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Extra Views

When Pol I Goes into High Gear

Processive DNA Synthesis by Pol I in the Cell

Manel Camps*
Lawrence A. Loeb

The Joseph Gottstein Memorial Cancer Research Laboratory; Department of Pathology; University of Washington; Seattle, Washington USA

*Correspondence to: Manel Camps; Email: mcamps@u.washington.edu

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ABSTRACT

Pol I is the most abundant polymerase in *E. coli* and plays an important role in short patch repair. In accordance with this role in the cell, the purified polymerase exhibits low processivity and high fidelity in vitro. Pol I is also the polymerase responsible for leader strand synthesis during ColE1 plasmid replication. In a previous publication, we described the generation of a highly error-prone DNA polymerase I. Expression of this mutant Pol I results in errors during the replication of a ColE1 plasmid. The distribution and spectrum of mutations in the ColE1 plasmid sequence downstream the ori indicates that Pol I is capable of more processive replication in vivo than previously accepted. Here, we review evidence suggesting that Pol I may be recruited into a replisome-like holoenzyme and speculate that processive DNA replication by Pol I may play a role in recombination-dependent DNA replication in the cell.

DNA polymerases catalyze the template-directed incorporation of deoxynucleotides into a growing primer terminus. In cells, polymerases are responsible for replicative DNA synthesis, and play a critical role in DNA repair by proofreading, by processing gaps or bypassing blocks in replication. The polymerase domain of Pol I represents the most abundant DNA polymerase activity in *E. coli* (>95%), and it was the first polymerase to be identified. Four additional polymerases have subsequently been found in bacteria: Pol II, Pol III, Pol IV, and Pol V. Pol III is responsible for replicative chromosomal DNA synthesis, and Pol II, IV and Pol V are involved in translesion DNA synthesis.

Strains with no or little Pol I activity have been isolated, which implies that Pol I is redundant with other enzymes. This finding was rather unexpected, given the abundance of Pol I in the cell. Pol I contains two distinct functional and structural units: a DNA polymerase domain and a 5' nuclease domain. These activities may be provided separately, although covalent linkage facilitates their coordinated action. The purified enzyme shows poor processivity (15-20 nt) and high fidelity.[1] In vitro, Pol I binds to nicked or gapped double-stranded DNA, and polymerase-catalyzed primer extension generates the substrate for the 5'→3' exonuclease, which results in a displacement of the nick along the duplex ("nick translation"). These properties of the enzyme in vitro appear to correlate with the role of Pol I in vivo as a central player in lagging-strand synthesis and in excision repair (reviewed in ref. 2).

Pol I is also involved in replication initiation in a number of plasmids, the best-characterized example of which is ColE1. ColE1 plasmid replication starts with the generation of a long RNA (~400 bp) by RNA polymerase. Once processed, this transcript is recognized by Pol I as a primer for DNA synthesis. The point where RNA sequence transitions into DNA is known as the origin of replication (ori). Melting of the double strand during leader strand synthesis reveals an n'-*pas* signal ~400 bp downstream of the ori. This signal initiates assembly of the Pol III holoenzyme, which completes the replication of the plasmid. Thus, two distinct stages can be distinguished in plasmid replication; an early, Pol I-dependent one, and a late, Pol III-dependent one (reviewed in ref. 3).

In a recent publication, we described a 2-plasmid system for random mutagenesis in *E. coli* (Fig. 1).[4] A highly error-prone Pol I containing point mutations in three critical determinants of fidelity is expressed from a Pol I-independent plasmid, pSC101. The host is JS200, a strain encoding a temperature-sensitive allele of Pol I. Reversion of an ochre stop codon in β-lactamase is used to monitor levels of mutagenesis. This reporter is encoded in a ColE1 plasmid and placed immediately downstream from the ori so it is replicated by Pol I. Expression of error-prone Pol I in JS200 cells at the restrictive temperature would be predicted to result in replication errors in the target plasmid during the synthesis of the 400-500 bp long leader strand. In agreement with this expectation, cells expressing

error-prone polymerase and an carrying a β-lactamase ochre codon located 230 bp downstream from the ori showed an 80,000-fold increase in the frequency of mutagenesis relative to cells expressing wild type Pol I.[4]

In our target plasmid, the n'-*pas* signal was deleted in order to bring the reporter in close proximity to the ori. In the absence of an n'-*pas* signal, the frequency of mutations did not decrease after the initial 400–500 bp from the ori, and remained constant for ~700 bp. Further, the spectrum of observed base pair substitutions did not significantly change comparing mutations in the first 500 bp relative to mutations in the 250 bp further downstream.[4] This provides strong evidence that these 700 bp are replicated by Pol I in the cell. Given the low processivity of Pol I in vitro, these observations argue Pol I likely recruits factors which enhance its processivity in the cell.

Further downstream from the ori, the frequency of mutagenesis does decrease (Fig. 2). The drop in mutation frequency at a distance of 700bp is sharp, rather than gradual, presumably reflecting a switch from Pol I to Pol III replication at around 700 bp from ori. The magnitude of the decrease in the frequency of mutations is in the order of 3-15 fold (depending on the experiment), but remains 3 orders of magnitude above background for at least 3.7 kb (Fig. 2).[4] The source of these distal mutations is unclear, especially given that the culture conditions used in these experiments (growing cell cultures to saturation) are known to be pro-mutagenic. Expression of Pol I variants carrying two point mutations in determinants of fidelity result in frequencies of mutation that are intermediate between those of our triple mutant and those of the wild-type (see "mutation frequency" column in Table 1). This argues against a threshold effect of saturation of repair or activation of mutagenic pathways. Strikingly, in cells expressing these moderately error-prone variants of Pol I, the decrease in the frequency of mutation beyond 700bp remains similar to that of the triple mutant, 3–19-fold (see "change in distal sequence" column in Table 1). No decrease was detected in the frequency of distal mutagenesis in cells expressing wild type, indicating that Pol I contributes little to ColE1 plasmid mutagenesis under normal circumstances, which agrees with the high fidelity exhibited by this enzyme in vitro. In sum, the ratio of distal versus proximal mutagenesis by Pol does not correlate with the efficiency of mutagenesis. This further supports the notion that, similar to proximal mutations, most distal mutations likely result from error-prone Pol I replication. If confirmed, replication of distal plasmid sequences by Pol I would imply that Pol I is capable of highly processive replication in vivo.

A parsimonious model that explains the distribution of mutations in the target plasmid associated with expression of error-prone Pol I is presented in Figure 3. Following synthesis of the leader sequence, Pol I and Pol III may compete for replisome components. As a result, we propose that a Pol I replisome-like structure may complete plasmid replication in ~5–30% of the plasmids, whereas Pol III would replicate the remaining 70–95%. The relative contribution of Pol I in the late stage of plasmid replication may be sensitive to culture conditions and other variables. A role of Pol I in completing plasmid replication may have been previously missed because of the poor resolution of the original genetic and biochemical experiments that defined the two stages in plasmid replication. Significant continued ColE1 plasmid replication in the absence of Pol III in vivo has indeed been reported, although it was originally attributed to leakiness in the polC^{ts} mutation.[5] Pol I has been shown to be present in replicating holoenzyme complexes.[6] Further, Pol I is essential for replication in certain polC^{ts} strains, suggesting Pol I

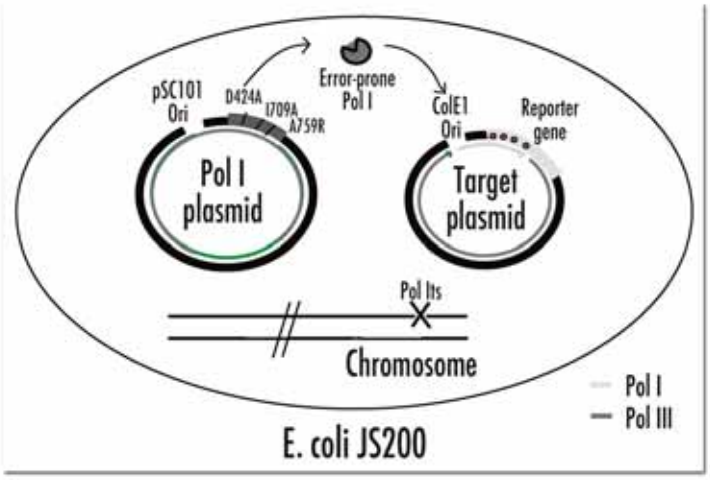

Figure 1. 2-Plasmid system of random mutagenesis in vivo. JS200 cells, encoding a temperature-sensitive mutant of Pol I, are transformed with two plasmids: a pSC101 (Pol I-independent) plasmid encoding an error-prone Pol I, and a ColE1 plasmid encoding the sequence of interest immediately downstream from the plasmid origin of replication. Pol I synthesizes a 400-500bp leader in the target plasmid. Errors during the replication of this plasmid by error-prone Pol I are expected to result in mutations in the 400-500 bp of sequence immediately downstream of the ori. Modified from Figure 1 in. Camps M, et al. Targeted gene evolution in Escherichia coli using a highly error-prone DNA polymerase I. Proc Natl Acad Sci USA 2003; 100:9727-32.

may be redundant with Pol III for replicative DNA synthesis in some exceptional conditions.[7] It is hard to imagine how Pol I may replicate large portions of the chromosome without invoking a replisome. The fact that only the polymerase domain is required for complementation in these experiments wouls also be consistent with a role of Pol I as a synthetic subunit in a replisome-like holoenzyme.[8]

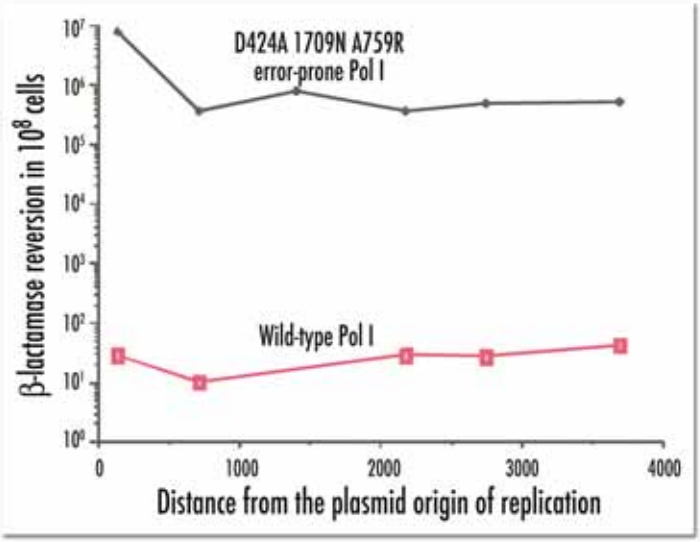

Figure 2. Mutagenesis as a function of distance from ori. Plasmids encoding a reporter β-lactamase gene placed at increasing distance from ori were transformed into cells expressing either wild type or error-prone Pol I. The graph presents the number of β-lactamase reversion events per 10^8 cells, as an average of two independent cultures plated in duplicate. Modified from Figure 4B in Camps M, et al. Targeted gene evolution in Escherichia coli using a highly error-prone DNA polymerase I. Proc Natl Acad Sci USA 2003; 100:9727-32.

Table 1 **Frequency of ColE1 Mutagenesis Replication by Different Error-Prone Pol1 Mutants, Comparing Reporters Proximal (230 bp) and Distal (2800 bp) to Ori**

Pol I	Distance to Ori (bp) of Reporter Stop	Mutation Frequency[a]	Standard Error[b]	Change in Distal Sequence[c]
Wild type	230	1.3 x 10	1.4 x 100	
Wild type	2800	4.4 x 10	ND	+2.2
I709F D424A	230	2.1×10^4	1.5×10^4	
I709F D424A	2800	4.2×10^3	5.4×10^2	-4.1
I709N D424A	230	3.4×10^4	8.9×10^3	
I709N D424A	2800	1.7×10^3	8.0×10^2	-19.0
A759R D424A	230	7.8×10^3	2.1×10^3	
A759R D424A	2800	1.7×10^3	ND	-3.5
I709N A759R D424A	230	2.1×10^5	4.2×10^4	
I709N A759R D424A	2800	4.5×10^4	ND	-3.6

[a]β-lactamase reversals in 10^8 cells, average of two independent cultures, each plated in triplicate.; [b]$p<0.05$; [c]Expressed as fold relative to the frequency of mutation in proximal sequence.

In sum, we propose that Pol I may perform processive DNA replication in vivo and that it may be recruited into a replisome-like holoenzyme in some cases. This calls for a reassessment of the role of Pol I, which constitutes the vast majority of the polymerase activity in the cell. Recombination-dependent DNA replication has been recently recognized as a mechanism that is central for double-strand break repair and for the restoration of inactive replication forks.[9,10] The two covalently linked domains of Pol I have potential for involvement in recombination-dependent DNA synthesis. The 5' nuclease domain is known to promote ATP-independent strand transfer and joint molecule formation, and the polymerase may use the 3' terminus of an invading strand to initiate strand synthesis as a first step for the assembly of a replication fork.[11,12] We propose Pol I may play a role in recombination-dependent replication in the cell. This role would be similar to initiation of ColE1 plasmid replication and would likely involve processive DNA replication. A role in recombination-dependent DNA replication would complement the role of Pol I in nick and gap processing, as these lesions typically lead to double strand breaks (reviewed in ref. 13), and would make Pol I a central player in DNA repair.

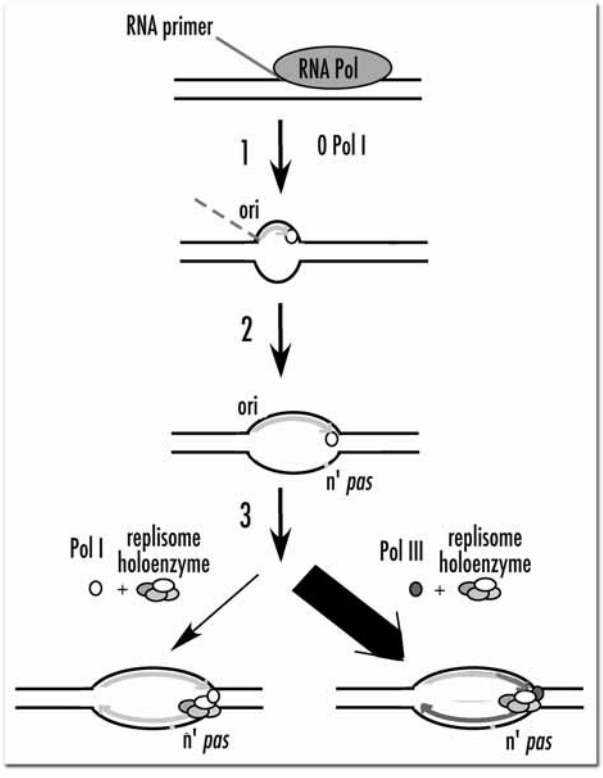

Figure 3. Model of initiation of ColE1 plasmid replication by Pol I. 1. Pol I synthesizes the leader strand using a processed RNA transcript as a primer. The 5′→3′ exonuclease activity of the enzyme degrades the primer at the 5′ end while the polymerase extends the 3′ end. 2. As leader strand synthesis progresses, an n′-*pas* signal becomes exposed as ssDNA. 3. This n′-*pas* signal initiates recruitment of a replisome holoenzyme that completes plasmid replication. We propose the existence of two different replisomes competing at the site of assembly depending on the active polymerase: a Pol I replisome and a Pol III replisome.

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