Biochimie 108 (2015) 25–32

Contents lists available at ScienceDirect

Biochimie

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

Review

Replication and re-replication: Different implications of the same mechanism

Michalina Mazurczyk, Dorota Rybaczek*

Department of Cytophysiology, Institute of Experimental Biology, Faculty of Biology and Environmental Protection, University of Łódź, Pomorska 141/143, 90-236 Łódź, Poland

ARTICLE INFO

Article history:
Received 8 May 2014
Accepted 27 October 2014
Available online 4 November 2014

Keywords:
Chromosome condensation
Endoreplication
Genome chaos
Replication
Re-replication

ABSTRACT

Replication is a process which provides two copies of genetic material to a mother cell that are essential for passing complete genetic information to daughter cells. Despite the extremely precise control of this process, regulation of replication can be impaired. This may trigger e.g. re-replication which leads to an increase in the total DNA content in a cell and, depending on the intensity, may result in gene amplification, genomic instability or apoptosis. Both replication and re-replication require pre-replication complex assembly, licensing, firing and initiation of DNA synthesis. Implications of each process in a cell are very different and all such possibilities are under intensive research because in both processes the same protein apparatus is used to carry out DNA synthesis. Therefore this article is meant to show the consequences of the same mechanism underlying two different processes.

1. Introduction

A mother cell must divide into two genetically identical daughter cells, therefore DNA replication must occur once and only once per cell cycle. This is crucial for both functioning of a cell and preservation of proper DNA content [1,2]. Replication is a process that is very strictly controlled by multiple pathway mechanisms and proteins which control a number of subsequent events essential for the cell cycle. Assembly of pre-replication complexes (pre-RCs) during the G1 phase is regulated by, amongst others, CDK level, cyclin A [3,4], expression of pre-RC composites (i.e. Cdc6), or their location (i.e. MCM2-7) [5]. Next, the initiation of the S-phase is triggered by a change in the concentration of proteins and their mutual equilibrium [4,6]. During the S-phase, the presence of double strand breaks within the DNA is checked (ATM and ATR are involved) to ensure that replication is not affected [7,8]. To date, a number of cell cycle perturbations have been described including the re-replication phenomenon, the replication of regions already replicated in a cell (>1 < 2 S phases per cell division; Table 1 and Fig. 1). Endoreplication (also called endoreduplication or endocycles), however, is the primary mechanism for developmentally programmed polyploidy (DPP) which results in more than one complete S phase per cell division (>4C, but in integral multiple of 4C, i.e. 8C, 16C, 32C, 64C etc.). Cells enter the first endoreplication S phase from G2 (e.g. in ORC-dependent endoreplication model described by Smith and Orr-Weaver [9]). However, in Asano's paper [10] the discussion of a possible ORC-independent endoreplication model can be found; the author indicates that cells arrested in G2 do not endoreplicate, whereas those arrested in prometaphase do endoreplicate. The final effect of endoreplication is always the same - the appearance of giant, nonproliferating but endocycling cells containing a single, polyploid nuclei (Table 1 and Fig. 1; [11]). Endoreplication is widespread among eukaryotes, but mainly in plants. It occurs in differentiating cells [12,13]. In animals, endoreplication supports tissue and organ growth and/or regeneration (e.g. damaged cardiomyocytes; [14]).

Re-replication does not occur naturally, it is an aberrant event [15,16]. Re-replication can be triggered and/or enhanced by various factors such as the ratio of Cdt1 to geminin or overexpression of Cdt1/Cdc6 [3], osmotic stress [17], and also vincristine, taxol, tetraethylthiuram disulfide [18]. Additionally, sequences susceptible to replication re-initiation have been observed in yeast origin [19].

Abbreviations: APC, Anaphase Promoting Complex; BIR, Break-induced replication; DPP, developmentally programmed polyploidy; GCS, generalized chromosome shattering; GINS, go-ichi-ni-san; MCM2-7, Mini-Chromosome Maintenance 2-7; NAHR, nonallelic homologous amplification; NHEJ, nonhomologous end joining; ORC, origin recognition complex; ori, origin; PALCC, parachute-like chromatin; Pre-RC, pre-replication complex; RC, replication complex; RRIGA, re-replication induced gene amplification.

* Corresponding author. University of of Łódź, Pomorska 141/143, 90-236 Łódź, Poland. Tel.: +48 42 6354735; fax: +48 42 3654514.
E-mail address: dorota.rybaczek@gmail.com (D. Rybaczek).

http://dx.doi.org/10.1016/j.biochi.2014.10.026
0300-9084/

Table 1
A summary of characteristics and emphasizing the differences between replication, re-replication and endoreplication.

	Replication	Re-replication	Endoreplication
Genetic material quantity	2C	2 < C < 4	>4C and integral multiple of 4C
Fragments that undergo doubling	Precise replication of whole genome	Randomly scattered fragments	Second (and following) round of precise replication of whole genome
Pre-PCs assembly	Late M/early G1	Re-assembly in G2/M	G2
Timing	S	G2/M	G⇄S
Cellular effect	Two viable, normal cells with the same genetic content	DNA damage, DNA damage response, checkpoint activation, genomic instability, and apoptosis	Terminally differentiated, viable, nonproliferating polyploidy cells that play important roles in animal and plant development

The direct cause of re-replication is the re-assembly of pre-replication complexes due to deregulation of factors which contribute to pre-RCs assembly once per cell cycle [6,20]. Tanny et al. [21] showed that most re-replication regions overlap with Mcm2-7 binding, therefore pre-RCs re-assembly is possibly directed by normal sites of pre-RC formation in G1. The re-assembly of RCs may be enhanced by deregulation of the level of CDK, the presence Cdt1 or MCM2-7 in the nuclei in G2 and M phases [6,20], geminin depletion, mutations in ORC, Cdc6 and MCM2-7 [8]. Although a great amount of research has been performed on the assembly of replication complexes and their functioning this process is still not fully understood [6,20].

This paper concerns the subsequent events leading to replication and re-replication. These events being pre-replication complexes assembly, licensing, firing and DNA synthesis initiation, are stages of the same mechanism. However, replication results in the doubling of genetic material while re-replication in the doubling of fragments of genetic material. Thus, the results of these processes are different and the direct implications of each process are distinct. The aim of this review is to emphasize that while replication, as a normal process, leads to cell division and proper chromosome condensation, re-replication, being an abnormal process, leads to genetic instability, cell cycle inhibition and/or apoptosis. It was interesting to compare the two different processes which have opposite implications although they are based on the same mechanism.

2. Function of the replication origin(s)

The replication process starts when pre-RCs are assembled at distinct sequences of a DNA strand. Bacteria, yeast and metazoa (including plant and human) DNA is organized into replicons – portions of DNA replicated from one origin (*ori*) of replication [22]. *Escherichia coli* has only one origin (OriC), *Saccharomyces cerevisiae* contains 500 origins, and human DNA around $5^{*}10^{4}$. Therefore, it would appear that larger genomes require more origins. This is further confirmed when the time limitations of the cell cycle and the efficiency of replication forks are taken into consideration [5]. Despite both *S. cerevisiae* and *Schizosaccharomyces pombe* having origins with ARS activity (conferring the ability to replicate when inserted into a plasmid without *ori*), *S. pombe* has no consensus sequence, while *S. cerevisiae* has a consensus sequence and is the best known example of eukaryotic origins [5,22]. The only sequence that may function as an origin of replication in plants is a non-transcribed spacer (NTS). NTS was revealed to contain an AT-rich region, but until its role is discovered it cannot be determined as a consensus sequence of a functional *ori* in higher plants [22]. In metazoa, replication origins are not yet well known, although it appears that metazoa origins do not have a consensus sequence. It has been suggested that in these organisms length, the structure of chromatin and gene transcription are more important for origin activity than the consensus sequence [5,22]. Interestingly, it has been demonstrated that in *Drosophila melanogaster* S2 cells, forced binding of an ORC subunit and the subsequent formation of pre-RC, induced DNA replication in sequences distinct from natural origins. Further investigation is necessary to determine how origins function and to what extent they are absolutely essential for DNA replication [23].

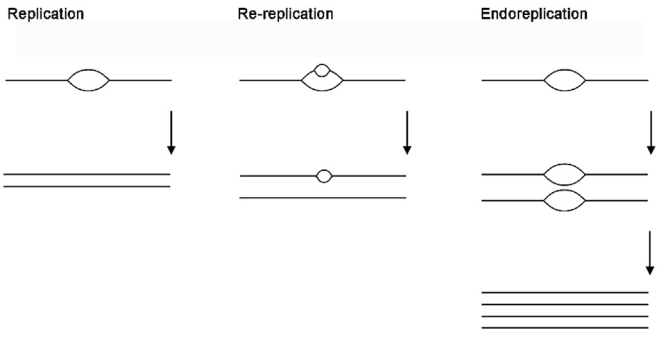

Fig. 1. A visual representation of the mechanisms and outcomes of replication, re-replication and endoreplication.

3. Pre-replication complexes: formation, initiation, timing, and firing

Replication complexes (RCs) need to be assembled in the late M or G1 phases, before replication can occur (Table 1; [1,6]). This process takes place in the presence of a very low level of mitotic-type cyclins (e.g. cyclin B1). Pre-RCs consist of origin recognition complex (ORC), Cdt1, Cdc6 (Cdc18 in yeast [1,6]) and Mini-Chromosome Maintenance 2–7 (MCM2-7) proteins [1,5,6,8,21,23–25]. Replication complexes (RCs) formation requires the following order of events: (1) ORC binding (ORC consists of six subunits - Orc1-6 [5]); (2) loading of Cdc6 [5]; (3) recruitment of Cdt1 and MCM2-7 [26–29]; and (4) loading of Cdc45 and GINS [5,22].

The energy released from hydrolysis of ATP is used by Cdc6 and ORC for binding MCM2-7 rings to DNA [22,26]. Cdt1 is required for loading of MCM2-7 and this process is directly related with licensing of pre-RCs [26–29]. MCM2-7 is necessary for both initiation of DNA replication and processing of replication [30]. Only two MCM2-7 complexes are needed to replicate a DNA strand in two directions. However, 10 to 40 MCM2-7 rings are loaded to form the pre-RC and can work as a backup [5,6]. Cdc45 and GINS provide activation of MCM2-7 in the G1 to S-phase. Cdc45, MCM2-7 and GINS form a complex which plays the role of a helicase to trigger