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DNA Polymerase β Can Substitute for DNA Polymerase I in the Initiation of Plasmid DNA Replication

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We previously demonstrated that mammalian DNA polymerase β can substitute for DNA polymerase I of *Escherichia coli* in DNA replication and in base excision repair. We have now obtained genetic evidence suggesting that DNA polymerase β can substitute for *E. coli* DNA polymerase I in the initiation of replication of a plasmid containing a pMB1 origin of DNA replication. Specifically, we demonstrate that a plasmid with a pMB1 origin of replication can be maintained in an *E. coli polA* mutant in the presence of mammalian DNA polymerase β. Our results suggest that mammalian DNA polymerase β can substitute for *E. coli* DNA polymerase I by initiating DNA replication of this plasmid from the 3′ OH terminus of the RNA-DNA hybrid at the origin of replication.

DNA polymerase I (Pol I) of *Escherichia coli*, encoded by the *polA* gene, functions in both DNA replication and DNA repair (3, 8). During DNA replication, Pol I joins the Okazaki fragments on the lagging strand of the replication fork (9, 11). Pol I participates in excision repair by filling gaps resulting from the excision of damaged DNA bases (1, 2). In addition, Pol I initiates the synthesis of certain plasmid DNAs in *E. coli*. Enterobacterial plasmids containing a ColE1 or pMB1 origin of DNA replication are not maintained in several *E. coli polA* mutants, including the *polA12* mutant (4, 7). Studies of plasmid DNA synthesis with purified proteins have demonstrated that the initiation of DNA replication at the ColE1 origin requires Pol I as well as RNA polymerase and RNase H (5). RNA polymerase transcribes RNA molecules, beginning approximately 555 bp upstream of the origin of DNA replication (6). The resulting RNA strand of the DNA-RNA hybrid is cleaved by RNase H, leaving a 3′ OH terminus that serves as a primer for the initiation of DNA replication (10). Pol I initiates DNA synthesis from these primers, and it is replaced with the Pol III holoenzyme at a primosome assembly site approximately 400 nucleotides downstream from the origin (12, 16).

We have previously shown that mammalian Pol β is able to substitute for Pol I in DNA replication and in base excision repair (13, 14). Pol β increases the rate of joining of Okazaki fragments in a *polA12*(Ts) mutant at the nonpermissive temperature, suggesting that the Pol β enzyme is able to fill gaps between the Okazaki fragments on the lagging strand (13). Expression of Pol β in a *polA12*(Ts) mutant of *E. coli* confers methylmethane sulfonate resistance to this otherwise methylmethane sulfonate-sensitive strain, suggesting that Pol β is able to fill gaps formed by the excision of alkylated bases (14). Therefore, we wished to determine whether Pol β could also substitute for Pol I in the initiation of DNA replication of a plasmid containing a pMB1 origin of replication.

The pβ plasmid is maintained in a *polA12* mutant. We initially observed that a plasmid that contains a pMB1 origin of replication, pMS119HE, was not maintained in a *recA718 polA12*(Ts) strain unless the strain was grown in medium containing ampicillin. However, the same plasmid carrying the rat Pol β cDNA, referred to as pβ, could be maintained in this strain in the absence of ampicillin. The presence of ampicillin selects for plasmids carrying the β-lactamase gene, including pMS119HE and pβ. Since the difference between the pMS119HE and pβ plasmids was the presence of the Pol β cDNA in pβ, we reasoned that expression of Pol β from the pβ plasmid was necessary for the plasmid to be maintained in the *recA718 polA12* strain.

To test this hypothesis, we inoculated approximately 1,000 Amp^r cells of the *recA718 polA12* strain, containing either the pMS119HE or pβ plasmid, into nutrient broth (13) that did not contain ampicillin. The JS262 strain carried the pMS119HE plasmid and the JS260 strain carried the pβ plasmid. These cultures were grown overnight at the nonpermissive temperature in the presence of isopropyl-β-D-thiogalactopyranoside (IPTG) to induce expression of Pol β from pβ. Each culture was diluted and plated onto E minimal medium supplemented with tryptophan (13) in the absence of ampicillin. After 2 days, the colonies were replica plated onto medium with and without ampicillin to determine if the pMS119HE or pβ plasmid was maintained in the *recA718 polA12*(Ts) strain. The JS239 and JS246 strains, which are *recA718* $polA^+$ and contain the pMS119HE and pβ plasmids, respectively, were tested in the same manner and served as controls.

The results of the plasmid maintenance experiments carried out at 42°C are shown in Table 1. Every colony of the $polA^+$ JS239 strain and of the $polA^+$ JS246 strain remained resistant to ampicillin in the absence of selective pressure, as expected. However, for the *polA12*(Ts) JS262 strain, which carried the pMS119HE plasmid, only 10 colonies in over 5,000 examined remained resistant to ampicillin. In contrast, all of the 4,300 colonies of the *polA12*(Ts) JS260 strain, which carried the pβ plasmid, were resistant to ampicillin after being grown in its absence. These data suggested that Pol β is necessary for high-efficiency maintenance of a plasmid carrying a pMB1 origin of replication in a *polA12 E. coli* strain at the nonpermissive temperature.

Ampicillin resistance is due to the presence of the pβ plasmid. In order to confirm that ampicillin resistance was indicative of the presence of a plasmid, PCR was used to amplify a

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TABLE 1. Plasmid maintenance[a]

Strain[b]	Mutant	Plasmid	Expt no.	No. of Amp^r colonies/total no. of colonies (%)
JS239	*polA*$^+$	pMS119HE	I	5,704/5,704 (100)
			II	971/971 (100)
			III	99/99 (100)
JS246	*polA*$^+$	pβ	I	2,949/2,949 (100)
			II	1,033/1,033 (100)
			III	104/104 (100)
JS262	*pol12*(Ts)	pMS119HE	I	0/2,849 (<0.03)
			II	9/1,780 (0.5)
			III	1/373 (0.3)
JS260	*pol12*(Ts)	pβ	I	2,450/2,450 (100)
			II	930/930 (100)
			III	926/926 (100)

[a] Bacterial strains were grown overnight in nutrient broth containing 35 μg of ampicillin per ml. An aliquot containing approximately 1,000 cells was inoculated into 5 ml of nutrient broth containing 1 mM IPTG to induce expression of Pol β from the *tac* promoter and was incubated overnight at 42°C. The cultures were then diluted into saline, plated onto E minimal medium supplemented with tryptophan and 1 mM IPTG (ETI), and incubated at 42°C for 2 days. Colonies were replica plated onto ETI containing 35 μg of ampicillin per ml and onto ETI, were incubated overnight at 42°C, and were counted.

[b] All bacterial strains are derivatives of SC18 (13) and have the following genotype: *recA718 srl*::Tn*10 lon sulA1 mal uvrA155 trpE65*. In addition, the JS239 strain contains the plasmid pMS119HE and the JS246 strain contains the pβ plasmid. The JS262 and JS260 strains contain the *polA12*(Ts) allele and carry the pMS119HE and pβ plasmids, respectively. The pMS119HE plasmid, a generous gift from E. Lanka, contains the pMB1 origin of replication. The pβ plasmid contains the pMS119HE backbone and the rat Pol β cDNA cloned into the polylinker region as described elsewhere (13).

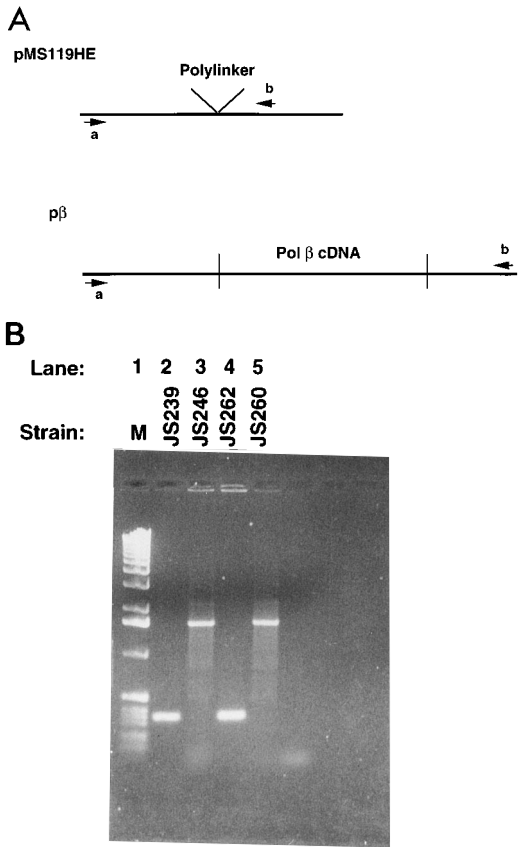

FIG. 1. Detection of plasmid DNA. Plasmid DNA was detected by PCR with the pMS3′ and pMS5′ primers. (A) Fragments of DNA from Amp^r colonies grown on replica plates. (B) Products of PCRs with Amp^r colonies. A band of approximately 312 bp appears in lanes 2 and 4 as expected, since both of these strains contain pMS119HE which lacks the Pol β insert. In lanes 3 and 5 a band of approximately 1,412 bp is visible; this is the size expected upon amplification of the pβ plasmid with the pMS3′ and pMS5′ primers. Lane 1, DNA markers.

fragment of plasmid DNA from Amp^r colonies grown on the replica plates (Fig. 1A). The pMS3′ (5′-ATCTTCTCTCATC CGCC-3′) and pMS5′ (5′-GGGCTTATCGACTGCACGG-3′) primers anneal to plasmid DNA located 3′ and 5′ of the polylinker region of both the pMS119HE and pβ plasmids. The pMS3′ and pMS5′ primers anneal to the pMS119HE plasmid at bases 67 to 83 and 3720 to 3738, respectively. When these primers are used to amplify plasmid DNA from strains carrying the pMS119HE plasmid, a 312-bp fragment will result. A 1,412-bp fragment will be observed upon amplification of the pβ plasmid, because the rat Pol β cDNA is inserted within the polylinker region. An example of the results of this assay is shown in Fig. 1B. Plasmid DNA was detected by PCR with the pMS3′ and pMS5′ primers in buffer containing 20 mM Tris-Cl (pH 8.0), 25 mM KCl, 1.5 mM $MgCl_2$, 0.05% Tween 20, and 2 U of *Taq* polymerase (Perkin-Elmer). Amp^r colonies were picked, directly added to the PCR buffer containing the primers, denatured at 96°C for 30 s, annealed at 48°C for 30 s, and extended for 3 min at 72°C. Thirty cycles of this program were performed on an MJ thermocycler. A 10-μl aliquot of each reaction mixture was resolved on a 1.2% agarose gel. Amplification of plasmid DNA from 20 Amp^r colonies of the JS260 strain, in which Pol β complements the Pol I-defective phenotype, invariably yielded a 1,412-bp fragment as expected (data not shown), suggesting that the Amp^r phenotype strongly correlates with the presence of the pβ plasmid in the *recA718 polA12* strain. Each of the 10 colonies that remained Amp^r in the absence of ampicillin during the growth of the JS262 strain (Table 1) were also tested in this manner. Fragments 312 bp in length were produced upon amplification of their plasmid DNA, demonstrating that the pMS119HE plasmid is maintained in the absence of selective pressure in approximately 0.5% of the colonies tested. This result is most likely due to the high frequency of reversion of the *recA718 polA12* strain (15a).

Pol β may participate directly in the initiation of plasmid replication. Our results demonstrate that the maintenance of a plasmid with a pMB1 origin of replication in a *polA12* mutant correlates with the presence of Pol β. The simplest interpretation of these data is that Pol β can substitute for Pol I in the initiation of plasmid DNA replication. Pol I normally initiates plasmid replication from the RNA-DNA hybrid at the origin of the ColEI type plasmid, as shown in Fig. 1A. Pol III then binds at or near the DNA at the primosome assembly site and completes synthesis of the plasmid. However, we envision that in the absence of functional Pol I, Pol β can substitute for Pol I by binding to the RNA primer of the RNA-DNA hybrid molecule at its 3′ OH terminus located at the plasmid's origin of replication, as shown in Fig. 2B. Pol β then initiates DNA synthesis at the origin and continues to synthesize DNA until it is replaced with the Pol III holoenzyme at or near a primosome assembly site approximately 400 nucleotides downstream from the origin. An alternative possibility is that Pol β replicates the entire plasmid; this is unlikely, because Pol β is a nonprocessive polymerase. To our knowledge, this is the first demonstration of a mammalian DNA polymerase functioning in the initiation of DNA replication from the origin of a prokaryotic plasmid.

Interestingly, with a system in which purified proteins were

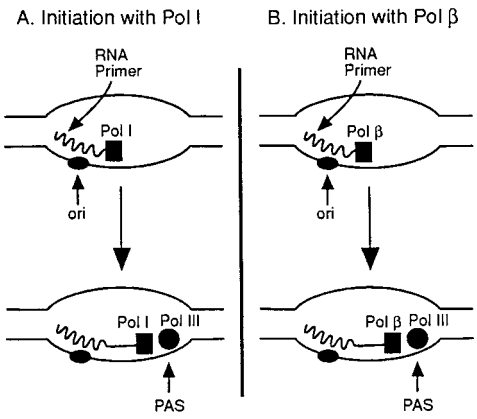

FIG. 2. Model of plasmid DNA replication catalyzed by Pol β. Initiation of plasmid replication with Pol I (A) and with Pol β (B). ori, plasmid origin of replication; PAS, primosome assembly site.

used, Itoh and Tomizawa have shown that the Klenow fragment of Pol I does not substitute for Pol I in the initiation of plasmid DNA replication (5). Since the Klenow fragment of Pol I does not exhibit a 5′-to-3′ exonuclease activity, Itoh and Tomizawa suggested that this activity was necessary to degrade RNA primers at the origin. Pol β does not possess a 5′-to-3′ exonuclease activity and is not able to degrade RNA primers in a nick translation assay. However, Pol β is also able to substitute for Pol I in filling gaps between Okazaki fragments during chromosomal DNA replication, a process which requires RNA primer degradation (15). Perhaps the PolA12 enzyme, which contains very little polymerase activity but small amounts of 5′-to-3′ exonuclease activity, is able to degrade the RNA primers during both the initiation of plasmid replication and the synthesis of the lagging strand during chromosomal replication. Alternatively, Pol β might substitute for Pol I by binding to the 3′ OH termini of the RNA primers and preventing their hydrolysis until either Pol II or III is able to participate in the initiation of plasmid DNA replication. The level of expression of Pol β is at least 1 order of magnitude greater than that of Pol I, supporting this possibility. However, a Pol β mutant protein that is able to bind to DNA but that is not able to substitute for Pol I in chromosomal DNA replication or repair in vivo (12a) is also unable to substitute for Pol I in plasmid replication. This suggests that the ability of Pol β to bind to a 3′ OH terminus is not sufficient for plasmid replication to occur.

The finding that Pol β can substitute for Pol I in the initiation of plasmid DNA synthesis suggests that one can insert a piece of target DNA, such as the *lacZ*α fragment, near the plasmid origin of replication, possibly placing synthesis of this target DNA under control of Pol β. Errors made by Pol β during the replication of the *lacZ*α fragment could be scored as white or light-blue plaques in a forward mutation assay, and their sequences could then be determined. This approach might afford the possibility of obtaining a spectrum of spontaneous mutations produced by Pol β in vivo.

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