

DNA Replication: Old Game, New Players

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Received: March 21, 2015

Revised: April 16, 2015

Accepted: April 21, 2015

Published online: June 6, 2015

ABSTRACT

Metazoans utilize multiple origins of replication in order to replicate their vast genome faithfully and expeditiously during each cell cycle. However, lesser known are the salient features defining these origins and their mechanism of selection for replication process. Here, we provide burgeoning evidences which suggest that transcription factors indeed play a major role in facilitating licensing of origins for firing during the S phase.

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Key words: DNA replication; Chromatin; Replication origin; Transcription Factors

Sidhu K, Kumar V. DNA Replication: Old Game, New Players. *Journal of Biochemistry and Molecular Biology Research* 2015; 1(2): 32-35 Available from: URL: <http://www.ghrnet.org/index.php/jbmr/article/view/1127>

EDITORIAL

All living things reproduce by faithfully replicating their genetic material. The process of DNA replication involves unwinding double-stranded DNA to recruit DNA polymerase complexes at

predesignated positions in the genome called origins of replication (oris) followed by replication of both DNA strands in opposing directions in a continuous (5'-3') or discontinuous (3'-5', Okazaki fragments) manner finally terminating at a specific site or by merging of progressing replicating forks. As genome size and complexity rises from prokaryotes to eukaryotes, the steps regulating replication also increase. For example, in eukaryotes, initiation of DNA replication is a regulated two-step process to ensure a single round of replication during a cell cycle (Figure 1). The first step, origin licensing, involves the sequential assembly of pre-replicative complex (pre-RC) to the oris in the G1 phase comprising of a multi-subunit DNA binding origin recognition complex (ORC) that marks ori sites followed by licensing factors Cdc6, Cdt1 that prevent re-replication and finally the helicase MCM 2-7. The second step marks the transition to the pre-initiation complex (pre-IC) in the S phase which involves the loading of MCM10, Cdc45, GINS, Dbf4, Sld3 and Treslin proteins^[1]. *Escherichia coli* with a genome size of 4×10^6 bp replicates its genome in less than 30 minutes compared to human cells that have only ~8 hours of S phase to replicate a genome of 3×10^9 bp, three orders of magnitude larger^[2,3]. Metazoans also face the arduous task of unpacking their compact and supercoiled DNA to access their genetic information for replication or transcription. Such enormous discrepancy in their genome complexity and replication time has been circumvented by utilizing multiple oris in a tightly regulated and sequentially coordinated manner. Unlike bacteria and budding yeast, oris in higher eukaryotes are not well-defined sequences but designated by DNA structural elements and chromatin context which navigate the interaction between initiator proteins and ori. Oris are generally G-C rich sequences with origin G rich element (OGRE) that form G-quadruplexes essential for initiation of DNA replication^[4]. There are ~100,000 predicted oris in humans of which ~30,000-50,000 are activated during each cell cycle^[5]. The spare oris perhaps form part of a contingency plan to support the replication program during extreme growth conditions, stalling of a progressing replication fork, or specific physiological state such as embryonic development during which S phase lasts for a short period of time and a lot more oris are engaged than in somatic cells. Further,

an excess of oris may allow the replication to coordinate with the transcription program and safeguard against DNA damage^[1]. As permissive chromatin environment is a prerequisite for transcription as well as replication, not surprisingly, oris are often found in the vicinity of actively transcribed gene promoters^[6]. Further, active transcription may influence ori selection in two ways, first, promoting replication by providing a nucleosome free region to allow assembly of pre-RC proteins or it may inhibit assembly of replication factors. Gene density correlates with early replicating segments whereas repetitive elements associated with heterochromatin, telomeres and fragile sites fall under late replication in S phase^[7].

Early replication oris are found associated with multiple marks of active chromatin including DNaseI hypersensitivity, H3K4 trimethylation and even RNA polymerase II association. Transcription factors (TFs) may, therefore, form a major class of trans-acting factors that can exert control on ori licensing, selection and timing of firing as illustrated briefly in Figure 1. Viruses such as SV40 and bovine papilloma virus, traditionally used model systems to study eukaryotic replication, are known to employ viral trans-activator proteins to recruit TFs to their oris to alter the local chromatin structure^[8,9]. Recent spurt of studies in metazoans strongly support this hypothesis and have established an unequivocal transcription-independent role for c-Myc in control of DNA replication. Early expressed c-Myc directly interacts with the components of pre-RC and its overexpression leads to replicative stress by increasing the density of early firing oris^[10]. Swarnalatha *et al.*^[11] deciphered the molecular underpinnings of c-Myc on the regulation of human β -globin ori. c-Myc transiently associates with unmethylated E-box on lamin B2 ori and recruits

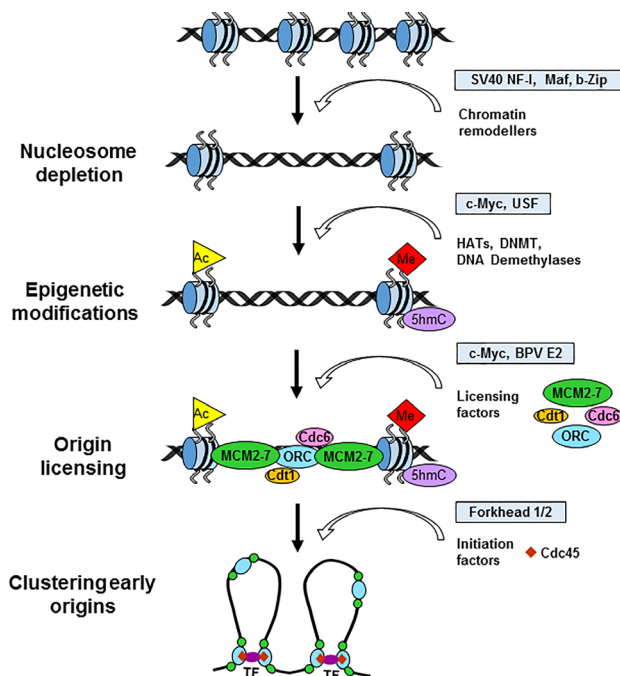


Figure 1 Integration of chromatin remodeling and DNA replication by TFs. Maf, bZip transcription factors and SV40 nuclear factor I (SV40 NF-I) promote ori licensing by recruiting chromatin remodelers and creating DNase I hypersensitive sites. c-Myc and USF orchestrate epigenetic modifications such as histone acetylation (Ac), histone methylation (Me) or DNA demethylation (5hmC) to promote early ori firing and influence replication timing, respectively. c-Myc and bovine papilloma virus E2 (BPV E2) proteins interact and facilitate the binding of pre-replication complex to license origins. Fork head transcription factors regulate ori timing by clustering early oris through direct interaction with ORC and recruitment of key initiation factor Cdc45.

chromatin modifying enzymes to initiate epigenetic modifications including hyperacetylation of histones which further enhanced the recruitment of licensing factors. Forkhead TFs are able to influence replication timing, independent of their transcription regulation role, by interacting with initiation factors Cdc45 and ORC to bring about clustering of early oris^[12]. Similarly, insertion of an USF binding sequence on either side of the β -globin replicator in an artificial construct is able to alter the replication timing^[13]. In *Xenopus* eggs, introduction of a TF and its cognate binding site template was able to form an ori with increased acetylation of histone lysines^[14]. Also, the duration of S phase in *Drosophila* and *Xenopus* extends during the late embryonic development due to firing of fewer oris as compared to firing of almost all oris until the mid-blastula stage^[15,16]. In *Drosophila*, the activated oris tend to lie in the vicinity of actively transcribed promoters^[17].

Whereas, early oris are associated with transcriptionally active regions and may be regulated by early expressing TFs such as c-Myc and Jun/Fos, the temporal positioning of late oris is considered a result of rate limiting levels of replication proteins which are cycled from early to late oris^[18]. Late ori associated ORC complexes understandably show correlation with silenced chromatin marks^[19]. In addition to TFs, a host of DNA modifying enzymes influence replication firing and timing. Histone deacetylase SIR2 acts a negative regulator of pre-RC formation while HBO1, a histone acetyltransferase and co-activator of Cdt1, remains essential for replication licensing^[20,21]. In addition to a nucleosome free central initiator region, histone modifications (H4K20 me1) and nucleosome remodelers (PR-Set7, SNF2H) also contribute to recruitment of pre-RC components at the ori^[22,23]. Interaction of ORC with chromatin and adjacent nucleosomes is a must for ori firing^[5]. Similarly DNA demethylase, TET2, is reported to actively demethylate DNA to maintain chromatin in active state^[24]. Replication proceeds in the nucleus in defined regions called replication foci which are organized from chromatin loops called replicons consisting of an average five licensed oris which further associate to form a cluster of replicons^[25]. A single ori is activated from each replicon during a cell cycle and all oris associated with a cluster of replicons are fired simultaneously showing a strong correlation between replication timing and spatial arrangement of oris^[5]. Recently, Rif-1 a telomere binding protein has been identified as a negative regulator of late oris while promoting early oris through its ability to regulate replication loop structures^[26].

Thus, replication and transcription can be seen as two different sports with similar rules being played on the same field with transcription having larger number of teams while replication having fewer but more adept teams. In order to economize the cell's efforts, replication often uses the groundwork laid down by transcriptional machinery delineated in Table 1. This crosstalk between the two

Table 1 Unique and shared features of transcription and replication processes.

	Events	Transcription	Replication
Unique	Requirements	Promoters (~10 kb)	Oris (~100 kb)
	Chromatin locus	TATA box, CAAT box, Enhancers	GC rich, CpG islands
	DNA elements	RNA polymerase I, II, III	DNA polymerase α , β , γ , δ , ϵ
	Polymerase	Transcription factors	ORC, replication factors
	Regulatory factors	Interphase	S phase
shared	Cell cycle phase	Histone H3 & H4 acetylation, Histone H3 & H4 monomethylation	
	Epigenetic signatures	Active chromatin, DNaseI hypersensitivity, nucleosome depletion	
	Chromatin region	Initiation, Elongation, Termination	
Process			

processes is also mediated by sharing of common players such as TFs, histone modifying enzymes or nucleosome remodeling complexes. However, a single conflict in their schedules has dire consequences on cell fate, leading to torsional stress, fork stalling and DNA double strand breaks which is otherwise prevented by stringent checkpoints in the cell cycle. The importance of faithful replication of genetic material is evident from the mostly lethal phenotype exhibited by mutants of the components of replication machinery. Nevertheless, some pathologies resulting from defects in DNA replication do exist such as Meier-Gorlin syndrome caused by mutations in the pre-RC components ORC1, ORC4, ORC6, Cdt1 and Cdc6 resulting in compromised DNA replication rate and manifestation of primordial dwarfism symptoms such as delayed development of kneecaps, ears and microcephaly^[27]. Besides, there is a well-established link between deregulation of replication control and cancer evident in some oncogenic viruses such as hepatitis B virus and human papilloma virus. Oncoproteins encoded by these viruses, such as HBx and E7, induce overexpression of replication factors PCNA, Cdt1, Cdc6 which correlates with re-replication or activation of incorrect oris leading to polyploidy, a characteristic of malignancy^[28,29]. The burgeoning evidences point towards regulation of replication by TFs and tempts us to speculate beyond the existing paradigm relating to DNA replication. The answers that have eluded us so far include criteria for selection of an ori during replication, mechanism distinguishing early vs. late oris and regulation of some excessive licensed oris in the S phase. The domain of late replicating heterochromatic regions and fragile sites remains largely untouched. Besides, the existence of distinct ori fingerprints during embryonic development, growth and differentiation or even viral infections is most likely a resultant of the coordination between ori firing and gene expression. Nevertheless, recent advances in molecular biology seems to suggest a profound synergy between these two independent and biologically distinct molecular processes apparently orchestrated by TFs.

CONFLICT OF INTERESTS

The Authors have no conflicts of interest to declare.

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