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Review

Coordinating DNA polymerase traffic during high and low fidelity synthesis

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ABSTRACT

With the discovery that organisms possess multiple DNA polymerases (Pols) displaying different fidelities, processivities, and activities came the realization that mechanisms must exist to manage the actions of these diverse enzymes to prevent gratuitous mutations. Although many of the Pols encoded by most organisms are largely accurate, and participate in DNA replication and DNA repair, a sizeable fraction display a reduced fidelity, and act to catalyze potentially error-prone translesion DNA synthesis (TLS) past lesions that persist in the DNA. Striking the proper balance between use of these different enzymes during DNA replication, DNA repair, and TLS is essential for ensuring accurate duplication of the cell's genome. This review highlights mechanisms that organisms utilize to manage the actions of their different Pols. A particular emphasis is placed on discussion of current models for how different Pols switch places with each other at the replication fork during high fidelity replication and potentially error-pone TLS.

1. Introduction

Our understanding of the mechanisms by which organisms copy and maintain the fidelity of their genomes has undergone striking changes in the last decade. In contrast to outdated models that envisioned high fidelity leading strand DNA polymerases (Pols) stably associated with the replication fork, and able to persist in a highly processive mode throughout replication, we now realize that the structure and composition of the replisome (the multiprotein complex that catalyzes DNA replication) can be extremely dynamic due in large part to Pol switching. The term Pol switching refers to the process by which one Pol replaces a second Pol at the 3′-OH end of a primed DNA template (Fig. 1). Pol switching is influenced by several factors, including the relative expression levels of the different Pols [1], changes in the sub-cellular localization of the different Pols [2], interactions of Pols with their cognate sliding clamp proteins [2,3], as well as with other Pols [2,3], posttranslational modification of Pols and clamp proteins [4], and the relative affinities of the different Pols for the DNA substrate in need of replication [5,6]. Additional as yet unappreciated factors likely also contribute. In this review, I discuss the factors involved in Pol switching, and have attempted to provide a framework for understanding the biological contexts in which Pol switching can influence genome integrity. A particular emphasis is placed on discussing current models for Pol switching in both prokaryotes and eukaryotes, with a special focus on *E. coli*. Finally, where appropriate, I refer the reader to other articles in this thematic issue that cover certain topics related to Pol switching in more detail than I do in this review.

2. Organisms possess multiple Pols with distinct fidelities, processivities, and catalytic abilities

Pols are organized into six distinct families based on phylogenetic relationships. Although some organisms, such as *Helicobacter pylori*, possess only two Pols, most contain far more. For example, the gram-negative bacterium *Escherichia coli* is currently known to possess five different Pols belonging to four distinct families, the yeast *Saccharomyces cerevisiae* has at least eight, and *Homo sapiens* possess at least fourteen (Table 1). Although many of these Pols are accurate, due in part to an intrinsic or associated 3′-to-5′ exonuclease proofreading activity (see article by Reha-Krantz), as many as half of the Pols in any given organism possess a more open active site, endowing them with the ability to replicate imperfect DNA templates, and are therefore referred to as 'specialized' Pols [7]. Although these specialized Pols participate in a growing number of biological transactions ([8–11]; see Table 1), they are best known for their ability to replicate over lesions that persist in the DNA and act as potent blocks to the normal DNA replication machinery *via* a process termed translesion DNA synthesis (TLS). As a result of this ability, these Pols typically display a reduced fidelity when replicating undamaged DNA. Likewise, many of these specialized Pols also display modest processivity. For example, the eukaryotic Y-family Pol ι, which lacks an associated proofreading activity, is essentially distributive and catalyzes ~seven misinsertions for every ten bases synthesized *in vitro*, on average [12,13]. In contrast, replicative Pols, such as the *E. coli* C-family Pol III, and the eukaryotic B-family Pol ε and Pol δ, are highly processive, and catalyze less than

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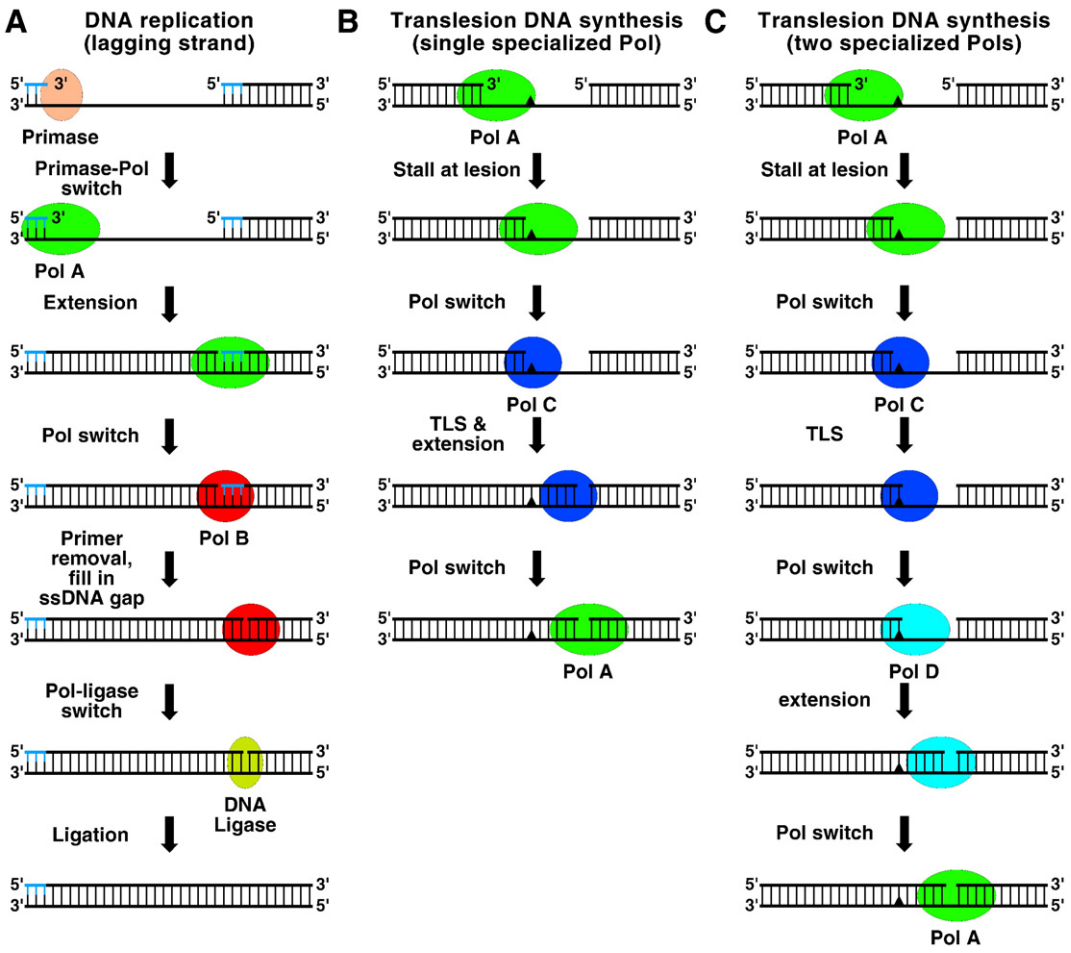

Fig. 1. Models describing Pol switching during high fidelity DNA replication and potentially error-prone TLS. (A) Model for Pol switching on lagging strand during maturation of Okazaki fragments. Primase (DnaG in *E. coli*, Pol α in eukaryotes) synthesizes a short RNA primer (blue), and then undergoes a switch with Pol A (Pol III in *E. coli*, Pol δ in eukaryotes) for elongation. When Pol A encounters the 5′-end of the previously synthesized Okazaki fragment, it undergoes a switch with Pol B (Pol I in *E. coli*, FEN1 and Pol δ in eukaryotes) which excises the RNA primer, and fills in the resulting ssDNA gap before switching with DNA ligase. (B and C) Models for Pol switching during TLS. The replicative Pol (Pol A), stalled at a lesion, undergoes a switch with a specialized Pol (Pol C) capable of bypassing the lesion. If Pol C is capable, it will extend the 3′-end of the nascent strand before handing it back to Pol A (B). If Pol C is unable to extend the 3′-end, it must undergo a switch with another specialized Pol (Pol D) for extension before the 3′-end is returned to Pol A (C).

one error for every 10,000 bases incorporated [14–16]. Structural features of replicative and specialized Pols responsible for their distinct behavior are discussed in articles by Beese and Pata in this thematic issue.

With their growing number came the obvious question — how do organisms manage the actions of their different Pols to ensure that the right Pol gets to the right place at the right time? This question takes on added complexity when one considers the fact that bypass of certain classes of DNA lesions requires the actions of multiple specialized Pols. In these cases, organisms must sequentially manage the actions of multiple Pols in order to mediate lesion bypass. Moreover, certain specialized Pols are capable of bypassing specific classes of DNA lesions in a relatively accurate manner, while others bypass the same lesion in a largely error-prone manner [5,17–19]. Thus, recruitment of the inappropriate specialized Pol during TLS could result in mutations or genome rearrangements. Taken together, these findings highlight the importance of Pol switching to genome integrity.

3. Biological transactions involving Pol switching

Several biological processes require the actions of multiple Pols, and thus involve Pol switching. For example, multiple Pols are involved in DNA replication, and the actions of these different Pols must be tightly coordinated to ensure the fidelity of this process (Fig. 1A). Likewise, organisms require a capacity to tolerate lesions that evade repair, or that for whatever reason cannot be repaired. In the absence of such a capacity, these lesions would serve as potent blocks to ongoing replication, resulting in cell death. Since specialized Pols are typically required for replicative bypass of these lesions, Pol switching is also crucial for coordinating high fidelity DNA replication with potentially error-prone TLS (Fig. 1B and C). Finally, certain Pols participate in specific DNA repair functions. In these cases, mechanisms must exist for both recruitment of the appropriate Pol, as well as to coordinate the actions of these Pols with the other proteins involved in the repair function. Although not formally a 'Pol switch,' the mechanisms underlying these Pol-partner switches are conceptually similar to Pol switches, and thus, the fundamental rules regarding Pol switching discussed in this review also apply to these types of switches.

4. Consequences of impaired Pol switching

Despite our relatively naive understanding of the molecular mechanisms underlying Pol switching, it is evident that recruitment of the inappropriate Pol to a replication fork can result in mutations that contribute to human disease, including cancer. One prominent example of this is the variant form of the human genetic disease Xeroderma pigmentosum (XP-V). Individuals afflicted with XP-V are predisposed to sunlight-induced skin cancers [20,21]. In 1999,