Published in final edited form as:

Transcr Open Access. 2015 ; 3(1): . doi:10.4172/2329-8936.1000e108.

Maintain Genomic Stability: Multitask of DNA Replication Proteins

Jing Hao and Wenge Zhu*

Department of Biochemistry and Molecular Medicine, The George Washington University Medical School, 2300 Eye Street N.W., Washington, DC 20037, USA

Editorial

Maintenance of genomic stability is critical for living organisms because it is crucial for cell survival and development, and it prevents the development of deleterious mutations. Overriding this control will cause genomic instability, a hallmark of cancer.

The genome is highly vulnerable to damage, especially during DNA replication because chromosome is decondensed and the replication forks are extremely sensitive to DNA damage agents. The eukaryotic replisome, which consists of a large number of replication fork-associated proteins, is essential for the elongation of replication forks during DNA replication. This complex contains DNA polymerases, MCM helicase, single stranded DNA (ssDNA) binding protein RPA, sliding clamp PCNA, Tipin, Timeless, Claspin, And-1, etc. In cells with DNA damage such as replication stress, replication forks are stalled Figure 1. At stalled replication forks, some of replisome components switch their role from facilitating DNA synthesis to inducing activation of DNA replication checkpoint, a signaling transduction pathway that is critical to maintain fork stability and triggers cell cycle arrest.

Specifically, DNA lesions induced by replication stress lead to replication fork stalling, and at stalled replication forks ssDNA and primer-template junctions are formed [1]. ssDNA is generated when DNA helicase and DNA polymerase activities become uncoupled from one another due to either physical obstructions or nucleotide deficiencies that block DNA polymerase progression [2]. The replication and checkpoint protein TopBP1 cooperates with the BACH1/FANCI helicase to promote loading of RPA onto ssDNA at the stalled replication forks [3]. The ATR kinase is then recruited to the stalled replication forks through a specific interaction between RPA and ATR-interacting protein ATRIP [4]. The ATR is activated by a mechanism involving Rad9/Hus1/Rad1 (9-1-1) clamp and TopBP1 [5,6]. Ultimately, coordinated actions of several replisome proteins, including Claspin, Timeless, and Tipin, bring Chk1 to the stalled forks to be phosphorylated by ATR [7,12].

Timeless and Tipin proteins form heterodimers and interact with MCM helicase [7,13]. Depletion of either one compromises S phase checkpoint activation [7,14]. Intriguingly,

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

*Corresponding author: Wenge Zhu, Department of Biochemistry and Molecular Medicine, The George Washington University Medical School, 2300 Eye Street N.W., Washington, DC 20037, USA, Tel: 202-994-3517; wz6812@gwu.edu.

Timeless-Tipin complex prevents ssDNA accumulation at unperturbed replication forks and facilitates activation of ATR-Chk1 pathway in cells experiencing replication stress [15]. Claspin binds to DNA at the replication origins and is important for DNA synthesis in unperturbed cells [16]. Importantly, Claspin is a Chk1-interacting protein and Claspin-Chk1 interaction is critical for Chk1 phosphorylation under conditions that causes replication stress [11]. Timely degradation of Claspin is needed to release the cells from checkpoint arrest, enabling them to resume DNA replication [17,18]. In addition, Claspin, Timless and Chk1 are also known to regulate PCNA ubiquitination to tolerate DNA damage and avoid catastrophic replication fork collapse [19,20].

Replication checkpoint can prevent synthesis of damaged DNA and maintain the stability of stalled replication forks. For example, nuclease Exo1 and helicase SMARCA1 are inhibited following activation of the replication checkpoint activation as a way to stabilize replication forks [21,22]. In cells with replication stress, checkpoint prevents DNA synthesis by inhibiting late origin firing [23,24]. As a key regulator of checkpoint activation, ATR has long been established to maintain replication fork stability and to prevent replication origin firing during replication stress. Recent study showed that ATR prohibits replication catastrophe by preventing global exhaustion of RPA [25]. However, it should be noted that RPA exhaustion in the presence of ATR can still lead to irreversible breakage of replication forks [25].

Replication proteins play multiple roles in cells. In addition to their role in DNA synthesis, replisome proteins are essential for DNA replication checkpoint activation and maintenance of fork stability. Although it is clear that replisome proteins are critical for replication checkpoint activation, it remains largely unknown that how replisome components coordinate with checkpoint proteins during checkpoint activation. It also remains to be determined how checkpoint pathway targets replisome proteins to maintain fork stability in cells with replication stress. Addressing these questions will significantly help us to understand the role of replication proteins in the regulation of genomic stability.

Acknowledgments

Wenge Zhu was supported by a Research Scholar Grant, RSG-13-214-01-DMC from the American Cancer Society and grants from NIH (CA177898 and CA184717 to W. Z). The authors would like to thank Dr. Zhiyong Han for reading this manuscript.

References

1. MacDougall CA, Byun TS, Van C, Yee MC, Cimprich KA. The structural determinants of checkpoint activation. *Genes Dev.* 2007; 21:898–903. [PubMed: 17437996]
2. Byun TS, Pacek M, Yee MC, Walter JC, Cimprich KA. Functional uncoupling of MCM helicase and DNA polymerase activities activates the ATR-dependent checkpoint. *Genes Dev.* 2005; 19:1040–1052. [PubMed: 15833913]
3. Gong Z, Kim JE, Leung CC, Glover JN, Chen J. BACH1/FANCI acts with TopBP1 and participates early in DNA replication checkpoint control. *Molecular cell.* 2010; 37:438–446. [PubMed: 20159562]
4. Zou L, Elledge SJ. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science.* 2003; 300:1542–1548. [PubMed: 12791985]

5. Kumagai A, Lee J, Yoo HY, Dunphy WG. TopBP1 activates the ATR-ATRIP complex. *Cell*. 2006; 124:943–955. [PubMed: 16530042]
6. Delacroix S, Wagner JM, Kobayashi M, Yamamoto K, Karnitz LM. The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. *Genes Dev*. 2007; 21:1472–1477. [PubMed: 17575048]
7. Chou DM, Elledge SJ. Tipin and Timeless form a mutually protective complex required for genotoxic stress resistance and checkpoint function. *Proc Natl Acad Sci*. 2006; 103:18143–18147. [PubMed: 17116885]
8. Yoshizawa-Sugata N, Masai H. Human Tim/Timeless-interacting protein, Tipin, is required for efficient progression of S phase and DNA replication checkpoint. *J Biol Chem*. 2007; 282:2729–2740. [PubMed: 17102137]
9. Unsal-Kacmaz K, Chastain PD, Qu PP, Mino P, Cordeiro-Stone M, et al. The human Tim/Tipin complex coordinates an Intra-S checkpoint response to UV that slows replication fork displacement. *Mol Cell Biol*. 2007; 27:3131–3142. [PubMed: 17296725]
10. Burrows AE, Elledge SJ. How ATR turns on: TopBP1 goes on ATRIP with ATR. *Genes Dev*. 2008; 22:1416–1421. [PubMed: 18519633]
11. Chini CC, Chen J. Human claspin is required for replication checkpoint control. *J Biol Chem*. 2003; 278:30057–30062. [PubMed: 12766152]
12. Kumagai A, Dunphy WG. Claspin, a novel protein required for the activation of Chk1 during a DNA replication checkpoint response in *Xenopus* egg extracts. *Mol Cell*. 2000; 6:839–849. [PubMed: 11090622]
13. Cho WH, Kang YH, An YY, Tappin I, Hurwitz J, et al. Human Tim-Tipin complex affects the biochemical properties of the replicative DNA helicase and DNA polymerases. *Proc Natl Acad Sci*. 2013; 110:2523–2527. [PubMed: 23359676]
14. Unsal-Kacmaz K, Mullen TE, Kaufmann WK, Sancar A. Coupling of human circadian and cell cycles by the timeless protein. *Mol cell biol*. 2005; 25:3109–3116. [PubMed: 15798197]
15. Smith KD, Fu MA, Brown EJ. Tim-Tipin dysfunction creates an indispensable reliance on the ATR-Chk1 pathway for continued DNA synthesis. *The Journal of cell biology*. 2009; 187:15–23. [PubMed: 19805627]
16. Lee J, Kumagai A, Dunphy WG. Claspin, a Chk1-regulatory protein, monitors DNA replication on chromatin independently of RPA, ATR, and Rad17. *Mol Cell*. 2003; 11:329–340. [PubMed: 12620222]
17. Mailand N, Bekker-Jensen S, Bartek J, Lukas J. Destruction of Claspin by SCFbetaTrCP restrains Chk1 activation and facilitates recovery from genotoxic stress. *Mol cell*. 2006; 23:307–318. [PubMed: 16885021]
18. Peschiaroli A, Dorrello NV, Guardavaccaro D, Venere M, Halazonetis T, et al. SCFbetaTrCP-mediated degradation of Claspin regulates recovery from the DNA replication checkpoint response. *Mol cell*. 2006; 23:319–329. [PubMed: 16885022]
19. Yang XH, Shiotani B, Classon M, Zou L. Chk1 and Claspin potentiate PCNA ubiquitination. *Genes & development*. *Genes Dev*. 2008; 22:1147–1152. [PubMed: 18451105]
20. Mailand N, Gibbs-Seymour I, Bekker-Jensen S. Regulation of PCNA-protein interactions for genome stability. *Nat rev Mol cell biol*. 2013; 14:269–282. [PubMed: 23594953]
21. Segurado M, Diffley JF. Separate roles for the DNA damage checkpoint protein kinases in stabilizing DNA replication forks. *Genes dev*. 2008; 22:1816–1827. [PubMed: 18593882]
22. Bansbach CE, Betous R, Lovejoy CA, Glick GG, Cortez D. The annealing helicase SMARCA1 maintains genome integrity at stalled replication forks. *Genes dev*. 2009; 23:2405–2414. [PubMed: 19793861]
23. Dimitrova DS, Gilbert DM. Temporally coordinated assembly and disassembly of replication factories in the absence of DNA synthesis. *Nature cell biology*. 2000; 2:686–694. [PubMed: 11025658]
24. Zachos G, Rainey MD, Gillespie DA. Chk1-deficient tumour cells are viable but exhibit multiple checkpoint and survival defects. *The EMBO journal*. 2003; 22:713–723. [PubMed: 12554671]

25. Toledo LI, Altmeyer M, Rask MB, Lukas C, Larsen DH, et al. ATR prohibits replication catastrophe by preventing global exhaustion of RPA. *Cell*. 2013; 155:1088–1103. [PubMed: 24267891]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.
Cells with DNA damage such as replication stress, replication forks are stalled