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Review

# Cellular responses to replication stress: Implications in cancer biology and therapy

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ABSTRACT

DNA replication is essential for cell proliferation. Any obstacles during replication cause replication stress, which may lead to genomic instability and cancer formation. In this review, we summarize the physiological DNA replication process and the normal cellular response to replication stress. We also outline specialized therapies in clinical trials based on current knowledge and future perspectives in the field.



**Contents**



*Abbreviations:* 53BP1, tumor protein p53 binding protein 1; 9-1-1, RAD9-RAD1-HUS1; AND1, acidic nucleoplasmic DNA-binding protein 1; ATR, ataxia telangiectasia and Rad3 related; ATRIP, ATR interacting protein; BIR, break-induced replication; BLM (RECQL3), Bloom Syndrome; CDC6/7/45, cell division cycle 6/7/45; CDK1/2/4/6/7, cyclin-dependent kinase 1/2/4/6/7; CDT1, chromatin licensing and DNA replication factor 1; CFSs, common fragile sites; CHK1/2, checkpoint kinase 1/2; CMG, CDC45-MCM-GINS; CTIP, CTBP-interacting protein; DDK, DBF4-dependent kinase; DNA2, DNA replication helicase/nuclease 2; DSBs, double strand breaks; dsDNA, double-stranded DNA; E2F6, E2 factor transcription factor 6; EXO1, exonuclease 1; FANCD2/M, Fanconi Anemia complementation group D2/M; GINS, Go-Ichi-Ni-San (termed from 5-1-2-3 in Japanese, SLD5-PSF1-PSF2-PSF3); HLTF, human helicase-like transcription factor; HR, homologous recombination; iPOND, isolate proteins on nascent DNA; MCM2-7/10, minichromosome maintenance 2–7/10; MEF1/2, FANCM-interacting histone-fold protein 1/2; MLL (KMT2A), lysine methyltransferase 2A; MPF, maturation promoting factor; MRE11, meiotic recombination 11; MRN, MRE11-RAD50-NBS1; MYT1, membrane-associated tyrosine/threonine 1; NBS1, Nijmegen breakage syndrome 1; ORC, origin recognition complex; PARI, PCNA-associated recombination inhibitor; PARP1, poly(ADP-ribose) polymerase1; PCNA, proliferation cell nuclear antigen; PLK1, polo like kinase 1; POLD3, polymerase delta 3; pre-IC, pre-initiation complex; pre-RC, pre-replication complex; RAD17/50/51, RAD17/50/51 recombinase; RB, retinoblastoma protein; RECQL2/3/4, RECQ helicase-like 2/3/4; RFC, replication factor C; RHINO, RAD9-RAD1-HUS1 interacting nuclear orphan; RIF1, replication timing regulatory factor 1; RPA, replication protein A; SHPRH, SNF2 histone linker PHD RING helicase; SLX4, synthetic lethal of unknown function 4; SMARCAL1, SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like 1; SSBs, single strand breaks; ssDNA, single-stranded DNA; TOPBP1, topoisomerase II binding protein 1; Tipin, Timeless-interacting protein; TLS, translesion synthesis; Treslin, TOPBP1-interacting checkpoint and replication regulator; UBC13, ubiquitin-conjugating enzyme complex 13; WRN (RECQL2), Werner Syndrome; ZRANB3, zinc finger, RAN-binding domain containing 3.

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## 1. Introduction

DNA replication is a general process for every proliferative cell. In order to ensure cell division into two identical daughter cells after replication, the whole process is tightly controlled by a complicated network. An accumulation of replication errors and damages can lead to serious diseases such as cancer. The acquired functional capabilities due to mutational processes caused by inaccurate DNA replication promote cancer survival, proliferation, and dissemination. In 2011, Hanahan and Weinberg added "genome instability and mutation" to their hallmarks of cancer [1]. The aim of this review is to apply our current knowledge in DNA replication, replication stress, and genome stability to cancer treatment.

## 2. DNA replication in eukaryotes

DNA replication starts from early G1 phase and ends before cells enter G2 phase (Fig. 1). In G1 phase, multiple replication origins spaced 30–100 kb apart are recognized by ORC [2,3]. ORC then recruits CDC6 and CDT1, which facilitate the loading of an inactive double-hexameric MCM (MCM2-7) helicase to form a pre-RC [4–6]. The process is known as "replication licensing" and requires little or no CDK activity [7]. By the increased level of CDKs, CDK4/6 forms an activated complex with its regulatory subunit cyclin D. The complex phosphorylates RB, which releases E2F transcription factors and triggers E2F-dependent transcription of *CCNE1* (encoding protein cyclin E) and other S-phase genes [8]. The cyclin E-CDK2 complex, subsequent to the cyclin D-CDK4/6 complex, promotes cell cycle progression from G1 to S phase [9,10].

During G1-S phase transition, the pre-RC is converted into the pre-IC, where the DNA helicase is activated. Assembly of the pre-IC involves the recruitment of proteins, including TOPBP1, Treslin, CDC45, GINS, RECQL4, MCM10, and the claspin-Timeless-Tipin complex, and it is followed by activation of the CMG (CDC45-MCM-GINS) helicase complex [11,12]. The whole process requires kinase activities of CDKs and DDK (CDC7-DBF4) [6,7,13]. For example, DDK promotes the association of CDC45 with origins while CDKs facilitate the recruitment of GINS to origins [6]; CDKs phosphorylate and activate RECQL4, which, with MCM10, is required for CMG formation [14]; phosphorylation of MCM subunits by DDK activates MCM helicase and enriches MCM-CDC45 on chromatin [15–17]; CDKs prevent MCM2-7 from reloading to the same origin to avoid re-replication [12]; and phosphorylation of Treslin at threonine 969 and serine 1001 by CDKs promotes Treslin interaction with TopBP1, which is essential for pre-IC formation and CMG helicase activation [4,11,13,18,19]. Once CMG helicase is activated, DNA synthesis begins at the replication origins, a process known as "origin firing" [11].

In S phase, activated CMG helicase unwinds the DNA double helix to form structures called "replication forks" and recruits other proteins to the forks. The recruited proteins and two MCM hexamers split from one MCM double-hexamer convert one pre-IC into two functional replisomes that carry out DNA replication at two replication forks emanating from the origin [11,20]. After DNA unwinding, RPA binds to ssDNA with a defined polarity (5′- to 3′-) and stabilizes the replication fork [21]. The tension from DNA helix unwinding is released by topoisomerase, which cuts either one (topoisomerase I) or both (topoisomerase II) strands of DNA double helix and re-anneals the strands. The ATR–ATRIP complex and its signaling-related proteins, such as claspin, the 9-1-1 complex, and CHK1, are also recruited after origin activation [22] to limit excessive origin firing under normal physiological conditions [23,24]. During the DNA elongation process, DNA polymerase α-primase stabilized by MCM10 and AND1 creates a small RNA primer to initiate DNA synthesis [25,26]. Later, with the help of RPA-ssDNA and the RFC-PCNA complex, DNA polymerase δ and ε replace polymerase α and keep synthesizing the complementary strand of DNA in the lagging strand and the leading strand [21,27]. PCNA, a DNA clamp, forms a complex with RFC, a DNA clamp loader, to prevent dissociation of DNA polymerase from the DNA template [28]. Once DNA replication is complete, cells will enter G2/M phase, which is controlled by the cyclin B1-CDK1 complex [23].

The cyclin B1-CDK1 complex is the MPF (maturation promoting factor) that triggers mitosis. The level of CDK1 is constant throughout the cell cycle, but the activity is regulated by protein phosphorylation and its binding partner cyclin B1. The increase of cyclin B1 from S phase and its disappearance at the end of mitosis affect the interaction of cyclin B1 and CDK1. The cyclin B1-CDK1 complex remains inactive until CDK1 is phosphorylated at threonine 161 by CDK7, and the phosphorylation at threonine 14/tyrosine 15 by MYT1-WEE1 is removed by CDC25 phosphatase [29,30]. The cyclin B1-CDK1 complex phosphorylates Bora, the cofactor of Aurora A, to facilitate PLK1 phosphorylation by Aurora A at threonine 210, and the phosphorylated PLK1 activates the complex by activating CDC25 and by inhibiting MYT1-WEE1 [31]. PLK1 also directly regulates the cyclin B1-CDK1 complex by controlling nuclear localization of cyclin B1 [29]. Thus, cell cycle progression is tightly controlled through the balance between mitotic kinase and phosphatase activities after DNA replication.

## 3. The cellular response to replication stress

DNA replication is highly regulated and requires multiple mechanisms to ensure its accuracy. Any obstacles may slow down or stall the DNA replication process before its completion, thereby causing "replication stress." The obstacles can be inappropriate origin firing, secondary DNA structures, unstable genomic regions such as chromosomal fragile sites, insufficient proteins and nucleotides for DNA replication, existing DNA damage lesions [32], collisions with transcription machineries due to accumulations of R loops or topological tension [33–38], or oncogene activation [39–41]. Prolonged replication stress leads to fork collapse (loss of replication competence, including the replisome), fork breakage or unscheduled cleavage of fork reversal (regression), and may further have serious implications for genome integrity if the cellular response to the replication stress is impaired [42].

Stress-activated signaling pathways cause cell cycle arrest and fork reversal, and further provide cells more time to repair the damage and restart replication. Many DNA replication proteins are also involved in the replication stress response, and these multifunctional proteins can be regulated either by post-translational modifications or protein-protein/protein-DNA interactions. For example, polyubiquitinated PCNA recruits ZRANB3 translocase to promote fork regression and restart following replication arrest [43,44], and SUMOylated PCNA interacts with PARI to inhibit hyper-recombination [45]. Unlike the activity of CDC45 in physiological DNA replication, during replication stress, CDC45 shows high affinity to stress-induced extensive ssDNA, which promotes CDC45 dissociation from MCM and further inhibits the function of CGM helicase [46]. A protein-mediated "hand-off" mechanism regulates conformational change of RPA and its DNA-binding modes characterized by the length of ssDNA and the number of ssDNA-binding domains involved [47]. The hand-off mechanism allows RPA-ssDNA to activate ATR in distinct ways toward different substrates: RPA32 phosphorylation at serine 33 by ATR is dependent on NBS1 and CTIP, but phosphorylation of the checkpoint regulator CHK1 by ATR is more dependent on RAD17 [21,48].

ATR activation is the primary cellular response to replication stress (Fig. 2a). When a cell faces replication stress, extensive ssDNA due to uncoupling of helicase and polymerase activities forms and