Extra Views

When Pol I Goes into High Gear
Processive DNA Synthesis by Pol I in the Cell

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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.
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 mutation 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 polC25 mutation.5 Pol I has been shown to be present in replicating holoenzyme complexes.6 Further, Pol I is essential for replication in certain polC25 strains, suggesting Pol I 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 would also be consistent with a role of Pol I as a synthetic subunit in a replisome-like holoenzyme.8

![Figure 1](image1.png)

**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.

![Figure 2](image2.png)

**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 10⁸ 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.
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. The two covalently linked domains of Pol I have potential for involvement in recombination-dependent DNA synthesis. The 5'→3' 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. 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.

References

Table 1  
FREQUENCY OF COLE1 MUTAGENESIS REPLICATION BY DIFFERENT ERROR-PRONE POL1 MUTANTS, COMPARING REPORTERS PROXIMAL (230 bp) AND DISTAL (2800 bp) TO ORI

<table>
<thead>
<tr>
<th>Pol I</th>
<th>Distance to Ori (bp) of Reporter Stop</th>
<th>Mutation Frequency</th>
<th>Standard Error</th>
<th>Change in Distal Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>230</td>
<td>1.3 x 10</td>
<td>1.4 x 100</td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>2800</td>
<td>4.4 x 10</td>
<td>ND</td>
<td>+2.2</td>
</tr>
<tr>
<td>I709 F D424 A</td>
<td>230</td>
<td>2.1 x 10^4</td>
<td>1.5 x 10^3</td>
<td>-4.1</td>
</tr>
<tr>
<td>I709 F D424 A</td>
<td>2800</td>
<td>4.2 x 10^3</td>
<td>5.4 x 10^2</td>
<td>-19.0</td>
</tr>
<tr>
<td>I709 N D424 A</td>
<td>230</td>
<td>3.4 x 10^3</td>
<td>8.9 x 10^3</td>
<td>-19.0</td>
</tr>
<tr>
<td>I709 N D424 A</td>
<td>2800</td>
<td>1.7 x 10^3</td>
<td>8.0 x 10^2</td>
<td>-19.0</td>
</tr>
<tr>
<td>A759 R D424 A</td>
<td>230</td>
<td>7.8 x 10^3</td>
<td>2.1 x 10^3</td>
<td>-19.0</td>
</tr>
<tr>
<td>A759 R D424 A</td>
<td>2800</td>
<td>1.7 x 10^3</td>
<td>ND</td>
<td>-3.5</td>
</tr>
<tr>
<td>I709 N A759 R D424 A</td>
<td>230</td>
<td>2.1 x 10^5</td>
<td>4.2 x 10^4</td>
<td>-3.6</td>
</tr>
<tr>
<td>I709 N A759 R D424 A</td>
<td>2800</td>
<td>4.5 x 10^4</td>
<td>ND</td>
<td>-3.6</td>
</tr>
</tbody>
</table>

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

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.