DNA REPLICATION

Focus and persistence: how Pol IV unblocks stalled DNA synthesis

The mechanisms by which translesion DNA polymerases mediate DNA repair are incompletely understood. A new study shows that *Escherichia coli* DNA polymerase IV is concentrated at the sites of arrested DNA synthesis by an interaction with SSB, the major single-stranded DNA-binding protein, specifically at stalled but not ongoing replication forks.

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All cells must deal with damage to DNA bases, which can be problematic during replication as the damage can stall the replicative polymerases. During replication, the normal approach of excising the damage is dangerous as the duplex has been unwound, removing the template required to restore the pre-existing sequence. Cells therefore resort to mechanisms that allow the damaged bases to be copied, returning the damage to double-stranded DNA so that excision repair can operate safely. This can be achieved either by borrowing an undamaged DNA template through a recombination-like mechanism known as template switching, or by replacing the stalled replicative DNA polymerase for one that is able to tolerate damaged or distorted bases, a process known as translesion synthesis (TLS). Although the specialized TLS polymerases can unblock DNA synthesis by taking an educated guess as to which base to incorporate opposite a lesion, this comes with a potential penalty: mutagenesis.

A still unresolved puzzle in the field is how these specialized polymerases are controlled so that they do not interfere with normal replication but are rapidly available for action when DNA synthesis is impeded by damage. In other words, how are these enzymes recruited to a primer terminus that is stuck and not to one that is being extended? Key to understanding how access to the replication fork is controlled are DNA sliding clamps, such as proliferating cell nuclear antigen (PCNA) in eukaryotes and the β clamp in prokaryotes. The clamps enable a huge range of enzymes involved in replication and repair, including the replicative and TLS polymerases, to be tethered but mobile while they work on the DNA. In *E. coli*, one of the most intensively studied systems of DNA replication, the major replicative polymerase is Pol III, while three additional polymerases — Pol II, Pol IV and Pol V — can perform TLS.

Because the β clamp is a homodimer with each protomer containing a hydrophobic cleft to which clamp-interacting proteins can bind, an attractive early suggestion was that the β clamp acts as a molecular 'toolbelt' that holds both type of polymerase at the ready[1]. However, subsequent structural analysis has shown that steric hindrance is likely to prevent a TLS polymerase binding β at the same time as an active Pol III, as two of the subunits of Pol III — the α catalytic subunit and ε exonuclease subunit — occupy both clefts simultaneously[2,3]. This indicates that binding of the replicative and TLS polymerases to the β clamp at a primer terminus alternate, a suggestions that has been backed up by single-molecule studies[4]. What promotes this exchange?

In *E. coli*, expression of Pol IV (DinB) is upregulated rapidly after DNA damage as part of the SOS response[5]. This provides a conceptually simple model of regulation in which a higher number of molecules of Pol IV simply increases the chance of successful displacement of Pol III from the β clamp. However, even before the tenfold SOS induction of Pol IV, there is already ten times more Pol IV, approximately 250 molecules per cell[6], than Pol III, of which there are only around 20 molecules per cell[7], giving Pol IV a numerical advantage even in uninduced cells. Thus, it might be expected that Pol IV would interfere with normal DNA synthesis. This is not observed as the error-prone Pol IV does not contribute notably to spontaneous mutagenesis[8–10], an observation explained by the fact that the ability of Pol III and Pol IV to access the clamp is not equal. For Pol IV to attach to the active clamp, it needs the weaker of the two interactions of Pol III with the β clamp — that is, the interaction with the ε subunit — to let go. In other words, to gain access to the primer terminus, Pol IV has to overcome the barrier imposed by the ε–β interaction. Interestingly, and as predicted by this model, modulating the strength of the ε–β interaction dictates the efficiency with which TLS is used. Mutations that reduce the affinity of ε for β favor TLS over the use of repriming to restart DNA synthesis downstream of the lesion[11].

So how can TLS be promoted in circumstances in which it is needed? In this issue of *Nature Structural and Molecular Biology*, Chang et al.[12] provide some intriguing answers and show how the bacterial TLS polymerase Pol IV is regulated at stalled replication forks. Pol IV also interacts with the major DNA single-stranded binding protein SSB, albeit weakly[13]. SSB rapidly binds to and protects any free single-stranded DNA (ssDNA) in the cell. ssDNA is formed during normal replication of the lagging strand, but is also generated as a consequence of the replisome encountering DNA damage when DNA continues to be unwound even when DNA synthesis has been arrested. The interaction between Pol IV and SSB is not strong, but with a footprint of only 30–70 nucleotides[14] even relatively short stretches of ssDNA can bind several molecules of Pol IV. The net result of this is to focus and concentrate Pol IV in the vicinity of the problem that has caused the arrest of DNA synthesis in the first place. This locally concentrated Pol IV is then able to effectively compete with the ε subunit for binding to the β clamp. The idea of a two-step approach to deliver TLS polymerases to the primer terminus has been around for some time[15]. However, evidence that the first step, increasing local concentration, is important has been less easy to establish. For example, deletion of motifs in the C terminus of human DNA polymerase η uncouple the formation of subnuclear foci, which are thought to reflect the concentration of the polymerase at sites of DNA damage, from its functional

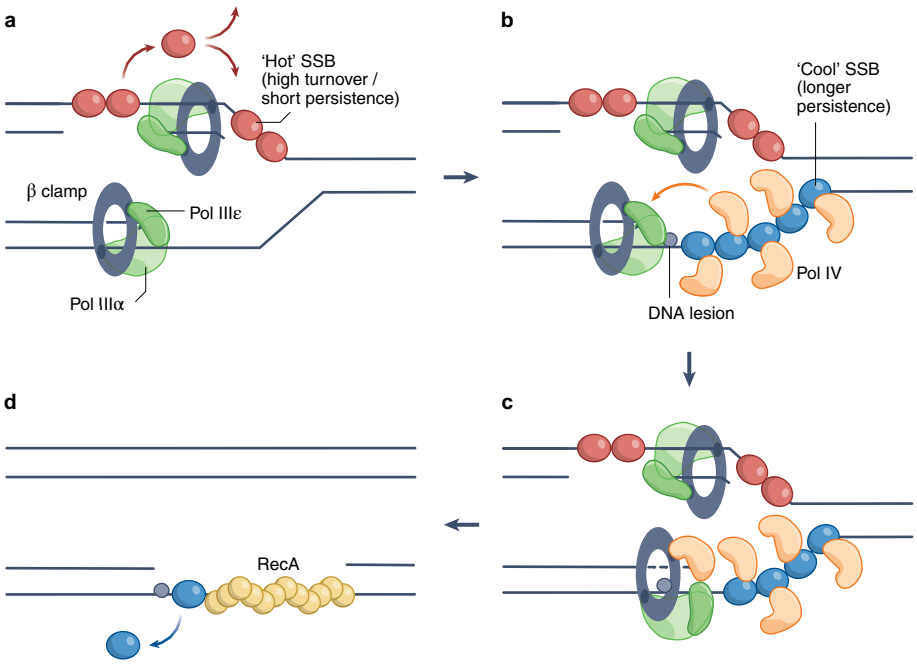

Fig. 1 | The two-step recruitment of Pol IV mediated by binding of SSB to ssDNA at sites of stalled DNA synthesis. a, During unperturbed replication, SSB binds to the ssDNA generated during discontinuous lagging strand synthesis. This 'hot' SSB is turned over rapidly on DNA as Okazaki fragments are matured. The replicative polymerase Pol III binds to both hydrophobic clefts on the β clamp via its catalytic α and proofreading ε subunits. **b**, When the replicative polymerase Pol III encounters a DNA lesion, it stalls. The replicative helicase (not shown for clarity) continues to unwind DNA, albeit at a slower rate, leading to the formation of ssDNA ahead of the stalled polymerase. This ssDNA is also bound by SSB. However, unlike the case of the Okazaki fragments, this 'cool' SSB is not rapidly displaced by DNA synthesis, allowing the low affinity interaction with Pol IV to concentrate the polymerase in the vicinity of the stalled primer terminus. This enables one Pol IV molecule from this high concentration 'cloud' to overcome the kinetic barrier posed by the β–ε interaction. Polymerase stalling on the lagging strand would also prevent the turnover of SSB, converting a normally 'hot' pool of SSB molecules to a 'cool' form that is able to recruit Pol IV. **c**, The binding of Pol IV to the hydrophobic cleft in the β clamp that has been vacated by the ε subunit puts it in a position to bypass the lesion, alleviating stalled DNA synthesis. **d**, If 'on-the-fly' bypass of the lesion proves difficult and leads to a delay in the resumption of DNA synthesis, repriming downstream confines the lesion to a post-replicative single-stranded gap within which SSB is replaced by RecA in preparation for recombinational bypass of the lesion.

ability to complement the loss of the enzyme in a mutant human cell[16]. What is striking in the present study is that a Pol IV mutant that exhibits decreased binding to SSB is defective in TLS. This strongly supports the idea that the concentration of Pol IV by SSB is not simply a refinement that may improve efficiency but instead is an important step in the regulation of pathway choice in lesion bypass.

It remains unclear why SSB bound to the lagging-strand template during normal replication does not have the same effect as SSB at a stalled replisome. A possible explanation is the persistence time of the ssDNA. *E. coli* Okazaki fragments are 1,000–2,000 nucleotides long, which would allow binding of up to around 70 SSB molecules. However, DNA synthesis is very rapid at approximately 700–1,000 nucleotides per second, meaning that these SSB molecules will persist for no more than 1–2 s on the DNA, leaving little opportunity for concentrating Pol IV (Fig. 1a). Indeed, this short half-life 'hot' SSB seems to be rapidly recycled from one Okazaki fragment to the next within the replisome[17]. However, at a stalled fork or post-replicative gap, the persistence of SSB will be longer. This 'cool', slower turnover of SSB allows a cloud of Pol IV to accumulate (Fig. 1b), maximizing the opportunity for the enzyme to jump in to take over one of the β clefts when the ε subunit of Pol III dissociates (Fig. 1c).

Notably, Chang et al.[12] hypothesize that this essentially kinetic model for regulating TLS recruitment may also explain the relative use of TLS versus homologous recombination at lesions of different 'difficulty' in terms of their replication. Thus, a small base lesion that is relatively easy to bypass would be quickly overcome by 'on-the-fly' TLS with limited helicase–polymerase uncoupling and ssDNA generation. A bulkier adduct that creates a more robust impediment to DNA synthesis would lead to greater accumulation of ssDNA, SSB and Pol IV, and may ultimately lead to repriming, with DNA synthesis restarting downstream of the lesion. In the resulting post-replicative gap, SSB is replaced by the RecA recombinase, which in turn will promote recombination-mediated gap filling (Fig. 1d).

Of course, many questions remain. For example, how does Pol IV transfer from SSB to the β clamp? The SSB-binding region of the enzyme and the clamp-interacting domain are spatially separated, so it may simply be a result of the higher affinity of Pol IV for β compared with SSB. More broadly, the principle of locally concentrating clamp-interacting proteins may be a general mechanism for regulating access to the clamp in all kingdoms of life. If this is the case, it will be important to determine which other accessory proteins provide the contextual clues that help to drive the selection of the most appropriate clamp-interacting partner. ❐

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Competing interests

The author has no competing interests.