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Interplay Among Replicative and Specialized DNA Polymerases Determines Failure or Success of Translesion Synthesis Pathways

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Living cells possess a panel of specialized DNA polymerases that deal with the large diversity of DNA lesions that occur in their genomes. How specialized DNA polymerases gain access to the replication intermediate in the vicinity of the lesion is unknown. Using a model system in which a single replication blocking lesion can be bypassed concurrently by two pathways that leave distinct molecular signatures, we analyzed the complex interplay among replicative and specialized DNA polymerases. The system involves a single *N*-2-acetylaminofluorene guanine adduct within the NarI frameshift hot spot that can be bypassed concurrently by Pol II or Pol V, yielding a −2 frameshift or an error-free bypass product, respectively. Reconstitution of the two pathways using purified DNA polymerases Pol III, Pol II and Pol V and a set of essential accessory factors was achieved under conditions that recapitulate the known *in vivo* requirements. With this approach, we have identified the key replication intermediates that are used preferentially by Pol II and Pol V, respectively. Using single-hit conditions, we show that the β-clamp is critical by increasing the processivity of Pol II during elongation of the slipped −2 frameshift intermediate by one nucleotide which, surprisingly, is enough to support subsequent elongation by Pol III rather than degradation. Finally, the proofreading activity of the replicative polymerase prevents the formation of a Pol II-mediated −1 frameshift product. In conclusion, failure or success of TLS pathways appears to be the net result of a complex interplay among DNA polymerases and accessory factors.

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Introduction

Cells deal with the huge diversity of DNA lesions potentially present in the template strands of their genome during replication by using a panel of so-called specialized DNA polymerases.[1,2] A key issue in this field is to unravel the mechanisms by which these specialized DNA polymerases are recruited during replication when a blocking lesion prevents synthesis by the replicative DNA polymerase.[3,4] Previous reports have revealed the complexity of bypass pathways *in vivo*, where the chemical nature of the lesion and/or the local sequence context determine the outcome.[5–11]

Recent data have demonstrated that when a lesion blocks DNA synthesis in one strand, the replicative helicase keeps opening the parental DNA strands and replication continues on the other, locally undamaged, strand.[12–14] As a consequence, single-stranded DNA accumulates downstream of the blocking lesion, until a new primer is synthesized. While re-priming of the lagging strand is a classical step in normal DNA synthesis, a mechanism for leading strand re-priming has been proposed only recently.[15,16] Indeed, DnaG that is recruited by the DnaB replicative helicase is capable of priming both

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Abbreviations used: AAF, *N*-2-acetylaminofluorene; SSB, single-stranded binding protein; ssDNA, single-stranded DNA.

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lagging and leading strands. Gaps up to several kilobases in length can thus form in both strands when damaged DNA is replicated,[17] as the replication machinery merely skips over the lesions,[18] leaving gaps to be filled subsequently by dedicated gap-filling reactions. In fact, this turns out to be the model proposed nearly four decades ago by Rupp and Howard-Flanders for damaged DNA replication in *Escherichia coli*.[19,20] However, the overall distance over which the fork skips lesions before coming to an arrest remains to be established.[21] The gap-filling reaction is accomplished either *via* a recombinational pathway (*recF* pathway) or by translesion synthesis. The β clamp that remains loaded onto the DNA when the replicative polymerase dissociates at the lesion sites acts as a platform for the recruitment of the polymerases that will fill-in the gap.[22] All three SOS-inducible DNA polymerases in *E. coli* absolutely require binding to the β-clamp to accomplish lesion bypass *in vivo*.[6,23] Specialized DNA polymerases synthesize a short patch of DNA (TLS patch) in order to shield the lesion and prevent degradation of the TLS patch upon subsequent binding of a proofreading-proficient polymerase (i.e. Pol III, Pol II, or Pol I that are likely to complete the gap-filling reaction owing their higher processivity).[24]

In this work, we investigate the interplay among the replicative DNA polymerase, Pol III holoenzyme and two specialized DNA polymerases Pol V and Pol II during the bypass of a single replication-blocking lesion G-AAF located within a −2 frameshift mutation hot spot, the NarI site.[25] The NarI site belongs to a family of related sequences that are all strong −2 frameshift mutation hot spots induced by the chemical carcinogen *N*-2-acetylaminofluorene (AAF) covalently bound to the C8 position of guanine residues,[26–28] and many related carcinogens, including human food carcinogens.[29] Within this sequence context, a single dG^{AAF} adduct can be bypassed in parallel by two distinct pathways, a pathway leading to −2 frameshift mutations mediated by Pol II (TLS-2) and another, error-free, pathway depending on Pol V (TLS0) (Figure 1).[5] Given that the two pathways exhibit distinct molecular signatures, this model system offers a unique occasion to study the interplay among DNA polymerases during lesion bypass. Here, we show that Pol II and Pol V preferentially use distinct replication intermediates as their favorite substrate. As a consequence, the two pathways proceed essentially independently. Furthermore, this work further highlights the complexity of TLS pathways that can be modulated by Pol III proofreading activity, interactions with the β-clamp or *via* RecA filament formation.

The NarI mutation hot spot: an *in vivo* overview

We initially discovered this mutation hotspot by serendipity when establishing a forward mutation spectrum induced by the chemical liver carcinogen AAF.[26] When covalently bound to G* in the NarI site, 5′-GGCG*CC-, AAF induces the loss of the G*pC dinucleotide at a frequency that is $\approx 10^7$-fold higher than the spontaneous frequency. *In vivo* studies showed that the NarI mutation hot spot is not restricted to the NarI sequence itself, or to the carcinogen AAF. Instead, the hot spot requires a sequence containing at least two GpC repeats and any of a family of aromatic amides and nitro aromatic compounds that form a large class of human carcinogens.[29–32] In a wild-type strain, under SOS-induced conditions, bypass of a single AAF adduct located within the NarI sequence ($GGCG^{AAF}CC$) (Figure 1), is performed by Pol V or Pol II yielding comparable levels of error-free (TLS0: GGCGCC) or −2 frameshift (TLS-2: GGCC) bypass products, respectively.[5,33] The frameshift pathway results from the extension by Pol II of a slipped replication intermediate, L0(−2), produced by isomerization of the replication intermediate L0 formed following insertion of dC opposite the dG^{AAF} adduct (Figure 1). This slippage intermediate is thermodynamically favored by an AAF-induced conformational change of the modified guanine residue from *anti* to *syn*, and by the local sequence context that allows the formation of two perfectly matched GC base-pairs at the 3′-end of the primer.[34,35] How are the two concurrent TLS pathways handled? The aim of this work was to dissect the interplay among the different DNA polymerases, Pol III, Pol II and Pol V during lesion bypass.

Results

Pol II and Pol V pathways are largely independent

Earlier, in order to investigate the process of TLS *in vitro*, we developed an assay based on a 2.7 kb

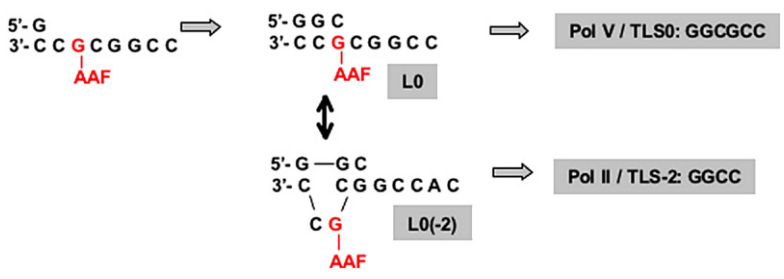

Figure 1. NarI lesion bypass pathways. The dG^{AAF}-containing NarI sequence 5′-$GGCG^{AAF}CC$ can be bypassed *via* two distinct pathways;[5] a Pol V-mediated error-free pathway (TLS0) and a −2 frameshift pathway (TLS-2) that requires Pol II. The frameshift pathway involves elongation by Pol II of a slipped intermediate, L0(−2) that results from isomerization of the L0 intermediate as shown.

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