager (Molecular Dynamics) to determine the polymerase activities for the mutant and wild-type Pol II.**

We assayed for potential trace contamination of Pol B100 by Pol I by preincubating purified Pol B100 on ice for 5 min in the presence of anti-Pol I antibody or by a mock preincubation serum in the absence of antibody, followed by an additional 1-min preincubation at room temperature. Primer elongation was then carried out at 37 °C for 5 min (14, 16), and the difference in elongation catalyzed by Pol B100 in the presence and absence of anti-Pol I antibody served as a measure of Pol I contamination. Kloenow fragment (2 units) was used as a positive control to show that the Pol I Ab was active; 1 unit catalyzes incorporation of 10 nmol of nt in 30 min at 37 °C. Control to show that the Pol I Ab was active; 1 unit catalyzes incorporation of 10 nmol of nt in 30 min at 37 °C. Trace contamination with Pol I was detected using a PhosphoImager (Molecular Dynamics) to determine the polymerase activities for the mutant and wild-type Pol II.

The earliest Pol II mutants were assigned to the polB locus (4, 5), but recent evidence indicates that the damage-inducible dinA gene is the structural gene coding for Pol II (7, 8) and is located counterclockwise of polB by approximately 1 min on the E. coli chromosome (7, 10)(Fig. 1). A clearly defined role for Pol II in either replication or repair has yet to emerge; therefore, it is important to ask if polB might be a negative regulator of Pol II expression, or alternatively, if polB and dinA represent the same gene. To address this issue, we have investigated the relationship between polB and dinA and have purified and characterized the Pol B100 gene product and compared it with wild-type Pol II.

**Pol B100 Has 10–20-Fold Lower Polymerase Activity Than Wild-type Pol II**—The strain HMS83, containing both the polB100 allele and an amber mutant in Pol I (polA1), was used to isolate Pol II. Crude cell lysates prepared from HMS83 contained a protein that cross-reacts with a high-titer, highly selective, anti-Pol II polyclonal antibody (11) and shows the same migration on a Western gel as purified wild-type Pol II. A mixture of Pol B100 and wild-type Pol II was purified from this strain by the standard Pol II purification method (14, 16) and assayed for polymerase and 3'-exonuclease activities as described above.

**RESULTS**

In Situ Two-dimensional Gel Analysis of 3' Exonuclease Activities—A 5'-32P-labeled 30-mer primer annealed to M13 DNA contained a two-base mismatch at the 3'-primer terminus. The labeled primer-template DNA was cast in a standard SDS-polyacrylamide gel, and purified Pol B100 was loaded on the gel. Electrophoresis, carried out in the first dimension, resolved proteins having different molecular weights. Removal of SDS by dialysis permitted protein renaturation to occur, thereby allowing exonuclease digestion of mismatched primer-template DNA in situ (19). Gel slices containing product DNA were recast in a second gel, and primer molecules of different lengths were resolved by electrophoresis carried out in the second gel dimension. Integrated band intensities corresponding to the presence of exonucleolytically degraded 32P-labeled primers of different lengths were quantified by phosphorimaging.

**Restoration of Pol II Activity in HMS83 (polB100)—**We introduced the wild-type dinA gene into HMS83 by growing HMS83 to mid-log phase (A260, 0.5–0.6), followed by infection with a lambda phage (AGG80) carrying wild-type dinA. The HMS83 strain, containing lambda, carries both wild-type Pol II and mutant Pol B100. A mixture of Pol B100 and wild-type Pol II was purified from this strain by the standard Pol II purification method (14, 16) and assayed for polymerase and 3'-exonuclease activities as described above.

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To isolate the gene coding for Pol II from HMS83, we used an oligonucleotide containing the wild-type dinA sequence to hybridize to HMS83 genomic DNA (see “Experimental Procedures”). We cloned and sequenced the dinA gene from HMS83, containing the polB100 allele, and found that it contained a single G → A base substitution at nucleotide position 1309 in the Pol B100 polypeptide and the fraction of activity attributable to contaminating exonucleases.

G. Gudmundsson and M. F. Goodman, unpublished results.

**DNA Polymerase II Mutant**

![Diagram](https://via.placeholder.com/150)

**FIG. 1. Location of polB and dinA genes on the E. coli chromosomal map.** Top, location of dinA, the structural gene coding for Pol II reported in 1990 by Bonner et al. (7) and mapped by Lewis et al. (10). Bottom, location of polB reported in 1974 by Campbell et al. (4).
dinA, causing a substitution of Gly → Asp at position 401 (G401E) in Pol II (7). The presence of a mutation in the structural gene encoding Pol II in the polB100 mutant strain strongly suggests that polB100 is this mutant dinA, i.e. polB is identical to dinA. We will, therefore, refer to the mutant Pol II protein (G401E) as Pol B100.

To study the effect of the Gly to Asp mutation on the enzymatic properties of Pol II, we made a site-directed G → A base substitution at nucleotide position 1309 in the dinA gene and placed it on a high copy number plasmid. We purified Pol B100 using as an assay its cross-reactivity against anti-Pol II antibody in Western gels (Fig. 2).

It is necessary to overexpress Pol B100 in a strain devoid of wild-type Pol II activity to investigate the polymerase and proofreading properties of the mutant enzyme. For reasons that are unclear, we found previously that the level of overproduction of an exonuclease-deficient mutant of Pol II on a plasmid (16) was roughly 50-fold less in a Pol II null-mutant background than in a wild-type background. The level of overproduction of Pol B100 was found to be compromised in a similar manner. Nevertheless, we were able to purify Pol B100 from the Pol II null-mutant background and assay its polymerase and proofreading activities distinct from either Pol I or Pol III. Clp A, a member of the Clp family of proteases (20), was found to copurify with Pol B100. Therefore, a clpA deletion strain, ZQ100, was used for purification of Pol B100 (see “Experimental Procedures”).

A standard 32P-labeled primer extension assay was used to compare deoxynucleotide incorporation activities of Pol B100 with wild-type Pol II (Fig. 3). Wild-type Pol II was observed to fill a 60-nt gap entirely, whereas Pol B100 added an average of about 10 nt.

Pol B100 has approximately 5–10% of the polymerase activity of wild-type Pol II, based on integration of the primer extension band intensities when equimolar concentrations of each enzyme were present in the primer extension assay (Fig. 3). 3' → 5' exonucleolytic degradation of the primers was observed for both polymerases, as shown by the appearance of lower molecular weight bands below the original primer band (Fig. 3). We will later present data showing that the proofreading activities of Pol B100 and wild-type Pol II are similar, if not identical.

Pol B100 has a 10–20-fold lower polymerase-specific activity than wild-type Pol II and cannot be overexpressed significantly in a null dinA mutant. Therefore, it is necessary to show that the low polymerase activity is attributable to Pol B100 and not to the presence of trace contaminants of either Pol I or Pol III. A primer extension assay run in either the presence or absence of polyclonal Ab prepared against Pol I was used to show that residual Pol B100 activity is not caused by Pol I (Fig. 4). Weak primer extension catalyzed by Pol B100 is unaffected by the presence of anti-Pol I Ab (Fig. 4: compare lane 2 – Pol I Ab, with lane 3, + Pol I Ab). The 10–20-fold higher rate of primer extension catalyzed by wild-type Pol II is also insensitive to the presence of anti-Pol I Ab (Fig. 4, lanes 4 and 5), in contrast to the strong inhibitory effect of anti-Pol I Ab on Pol I Klenow fragment activity (Fig. 4, lanes 6 and 7).

A hallmark property of Pol II is its ability to interact with the β processivity clamp protein in the presence of the γ clamp loading complex (17, 21, 22). The β, γ complex are components of the Pol III holoenzyme, a complex containing a Pol III core and the β sliding clamp and five-protein subunit clamp loading γ complex (23). Both Pol II and Pol III core synthesize DNA processively in the presence of β, γ complex and SSB (Fig. 5, lanes 5 and 8, respectively). Synthesis by either Pol II or Pol III core alone is considerably less processive (Fig. 5, lanes 6 and 9), as shown by the presence of many more intermediate bands migrating between the unextended primer (Fig. 5, lane 1) and 60-nt full-length product DNA (Fig. 5, lanes 6 and 9). The mutant Pol B100 was stimulated to synthesize full-length product DNA in the presence of β, γ complex and SSB (Fig. 5, lane 2), whereas synthesis was weakly distributive in the absence of the processivity factors (Fig. 5, lane 3). Thus, the mutant Pol B100 retained the ability to synthesize DNA processively in the presence of β, γ complex + SSB, despite its compromised ability to synthesize DNA.

The presence of SSB in the reaction, in the absence of β, γ complex, provides a means to distinguish between primer extension catalyzed by either Pol II or Pol III. Pol III core activity is inhibited strongly by SSB, whereas Pol II is insensitive to
SSB on a gapped DNA template (18). Primer extension products catalyzed by Pol III core were significantly reduced in size and intensity in the presence of SSB (Fig. 5, lane 10). In contrast to the strong inhibitory effect of SSB on synthesis by Pol III core, DNA synthesis by wild-type Pol II was slightly stimulated in the presence of SSB (Fig. 5, lane 7). The key finding is that synthesis by Pol B100 was essentially unaffected (Fig. 5, lane 4). Thus, it is unlikely that trace contamination by Pol III core is responsible for the primer extension activity observed in the purified Pol B100 fraction.

Pol B100 and Wild-type Pol II Have Similar 3'-Exonuclease Activities—To demonstrate that 3'-exonuclease activity is an integral part of Pol B100, we ran an in situ two-dimensional polyacrylamide gel (19), which allows identification of exonucleolytic degradation products and the relative size of the 3'-exonuclease that forms each product. A 32P-labeled primer annealed to ssM13 DNA was cast within a SDS-polyacrylamide gel prior to loading Pol B100. The gel was run under denaturing conditions in the first dimension to separate proteins having different molecular mass. Exonucleolytic degradation of the primer molecules occurred in situ following removal of the denaturant by diffusion (see "Experimental Procedures"). Lanes cut from the original gel were run on a separate gel, in a second dimension, to resolve primers of different lengths. A detailed technical description of the in situ activity assay is given by Longley and Mosbaugh (19).

The intense horizontal band observed on the gel corresponds to nondegraded 30-mer primer molecules (Fig. 6). Exonucleolytic degradation products, ranging from 29 to 23 nt, are present as less intense bands running below the undigested primer band (Fig. 6). The location of each of the product bands along the horizontal direction is determined by the molecular mass of the 3'-exonuclease.

Wild-type Pol II gives rise to a single product band (Fig. 6a) at a position corresponding to the 89.9-kDa molecular mass of Pol II (7, 8). Two sets of primer degradation bands were observed for Pol B100 (Fig. 6b); one at the 89.9-kDa location of Pol II, and the other generated by a contaminating exonuclease having a molecular mass of about 30 kDa. When Pol B100 and wild-type Pol II were mixed together and assayed in situ (Fig. 6c), the banding pattern was identical to that for Pol B100 (Fig. 6b). The mixing data support the idea that Pol B100 contains an associated 3'-exonuclease activity. We measured the rate of primer degradation and found that Pol B100 has approximately the same level of exonuclease activity as wild-type Pol II. Note that in the gel shown in Fig. 6, the amount of wild-type Pol II used was 10 times greater than Pol B100.

Pol I Klenow fragment (Fig. 6d) was run in a separate control lane to verify that its degradation products ran at a location consistent with the molecular mass (68 kDa) of the enzyme. We also verified that the degradation products of Pol I ran at a molecular mass of 109 kDa, but the bands were less intense because of a loss of signal emanating from the removal of the 5'-32P label by Pol I-associated 5' → 3' exonuclease activity (data not shown).
Fig. 6. *In situ* detection of Pol B100-associated 3’ to 5’ exonuclease activity. A two-dimensional *in situ* gel assay (see “Experimental Procedures”) was used to measure 3’-exonuclease activity for Pol B100. a, purified wild-type Pol II (8 μg); b, purified Pol B100 (0.8 μg); c, a mixture of wild-type Pol II (8 μg) and Pol B100 (0.8 μg); d, Klenow fragment (2 units). A single exonuclease activity is found for wild-type Pol II migrating with a molecular mass of about 89.9 kDa and for Klenow fragment migrating with a molecular mass of about 68 kDa. Two exonuclease activities are found in Pol B100; one of the activities migrates with a molecular mass of about 89.9 kDa, corresponding to the Pol B100-associated exonuclease activity, whereas a non-associated contaminant exonuclease activity migrates with a molecular mass of about 30 kDa.

Restoration of Wild-type Pol II Polymerase Activity in the polB100 Strain (HMS83) — The strain carrying polB100 was originally characterized as having no detectable Pol II activity (4). Although we have shown that Pol B100 purified from the polB100 (HMS83) mutant strain contains a Gly401 → Asp401 replacement causing a large reduction in polymerase activity, there remains a remote possibility for the existence of an additional mutation in HMS83 that might suppress or inactivate Pol II. To investigate this possibility, we infected HMS83 with a lambda phage containing a wild-type copy of Pol II (see “Experimental Procedures”) and carried out the standard Pol II purification.

A 32P-labeled primer annealed to M13 DNA was extended using either 10 ng of Pol II purified from polB100 (HMS83(λGG13)), which carried a single copy of the dinA gene on lambda and a single chromosomal copy of polB100, (Fig. 7, lane 2), or extended using 5 ng of purified wild-type Pol II (Fig. 7, lane 3). DNA synthesis in lane 2 results from the combined action of wild-type Pol II and mutant Pol B100, present presumably at approximately equimolar concentrations. Pol B100 makes a negligible contribution to the integrated band intensities in lane 2 because the mutant polymerase is 10–20 times less active than wild-type Pol II. The primer extension rates are roughly similar in lanes 2 and 3, and because twice as much protein was present in the lane 2 reaction, it follows that the specific activities were similar for wild-type Pol II purified from the λ-infected polB100 and wild-type polB + strains. We, therefore, conclude that the 10–20-fold reduction in Pol II activity in the polB100 mutant is caused by replacement of a single amino acid, Gly401 → Asp401, in the dinA gene rather than by the presence of an additional mutation in a “putative” polB control gene.

DISCUSSION

In the 27 years since E. coli Pol II was discovered in 1970 (1), a specific role for this enzyme in DNA replication or repair has not been defined. The structural gene for Pol II was originally characterized as having no detectable Pol II activity (4). Although we have shown that Pol B100 purified from the polB100 (HMS83) mutant strain contains a Gly401 → Asp401 replacement causing a large reduction in polymerase activity, there remains a remote possibility for the existence of an additional mutation in HMS83 that might suppress or inactivate Pol II. To investigate this possibility, we infected HMS83 with a lambda phage containing a wild-type copy of Pol II (see “Experimental Procedures”) and carried out the standard Pol II purification.

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**polB and dinA Are the Same Gene** — We found a single G → A base substitution at nucleotide position 1309 in the dinA gene cloned from the E. coli strain HMS83 carrying the polB100 mutant allele. This mutation results in the substitution of Gly to Asp at amino acid position 401 in Pol II. Sequence analysis of the upstream region of dinA from HMS83 showed that the promoter element was located two nucleotides downstream from the 3’ end of araD, as shown previously for wild-type dinA (7), and a comparison of sequences surrounding dinA from HMS83 found them to be identical to their wild-type counterpart (7, 8). The observation that the polB100 mutant strain HMS83 contains a mutant dinA gene having the same surrounding sequences as wild-type dinA is conclusive evidence that polB and dinA are identical.

The Pol B100 Mutant Polymerase Has a Defective Polymerase and Normal Proofreading Exonuclease — The polB100 mutant strain (4).
was originally identified by the absence of Pol II polymerase activity in cell lysates prepared from the mutant strain (2). We made a site-directed G → A base substitution at nucleotide position 1309 in the dinA gene, placed it on a high copy number plasmid, and purified the mutant Pol II protein (G401E), which we refer to as Pol B100, on the basis of its cross-reactivity against anti-Pol II antibody in Western gels (Fig. 2). We found that Pol B100 contains about 5–10% of the wild-type Pol II polymerase activity (Fig. 3). It is noteworthy that the substitution of Gly401 → Asp401 causes a dramatic reduction in nucleotide incorporation activity because this mutation is located in any of the five conserved domains characteristic of the group B ("α-like") polymerases (24, 25). Replacement of the nonpolar Gly by the polar Asp probably does not cause a dramatic change in the overall physical properties of Pol B100 compared with wild-type Pol II because the purification scheme used for wild-type Pol II (14, 16) was used successfully in purifying the mutant enzyme. However, a large change may be occurring in the active conformation of the mutant polymerase, which will be interesting to investigate by x-ray crystallography (26).

We showed that residual polymerase activity in the purified Pol B100 preparation was not attributable to contamination by either Pol I or Pol III. Incubation of Pol B100 in the presence of anti-Pol I antibody had no measurable effect on deoxyribonucleotide incorporation, demonstrating that Pol I was not responsible for the low level of polymerase activity in purified Pol B100 fractions (Fig. 4, lanes 2 and 3). Contamination by Pol III core was ruled out by showing that SSB inhibited primer elongation by Pol III core (Fig. 5, lanes 9 and 10) but not by Pol B100 (Fig. 5, lanes 3 and 4).

Both Pol III and Pol II were shown to carry out processive DNA synthesis in the presence of the β sliding clamp and clamp loading γ complex (17, 21). The weak DNA synthesis catalyzed by Pol B100 was primarily distributive in the absence of β and γ complex (Fig. 5, lane 3). However, despite its low polymerase activity, Pol B100 was able to catalyze processive DNA synthesis in the presence of β, γ complex + SSB (Fig. 5, lane 2), demonstrating that Pol B100 retained Pol II-like biochemical properties. Pol II contains an active 3′ → 5′ exonuclease proofreading activity (16). In marked contrast to the strongly detrimental effect of the Gly401 → Asp401 replacement on polymerase activity, the mutant enzyme retained a normal level of 3′ → 5′ exonuclease proofreading activity.

Relating Proofreading of Pol II to Mutagenesis in E. coli—A mutator phenotype resulted when Pol II was replaced on the E. coli chromosome by a proofreading-defective Pol II allele (27). The weak DNA synthesis catalyzed by Pol B100 retained Pol II-like biochemical properties. Pol II contains an active 3′ → 5′ exonuclease proofreading activity (16). In marked contrast to the strongly detrimental effect of the Gly401 → Asp401 replacement on polymerase activity, the mutant enzyme retained a normal level of 3′ → 5′ exonuclease proofreading activity.

Relating Proofreading of Pol II to Mutagenesis in E. coli—A mutator phenotype resulted when Pol II was replaced on the E. coli chromosome by a proofreading-defective Pol II allele (27). We found an increase in forward spontaneous mutations rates at polB and gyrA loci in a Pol III mutantator (dnaE915) background in normally dividing cells, and a measurement of the spectrum of polB chromosomal mutations revealed a G → A transition hot spot specific to the proofreading-defective Pol II exo. There was also an increase in reversion rates of F'(lacZ) base substitutions and frameshifts in the presence of wild-type Pol III (27). In addition to the mutator effects of Pol II in dividing cells, a loss of Pol II proofreading also resulted in an increased reversion rate of an F'(lacZ) frameshift mutation in nondividing cells (28). These results document the importance of Pol II-associated proofreading in controlling chromosomal and episomal mutagenesis in vivo. Therefore, the difficulty in defining a phenotype for the polB100 strain may be attributed to the presence of a wild-type proofreading activity for the mutant Pol B100 (Fig. 6).

Although a well defined role for Pol II in replication or repair has not yet been defined, it has now been established that Pol II is involved in controlling mutation rates in vivo (27, 28). The ability of Pol II to share polymerase accessory proteins with Pol III to achieve high processivity offers the potential for Pol II to “take over” for Pol III, perhaps to carry out specialized tasks requiring moderate to high processivity that cannot be performed by Pol I. We have found that the number of Pol II molecules per cell is in the range of 30–50.3 Because there are only about four Pol III molecules per cell (18), one might expect to find conditions in which Pol II might compete favorably against Pol III for access to the β processivity clamp.

Pol II levels are induced by 7-fold following induction of SOS (6), whereas Pol III levels increase by only 2-fold, at most (6). Thus, in the presence of DNA damage, the increase in intracellular Pol II concentrations might enable the enzyme to recruit the processivity subunits to fill in repair patches numbering perhaps hundreds to thousands of bases. We speculate that Pol II can substitute for Pol III and vice versa in catalyzing long-patch repair. However, the small number of Pol III molecules might place stringent restrictions on the sole use of Pol III to carry out both semi-conservative replication and repair DNA synthesis. It may then fall to Pol II to “pick up the slack” in either or both processes, particularly when SOS is turned on.

Now that Pol II has been shown to play a role in the synthesis of chromosomal and episomal DNA in dividing and nondividing cells, it is timely and important to eliminate ambiguities arising from the different map locations of polB and dinA. Data presented in this paper, documenting that polB and dinA are the same gene, eliminate an uncertainty in the pathway governing the regulation of Pol II.

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