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Review Article

DNA replication stress: Causes, resolution and disease

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ABSTRACT

DNA replication is a fundamental process of the cell that ensures accurate duplication of the genetic information and subsequent transfer to daughter cells. Various pertubations, originating from endogenous or exogenous sources, can interfere with proper progression and completion of the replication process, thus threatening genome integrity. Coordinated regulation of replication and the DNA damage response is therefore fundamental to counteract these challenges and ensure accurate synthesis of the genetic material under conditions of replication stress. In this review, we summarize the main sources of replication stress and the DNA damage signaling pathways that are activated in order to preserve genome integrity during DNA replication. We also discuss the association of replication stress and DNA damage in human disease and future perspectives in the field.

Contents

Abbreviations: pre-RC, pre-replicative complex; CFS, common fragile site; DSB, DNA double strand break; CMG complex, Cdc45.Mcm2–7.GINS; ERFS, early-replicating fragile site; DDR, DNA damage response; ssDNA, single-stranded DNA; IR, ionizing radiation; HGPS, Hutchinson–Gilford progeria syndrome; SIOD, Schimke immune-osseous dysplasia; AOA1, Apraxia Oculomotor Ataxia 1; FA, Fanconi anemia; iPOND, Isolation of protein on nascent DNA; BLESS, direct in situ breaks labeling, enrichment on streptavidin and next-generation sequencing; CRISPR, Clustered regulatory interspaced short palindromic repeats

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Introduction

Several exogenous and endogenous sources constantly challenge the integrity of replicating DNA, and can pose a serious threat to chromosomal stability by interfering with progression, stability and proper resumption of replication after fork arrest. DNA damage generated endogenously by errors during DNA replication is often referred to as replication stress and particularly affects genomic loci where progression of replication forks is slow or problematic. Cells have evolved a panoply of mechanisms to deal with different kinds of DNA damage that ensure the integrity of the genome during replication. Various repair mechanisms and different checkpoint machineries exist, which stop or slow down cell cycle progression until the damage is repaired. These DNA replication, repair and checkpoint activation pathways are highly regulated and coordinated. Defects in any of these functions leads to genomic instability and may lead to cancer, premature ageing or disorders associated with loss of genomic integrity.

Overview of DNA replication

DNA replication is initiated at defined loci known as replication origins. In the eukaryotic genome, replication begins at multiple origins, ranging from a few hundred in yeast to thousands in humans. These are distributed along the length of each chromosome [1]. Initiation of replication comprises a two-step process: origin licensing and firing. Origin licensing starts as early as late M or early G1 with the assembly of a pre-replicative complex (pre-RC) at each origin (early or late). The pre-RC consists of the origin recognition complex (ORC1–6 proteins), cell division cycle 6 (Cdc6), cell division cycle 10-dependent transcript 1 (Cdt1) and the core replicative helicase component Mcm2–7, consisting of the minichromosome maintenance proteins 2–7 (Mcm2–Mcm7) [2,3]. The second step, origin firing, involves the activation of the Mcm2–7 complex which is restricted to S phase and culminates in the formation of a pair of oppositely oriented replication forks that contain a single Mcm2–7 helicase hexamer complex at the apex of each fork [4]. Cyclin dependent kinases (CDKs) and Dbf dependent kinases (DDKs) promote the conversion of the pre-RC complex into a pre-initiation complex capable of unwinding DNA and carrying out DNA synthesis [5]. At the G1/S transition, when CDK activity rises, numerous additional factors cooperate to convert the MCM2–7 double hexamer into two CMG (Cdc45. Mcm2–7.GINS) complexes [6]. In particular, Cdc7–Dbf4 protein kinase (DDK) phosphorylates MCM2–7. CDK phosphorylates Sld2 (sharing homology to human RECQ4) and Sld3 (the yeast homolog of Treslin in human), promoting their interaction with Dpb11 (the yeast homolog of TopBP1 in human). The Sld3–Sld2–Dpb11 complex enables the stable binding of Cdc45 and GINS to phosphorylated MCM2–7. Once formed, CMG unwinds the origin, allowing replisome assembly. Replication forks then travel bidirectionally outwards from the origin until the entire genome is replicated [7–10].

Sources of DNA replication stress

Replication stress is defined as slowing or stalling in replication fork progression. It arises from many different sources, which are considered as replication barriers such as telomeres, repetitive sequences, DNA lesions and misincorporation of ribonucleotides, secondary DNA structures, DNA–RNA hybrids, dormant replication origins, collisions between replication and transcription complexes, hypo-acetylation and compaction of chromatin, early-replicating fragile sites (ERFSs) and common fragile sites (CFSs). Finally overexpression or constitutive activation of oncogenes such as HRAS, c-Myc and cyclin E is an emerging source of replication stress. Following, we discuss some of the most relevant sources of replication stress in more detail (see Fig. 1). We refer readers to the following review for an overall picture of agents than induce replication stress [11].

Fragile sites

Certain loci in the human genome are particularly difficult to replicate, hence rendering them prone to fragility. Most prominent amongst these are the so-called fragile site loci. As mentioned above, fragile sites can be classed CFSs or ERFSs. The former have a high A/T content, occur at sequences prone to form secondary structures, possess a condensed chromatin structure and replicate late. In contrast ERFSs are G/C rich, have an open chromatin state and replicate early.

Fragile sites are defined as being either common or rare; the former, CFSs, are present in all individuals, whereas rare fragile sites are found in less than 5% of the population [12]. There are over 200 CFSs in the human genome and these regions are quite large, ranging from just under 1 Mb to over 10 Mb in size. CFSs are prone to replication stress-induced DNA double-strand breaks (DSBs) visible in condensed metaphase chromosomes and their occurrence is dependent on the endonuclease activity of MUS81-EME1, in synergy with the resolving action of the BLM helicase to prevent chromosome breakage [13,14]. The most typical inducer of CFSs used experimentally is aphidicolin, an inhibitor of the replicative DNA polymerases α, δ, and ε [15]. The three most frequently expressed CFSs are FRA3B, FRA16D, and FRA6E [16–18]. Several studies in cell culture models have shown that under conditions that induce replication stress, fragile sites are hotspots for sister chromatid exchange, translocations and deletions [19]. The frequent alterations within these regions in multiple cancers have led to the identification of a number of extremely large genes contained within CFSs. Several of these large genes have