Human PrimPol1: a novel guardian of stalled replication forks

Jun-Sub Im*, Kyung Yong Lee*, Laura W. Dillon & Anindya Dutta

The successful duplication of genomic DNA during S phase is essential for the proper transmission of genetic information to the next generation of cells. Perturbation of normal DNA replication by extrinsic stimuli or intrinsic stress can result in stalled replication forks, ultimately leading to abnormal chromatin structures and activation of the DNA damage response. On formation of stalled replication forks, many DNA repair and recombination pathway proteins are recruited to resolve the stalled fork and resume proper DNA synthesis. Initiation of replication at sites of stalled forks differs from traditional replication and, therefore, requires specialized proteins to reactivate DNA synthesis. In this issue of *EMBO reports*, Wan *et al* [1] introduce human primase-polymerase 1 (hPrimPol1)/CCDC111, a novel factor that is essential for the restart of stalled replication forks. This article is the first, to our knowledge, to ascertain the function of human PrimPol enzymes, which were originally identified as members of the archaeo-eukaryotic primase (AEP) family [2].

Single-stranded DNA (ssDNA) forms at stalled replication forks because of uncoupling of the DNA helicase from the polymerase, and is coated by replication protein A (RPA) for stabilization and recruitment of proteins involved in DNA repair and restart of replication. To identify novel factors playing important roles in the resolution of stalled replication forks, Wan and colleagues [1] used mass spectrometry to identify RPA-binding partners. Among the proteins identified were those already known to be located at replication forks, including SMARCA1/HARP, BLM and TIMELESS. In addition they found a novel interactor, the 560aa protein CCDC111. This protein interacts with the carboxyl terminus of RPA1 through its own C-terminal region, and localizes with RPA foci in cells after hydroxyurea or DNA damage induced by ionizing irradiation. Owing to the presence of AEP and zinc-ribbon-like domains at the amino-terminal and C-terminal regions, respectively [2], CCDC111 was predicted to have both primase and polymerase enzymatic activities, which was confirmed with

in vitro assays, leading to the name hPrimPol1 for this unique enzyme.

The most outstanding discovery in this article is that hPrimPol1 is required for the restart of DNA synthesis from a stalled replication fork (Fig 1). With use of a single DNA fibre assay, knock down of hPrimPol1 had no effect on normal replication-fork progression or the firing of new origins in the presence of replication stress. After removal of replication stress, however, the restart of stalled forks was significantly impaired. Furthermore, the authors observed that hPrimPol1 depletion enhanced the toxicity of replication stress to human cells. Together, these data suggest that hPrimPol1 is a novel guardian protein that ensures the proper re-initiation of DNA replication by control of the repriming and repolymerization of newly synthesized DNA.

Eukaryotic DNA replication is initiated at specific sites, called origins, through the help of various proteins, including ORC, CDC6, CDT1 and the MCM helicase complex [3]. On unwinding of the parental duplexed DNA, lagging strand ssDNA is coated by the RPA complex and used as a template for newly synthesized daughter DNA. DNA primase, a type of RNA polymerase, catalyses short RNA primers on the RPA-coated ssDNA that facilitate further DNA synthesis by DNA polymerase. While the use of a short RNA primer is

Fig 1 | The role of hPrimPol1 in stalled replication fork restart. (A) Under normal conditions, the replicative helicase unwinds parental DNA, generating ssDNA that is coated by RPA and serves as a template for leading and lagging strand synthesis. Aside from interacting with RPA bound to the short stretches of ssDNA, the role of hPrimPol1 in normal progression of replication forks is unknown. (B) Following repair of a stalled replication fork, (1) hPrimPol1 rapidly resumes DNA synthesis of long stretches of RPA-coated ssDNA located at the stalled fork site. Later, the leading-strand polymerase (2) or lagging-strand primase and polymerase (3) replace hPrimPol1 to complete replication of genomic DNA. RPA, replication protein A; ssDNA, single-stranded DNA.

occasionally necessary to restart leading-strand replication, such as in the case of a stalled DNA polymerase, it is primarily utilized in lagging-strand synthesis for the continuous production of Okazaki fragments. The lagging-strand DNA polymerase must efficiently coordinate its action with DNA primase and other replication factors, including DNA helicase and RPA [4]. Cooperation between DNA polymerase and primase is disturbed after DNA damage, ultimately resulting in the collapse of stalled replication forks. Until now, it was believed that DNA primase and DNA polymerase performed separate and catalytically unique functions in replication-fork progression in human cells, but this report provides the first example, to our knowledge, of a single enzyme performing both primase and polymerase functions to restart DNA synthesis at stalled replication forks after DNA damage (Fig 1).

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A stalled replication fork, if not properly resolved, can be extremely detrimental to a cell, causing permanent cell-cycle arrest and, ultimately, death. Therefore, eukaryotic cells have developed many pathways for the identification, repair and restart of stalled forks [5]. RPA recognizes ssDNA at stalled forks and activates the intra-S-phase checkpoint pathway, which involves various signalling proteins, including ATR, ATRIP and CHK1 [6]. This checkpoint pathway halts cell-cycle progression until the stalled forks are properly repaired and restarted. Compared with the recognition and repair of stalled forks, the mechanism of fork restart is relatively elusive. Studies have, however, begun to shed light on this process. For instance, RPA-directed SMARCAL1 has been discovered to be important for restart of DNA replication in bacteria and humans [7]. Together with the identification of hPrimpol1, these findings have helped to expand the knowledge of the mechanism of restarting DNA replication. Furthermore, both reports raise many questions regarding the cooperative mechanism of hPrimpol1 and SMARCAL1 with RPA at stalled forks to ensure genomic stability and proper fork restart [7].

The discovery of hPrimpol1 is also important in an evolutionary context

First, these findings raise the question of why cells need the specialized hPrimpol1 to restart DNA replication at stalled forks rather than using the already present DNA primase and polymerase. One possibility is that other DNA polymerases are functionally inhibited due to the response of the cell to DNA damage. Although the cells are ready to restart replication, the impaired polymerases might require additional time to recover after DNA damage, necessitating the use of hPrimpol1. In support of this idea, we found that the p12 subunit of DNA polymerase δ is degraded by CRL4^{CDT2} E3 ligase after ultraviolet damage [8]. As a result, alternative polymerases, such as hPrimpol1, could compensate for temporarily non-functioning traditional polymerases. A second explanation is that the polymerase and helicase uncoupling after stalling of a fork results in long stretches of ssDNA that are coated with RPA. To restart DNA synthesis, cells must quickly reprime and polymerize large stretches of ssDNA to prevent renewed fork collapse. By its constant interaction with RPA1, hPrimpol1 is present on the ssDNA and can rapidly synthesize the new strand of DNA after the recovery of stalled forks. Third, the authors found that the association of hPrimpol1 with RPA1 is independent of its functional AEP and zinc-ribbon-like domains and occurs in the absence of DNA damage. These results might indicate a role for hPrimpol1 in normal replication fork progression, but further work is necessary to determine whether that is true.

Several questions remain. First, what is the fidelity of the polymerase activity? Other specialized polymerases that act at DNA damage sites sometimes have the ability to misincorporate a nucleotide across from a site of damage, for example pol- η and - ζ [9]. It will be interesting to know whether hPrimpol1 is a high-fidelity polymerase or an error-prone polymerase. Second, is the polymerase only brought into action after fork stalling? If hPrimpol1 is an error-prone polymerase, one could envision other types of DNA damage that can be bypassed by hPrimpol1. Third, is the primase selective for ribonucleotides, or can it also incorporate deoxynucleotides? The requirement of the same domain—AEP—for primase and

polymerase activities raises the possibility that NTPs or dNTPs could be used for primase or polymerase activities.

The discovery of hPrimpol1 is also important in an evolutionary context. In 2003, an enzyme with catalytic activities like that of hPrimpol1 was discovered in a thermophilic archaeon and in Gram-positive bacteria [10]. This protein had several catalytic activities *in vitro*, including ATPase, primase and polymerase. In contrast to these Primpol enzymes, those capable of primase and polymerase functions had not been found in higher eukaryotes, which suggested that evolutionary pressures forced a split of these dual-function enzymes. Huang *et al*'s report suggests, however, that human cells do in fact retain enzymes similar to Primpol. In summary, the role of hPrimpol1 at stalled forks broadens our knowledge of the restart of DNA replication in human cells after fork stalling, allowing for proper duplication of genomic DNA, and provides insight into the evolution of primases in eukaryotes.

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*These authors contributed equally to this work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Wan L *et al* (2013) *EMBO Rep* [Epub 15 Oct] doi:10.1038/embor.2013.159
2. Frick DN, Richardson CC (2001) *Annu Rev Biochem* **70**: 39–80
3. Bell SP, Dutta A (2002) *Annu Rev Biochem* **71**: 333–374
4. Patel SS, Pandey M, Nandakumar D (2011) *Curr Opin Chem Biol* **15**: 595–605
5. Jones RM, Petermann E (2012) *Biochem J* **443**: 13–26
6. Cimprich KA, Cortez D (2008) *Nat Rev Mol Cell Biol* **9**: 616–627
7. Betous R *et al* (2013) *Cell Rep* **3**: 1958–1969
8. Terai K, Shibata E, Abbas T, Dutta A (2013) *J Biol Chem* **288**: 30509–30514
9. McCulloch SD, Kunkel TA (2008) *Cell Res* **18**: 148–161
10. Lipps G, Rother S, Hart C, Krauss G (2003) *EMBO J* **22**: 2516–2525

**Jun-Sub Im, Kyung Yong Lee, Laura W. Dillon and Anindya Dutta are at the Department of Biochemistry and Molecular Genetics, University of Virginia School of Medicine, Charlottesville, Virginia, USA.
E-mail: ad8q@virginia.edu**

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