Cell Cycle 12:24, 3713–3714; December 15, 2013; © 2013 Landes Bioscience

Pol κ in replication checkpoint

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Cells have evolved several mechanisms to deal with the constant challenge of DNA replication fork arrest during the S phase of the cell cycle. One important part of the cellular response to replication arrest or stalling by DNA damage is the induction of the ATR replication checkpoint pathway, which senses stalled replication forks and allows the independent translocation of multicomponent protein complexes, leading to the phosphorylation of the main ATR effector, the protein kinase Chk1.[1] In addition, activation of the DNA damage tolerance pathway that includes translesional (TLS) Y-family DNA polymerases (Pol η, Pol κ, Pol ι, Rev1)[2] facilitates bypass of DNA lesions. TLS polymerases promote damage tolerance through their ability to insert nucleotides opposite DNA lesions that block the replicative DNA polymerases or to fill in postreplication gaps containing lesions left behind replication forks.

Although both TLS and replication checkpoint choreograph the response to fork stalling, whether these pathways are coordinated has remained an open question. We addressed this issue by focusing on Pol κ, one of the most highly conserved TLS DNA polymerases.[3] We reasoned that the ubiquity of Pol κ, and the fact that the mice defective for the *POL* κ gene manifest a spontaneous genetic instability phenotype in absence of external stress, argue that this protein may contribute to additional aspects of cell physiology in addition to its role in TLS. We described a previously unrecognized and unexpected role for Pol κ in response to replication stress using 2 different experimental systems, *Xenopus* cell-free extracts and mammalian cultured cells.[4] We have found that Pol κ is required for checkpoint activation after replication fork stalling with DNA polymerases inhibitors such as hydroxyurea or aphidicolin, or in the presence of UV-blocking lesions. These effects appear to be specific to Pol κ, since removal of another member of the Y-family, Pol η did not affect the efficiency of Chk1 phosphorylation. This novel function appears to depend upon Pol κ catalytic activity, and we showed that Pol κ fulfils this checkpoint function by participating in DNA synthesis on ssDNA at stalled replication forks. Indeed, recent work has demonstrated that short DNA products accumulate on ssDNA templates in response to fork stalling by aphidicolin and strongly contribute to checkpoint activation.[5] These DNA products are longer than the size normally synthesized by Pol α, and a subset of them are generated by Pol δ, most probably on the lagging strand. The leading-strand replicative polymerase Pol ε, in contrast, does not appear to play a significant role in the synthesis of these DNA products.[5] Pol κ was found to contribute with replicative Pol α and δ to the synthesis of these short DNA intermediates, which, in turn, may facilitate recruitment of the 9-1-1 complex at stalled forks and consequently contribute to efficient activation of the replication checkpoint[4] (see also **Fig. 1**). Further studies will be required to determine the molecular bases of Pol κ recruitment at stalled forks for its checkpoint function. It will be of interest to explore whether the Pol κ domains required for TLS are similar to the critical domains involved in the checkpoint function. Whether Pol κ is recruited to particular chromosomal regions is also an interesting question. Pol κ has been recently shown to perform accurate DNA synthesis at microsatellite,[6] one type of interspersed tandem repeat ubiquitously present throughout the genome that constitutes natural fork barriers. Pol κ could potentially promote microsatellite stability and limit microsatellite allele length variation by its recruitment at stalled forks and its checkpoint associated DNA synthesis.

We have also observed that Pol κ downregulation in mammalian results in accumulation of DNA damage, thus revealing a function for Pol κ during DNA replication in unperturbed cells and further extending the role of this DNA polymerase outside TLS.[4] Interestingly, in the absence of Pol κ, ssDNA persists upon recovery from a hydroxyurea block, suggesting that Pol κ may be also required for replication fork restart. This latter observation may suggest that Pol κ could play a role in repriming replication forks. Since replication intermediates are already present on the lagging strand, it is likely Pol κ may function on the leading strand, alone or in combination with an as-yet-unknown DNA primase (**Fig. 1**), such as the very recently identified Polprim.[9,10]

Our work illustrates how 2 major pathways that respond to stalled replication forks could be coordinated to ensure high cell viability and genomic stability. It reinforces also the emerging concept that TLS may not be the sole function assigned to the Y-family TLS DNA polymerases. Pol η, best known for its role in responding to lesions generated by UV irradiation, has been also found to hold another function outside TLS in the stability of common fragile site (CFS) during unperturbed S phase.[7,8]

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Submitted: 08/22/2013; Accepted: 09/25/2013
http://dx.doi.org/10.4161/cc.26976
Comment on: Bétous R, et al. EMBO J 2013; 32:2172-85; PMID:23799366; http://dx.doi.org/10.1038/emboj.2013.148

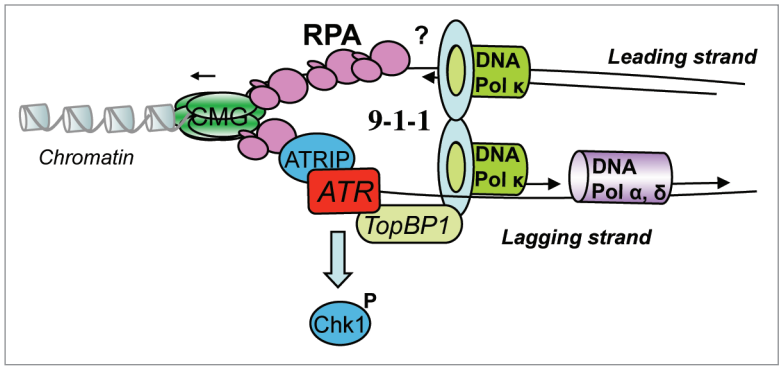

Figure 1. Speculative model of Pol κ function in replication checkpoint. Upon replication fork stalling with replicative DNA polymerases inhibitors (hydroxyurea, aphidicolin) or UV-blocking lesions, ssDNA is generated by the action of the helicase (CMG complex). Replicative DNA polymerases α and δ as well as TLS polymerase Pol κ contribute to synthesis and/or stabilization of small replication intermediates. These structures are bound by the checkpoint clamp 9-1-1 complex. DNA Pol κ may interact with the 9-1-1 complex on chromatin, thus facilitating local formation of the active ATR complex that include ATRIP and ToPBP1. Pol κ may also be implicated in replication fork restart by repriming (question mark).

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