Seminars in Cancer Biology 37-38 (2016) 3–15

Contents lists available at ScienceDirect

Seminars in Cancer Biology

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

Review

Exploring and exploiting the systemic effects of deregulated replication licensing

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ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form
10 December 2015
Accepted 15 December 2015
Available online 17 December 2015

Keywords:
Cdc6
Cdt1
Replication licensing factors
Replication stress
Cancer

ABSTRACT

Maintenance and accurate propagation of the genetic material are key features for physiological development and wellbeing. The replication licensing machinery is crucial for replication precision as it ensures that replication takes place once per cell cycle. Thus, the expression status of the components comprising the replication licensing apparatus is tightly regulated to avoid re-replication; a form of replication stress that leads to genomic instability, a hallmark of cancer. In the present review we discuss the mechanistic basis of replication licensing deregulation, which leads to systemic effects, exemplified by its role in carcinogenesis and a variety of genetic syndromes. In addition, new insights demonstrate that above a particular threshold, the replication licensing factor Cdc6 acts as global transcriptional regulator, outlining new lines of exploration. The role of the putative replication licensing factor ChlR1/*DDX11*, mutated in the Warsaw Breakage Syndrome, in cancer is also considered. Finally, future perspectives focused on the potential therapeutic advantage by targeting replication licensing factors, and particularly Cdc6, are discussed.

Abbreviations: RS, replication stress; DDR, DNA damage response; RLM, replication licensing machinery; pre-RC, pre-replication complex; CDK, cyclin-dependent kinase; ORC, origin recognition complex; Cdc6, cell division cycle 6; Cdt1, cell division cycle 10-dependent transcript 1; MCM, minichromosome maintenance; DDK, Dbf4-dependent kinase; pre-IC, pre-initiation complex; RLFs, replication licensing factors; pRb, phospho-retinoblastoma; UTR, untranslated region; miRs, micro-RNAs; MGS, Meier–Gorlin syndrome; DSBs, double-strand breaks; RPA, replication protein A; CFSs, common fragile sites; HBECs, human bronchial epithelial cells; ATM, ataxia telangiectasia mutated; ARF, alternative reading frame; NK, natural killer; PCNA, proliferating cell nuclear antigen; EMT, epithelial to mesenchymal transition; ESC/iPSC, embryonic stem cell/induced pluripotent stem cell; SDF-1, stromal derived factor-1; ICAM1, intercellular adhesion molecule-1; ChIP, chromatin immune-precipitation; LC–MS/MS, liquid chromatography–tandem mass spectrometry; AAA^+, ATPases associated with a variety of cellular activities; TK, tyrosine kinases; TKIs, tyrosine kinase inhibitors; TSS, transcription start site.

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http://dx.doi.org/10.1016/j.semcancer.2015.12.002
1044-579X/

1. Introduction: Replication stress as driving force for cancer development

Cancer is a complex and heterogeneous disease with many features still remaining obscure. Unraveling all facets of the "molecular machinery" that drives cancer is of vital importance in designing personalized therapeutic strategies. According to a cancer development model that we recently proposed [1], activated oncogenes disrupt normal proliferation, compromising the replication process by causing "replication stress" (RS). During RS, the timely and error-free completion of S-phase is mislaid, and the DNA damage response (DDR) signaling cascade is induced. DDR is activated from early stages of carcinogenesis (precancerous stage) setting in motion the anti-tumor barriers of apoptosis and senescence, eliminating incipient cancer cells. However, continuous accumulation of DNA damage, due to RS, overwhelms the cells' anti-tumor resistance, leading to selective loss of key checkpoint proteins, such as p53, paving the way for genomic instability and cancer progression [1–4].

A prediction of the above scenario is that the elements comprising the replication machinery must be accurately controlled to avoid RS. As the replication licensing machinery (RLM) is an essential constituent of the replication apparatus and responds to incoming oncogenic signals (Fig. 1a), its deregulation may be critical for the oncogene-induced replication stress model of cancer development. Here we will present and discuss evidence supporting this working hypothesis.

2. The replication licensing apparatus: Precise as a clock

DNA replication represents a central event in each cell cycle, being the prerequisite for accurate chromosome segregation that ensures that each daughter cell receives a complete and faithful copy of the organism's genome. The process is mediated by a dynamic protein complex called replisome [5], and is comprised of three steps, initiation, elongation and termination. The details of elongation and termination are out of the scope of the present work. However, we note that the mechanistic basis of initiation and elongation is quite similar between prokaryotes and eukaryotes, whereas the procedure of termination differs. Prokaryotes harbor circular DNA requiring the presence of the Tus (Terminus utilization substance) proteins for termination [6], while DNA in eukaryotes is linear and termination at chromosome ends is telomerase-dependent [7]. The accuracy of DNA duplication is overseen by regulatory protein networks, called checkpoints, which are activated when DNA lesions impede symmetrical bi-directional replication fork movement and single stranded (ss) DNA accumulates. Activated checkpoint proteins facilitate fork reactivation and DNA damage tolerance and are crucial for genome stability [8].

The initiation step of DNA replication starts at elements termed origins (being organized in replication units or replicons). Their number depends on the size of the genome, a parameter that varies between different species. For instance, bacteria and most archaea contain a sole circular chromosome and possess a single replication origin [9,10]. Conversely, in eukaryotes the amount of origins increases noticeably, ranging from 400 in yeasts to 30 000–50 000 in humans, as timely duplication of the larger linear chromosomes requires replication initiation from multiple sites [11,12]. Another feature worth mentioning is that the sequence of origins in yeasts is specific, whereas in metazoa is less defined [13] providing a flexibility for replication to initiate, under stressful conditions, from a broader spectrum of regions (activation of dormant origins). Within this context, it is estimated that only one out of five potential replication origins, within a replicon, is used in any cell during a given cell cycle [14].

Replication starts when pre-replicative complexes (pre-RCs) are assembled at the origins, through a process known as licensing [15] (Fig. 1b). This is a tightly regulated procedure driven by the periodic oscillations of cyclin-dependent kinase (CDK) activity, ensuring that the duplication of the genome occurs once per cell cycle. Replication licensing occurs from late mitosis throughout G1-phase, during which the hexameric ORC complex (origin recognition complex) serves as a platform to recruit Cdc6 (cell division cycle 6) and Cdt1 (cell division cycle 10-transcript 1), which in turn cooperate to load the hexameric MCM2-7 helicase complex (minichromosome maintenance) around double strand DNA in an inactive double head-to-head form. It is noteworthy that the MCMs loaded on the DNA are always in excess, compared to those used for normal origin firing, to act as a back- up pool in case of RS. The resulting multi-protein complex is termed pre-replication complex (pre-RC) [15] (Fig. 1b). There are indications that the assembly of the pre-RC is monitored by the so-called "licensing checkpoint" that reduces S-phase Cyclin E/CDK2 activity when licensing inhibition occurs and prevents S-phase entry until the functional flaw is fixed [16–19]. The "licensing checkpoint" seems to be a p53-dependent process, but many aspects require further investigation. Moreover, evidence from yeast suggests that a Cdc6-derived signal, the nature of which is still unknown, prevents entry into mitosis contributing to regulated coupling of S and M phases [20].

As the cell enters S-phase (G1/S transition) the activity of S-phase CDK and Cdc7–Dbf4 protein kinase (DDK) rises, driving the conversion of pre-RC into the pre-initiation complex (pre-IC). This involves assembly of additional factors, including Cdc45, Sld2 (sharing homology to human RECQ4), Sld3 (the yeast homolog of Treslin in human), Dpb11 (the yeast homolog of TopBP1 in human), the GINS complex (from the Japanese go-ichi-ni-san meaning 5-1-2-3, after the four related subunits of the complex Sld5, Psf1, Psf2 and Psf3 [21]), MCM10 and DNA polymerases α/ε [22]. The switch of the inactive double MCM2-7 complex (pre-RC) into two active CMG (Cdc45/MCM2–7/GINS) complexes (pre-IC) is called origin firing and is defined by origin unwinding allowing a symmetrical bidirectional replisome movement until the entire genome is duplicated [23] (Fig. 1b). At the same time, the replication licensing factors (RLFs) ORC, Cdc6 and Cdt1 are neutralized by a panel of E3-ubiquitin ligases and Geminin, a specific Cdt1 inhibitor, rendering the genome in an un-licensed state (Fig. 1c—details in legend). Removal of ORC, Cdc6 and Cdt1 from the replication scenery takes place when CDK and DDK activity rises following a reverse periodic expression pattern. Under physiological conditions the production and degradation phases of RLFs are temporally separated leading to oscillatory fluctuations in which the ascending slope of the curve (production phase) corresponds to G1 phase and the descending one to S/G2 and M phases of the cell cycle [24–28] (Suppl. Fig. 1). This periodicity ensures that origin firing happens once per cell cycle, protecting the genome from re-replication, a form of RS with deleterious consequences as we will discuss in the following sections [29].

Notably, some cells are developmentally programmed to exit their mitotic cell cycle and differentiate in non-proliferating polyploid ones. Unlike DNA re-replication, this procedure results in cells with DNA content bigger than 4n, but always in integral multiples of 4n (8n, 16n, 32n, etc), meaning that multiple S-phases take place in the absence of cytokinesis. Developmentally programmed polyploidy, which is common in ferns, plants, amphibians and fish, but rare in mammals, occurs by at least four different mechanisms [30] named as; (a) "acytokinetic mitosis", which is the failure to undergo cytokinesis after mitosis in syncytial blastoderm of flies and some hepatocytes, (b) "cell fusion", observed in skeletal muscle myoblasts for instance, (c) "endoreplication", the exit of mitosis in G2 phase and subsequent multiple S-phases, observed in