available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/dnarepair

The DNA damage response during an unperturbed S-phase

Merav Ben-Yehoyada, Jean Gautier, Aude Dupré*

Columbia University, Department of Genetics and Development, Irving Cancer Research Center, 1130 St Nicholas Avenue Room 302, New York, NY 10032, USA

ARTICLE INFO

Article history:
Published on line 19 March 2007

Keywords:
ATM
ATR
Checkpoints
Replication
Double-strand breaks DNA
Single-stranded DNA

ABSTRACT

DNA replication is a highly conserved and controlled process. To maintain genome integrity, the DNA must be faithfully duplicated once before chromosomes are segregated to daughter cells. Experimental insults to cells during DNA replication trigger an array of responses to help cells cope with DNA damage and replication stress. This has been coined the DNA damage response. During an unperturbed S-phase, DNA lesions and aberrant DNA structures arise as a consequence of normal DNA replication. Recent data suggest that the same pathways regulating the response to acute DNA damage also operate during normal S-phase to maintain genome integrity in the face of low levels of damage. This review will focus on the role of key proteins and signaling pathways, originally identified by their requirement to maintain genome stability during DNA replication following experimental insults, in the regulation of progression through normal S-phase.

1. Targets of the DNA damage response during S-phase

Successful progression through S-phase requires coordination of complex DNA transactions responsible for the exact duplication of the entire genome at each cell cycle and monitoring any deviation from this rule. Notably, the complex molecular machines that assemble on chromatin at the onset of S-phase to support DNA unwinding and DNA replication play an active role in monitoring the faithfulness of DNA replication and are also targets for inhibition by the DNA damage response pathways (Fig. 1).

1.1. *Origin assembly and activation*

Although the transition from G1 to S-phase is morphologically indistinguishable, dramatic changes occur at the molecular level to signal the cell to start DNA replication [1]. In prokaryotes, replication begins from a single origin until completion of the entire genome. In contrast, higher eukaryotes initiate DNA replication at multiple, less defined, loci that are termed 'origins of replication'. First, the origin recognition complex (ORC) binds DNA origins and serves as an assembly platform for the Cdt1- and Cdc6-dependent loading of the hexameric Mini-Chromosome Maintenance (MCMs) protein

* *Corresponding author*. Tel.: +1 212 851 4564; fax: +1 212 851 5284.
E-mail address: jg130@columbia.edu (J. Gautier).

Abbreviations: A–T, Ataxia–Telangiectasia; ATM, Ataxia–Telangiectasia Mutated; ATR, ATM and Rad3 related (mec1); DSBs, double-strand breaks; ssDNA, single strand DNA; PIKK, phosphoinositol kinase like kinase; IR, ionizing radiation; UV, ultraviolet; HU, hydroxyurea; MMC, Mitomycin C; MCM, Mini-Chromosome Maintenance protein; ORC, origin replication complex; Chk, checkpoint proteins; BLM, Bloom; FANC, Fanconi anemia proteins; MDC1, mediator of DNA damage checkpoint protein; FHA, Forkhead associated domain; ATRIP, ATR-interacting protein; CDK, cyclin-dependent kinase; RDS, radio-resistant DNA synthesis; MRN, Mre11-Rad50-Nbs1; APH, Aphidicholin

1568-7864/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2007.02.005

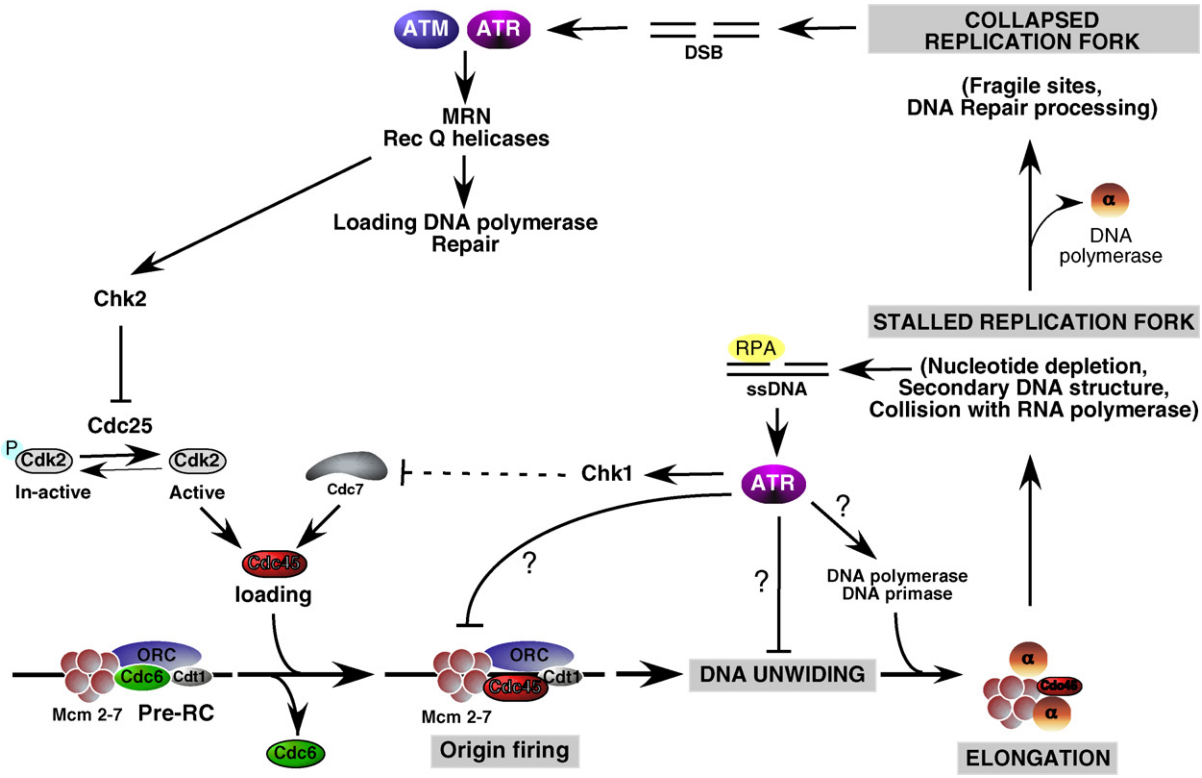

Fig. 1 – DNA replication is initiated by the loading of the pre-RC containing the origin recognition complex (ORC), Cdc6, Cdt1 and Mini-Chromosome Maintenance proteins (MCM). Cdk2 and Cdc7 activities are then required to promote the loading of Cdc45 onto chromatin, and unwind DNA by MCM helicase. The subsequent loading of the DNA polymerases allows the formation of the replisome at the replication fork, which coordinates the synthesis of DNA between leading and lagging strand. During unperturbed S-phase lesions and aberrant DNA structures arise as a consequence of normal DNA replication. In response to nucleotide depletion, secondary DNA structure, collision with RNA polymerase and endogenous metabolism the replication fork can be stalled. Under these conditions ATR is activated to stabilize the replication fork by phosphorylating component of the replisome and by blocking late origin firing through the inhibition of the S-phase protein kinases Cdc7 and Cdk2. However, if the replisome is not stabilized, the DNA polymerase dissociates from the fork causing the fork to collapse. Under these conditions, an ATM/ATR dependent checkpoint is initiated to repair the DNA and allow reloading of the DNA polymerase. Activation of this pathway also leads to the inhibition of unfired origins. Fork collapse can also occur spontaneously at fragile sites and due to DNA repair processing. Finally, ATM and/or ATR have the ability to phosphorylate and possibly inhibit directly the activity of the MCM DNA helicase and DNA polymerases (pathways marked with?).

complex, which altogether form the pre-replication (pre-RC) complex [2]. This highly regulated and organized assembly on DNA origins occurs during the G1 phase of the cell cycle. As cell cycle progresses, S-phase kinases Cdk2 and Cdc7 accumulate in the nucleus and are activated to initiate DNA replication by phosphorylating target proteins at the origin of replication. In turn, these phosphorylation events promote the loading of additional proteins including Cdc45, GINS, and MCM10 onto chromatin allowing DNA unwinding by the MCM helicase to take place. The melting of the DNA enables the loading of the DNA polymerases and the DNA primase to form an active replication fork. This active fork structure allows the coordinated synthesis of both leading and lagging DNA strands [2]. Inhibition of Cdk2 or Cdc7 is a prominent mechanism to regulate origin activity and subsequent S-phase progression.

1.2. *The replication fork*

The replication fork is a structure with the potential to trigger a DNA damage response. DNA replication can be blocked or stalled by variety of endogenous and exogenous lesions. DNA–protein complexes, DNA secondary structures, collision with RNA polymerases and nucleotide depletion are some of the endogenous barriers that may cause a replicating fork to stall. Fork progression can also be interrupted by lesions to DNA template, such as, single (ssDNA) and double strand breaks (DSBs), intra- and inter-strand cross-links, or by chemical inhibitors like aphidicholin (APH) and hydroxyurea (HU) [3]. In addition, there is increasing evidence that lesions and presumably replication blocks occur spontaneously, and at a considerable frequency, during normal DNA replication [4–6]. Altogether, these insults may pose a significant challenge for