Review

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Replication timing regulation of eukaryotic replicons: Rif1 as a global regulator of replication timing

Satoshi Yamazaki, Motoshi Hayano, and Hisao Masai

Department of Genome Medicine, Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamkitazawa, Setagaya-ku, Tokyo 156-8506, Japan

Origins of DNA replication on eukaryotic genomes have been observed to fire during S phase in a coordinated manner. Studies in yeast indicate that origin firing is affected by several factors, including checkpoint regulators and chromatin modifiers. However, it is unclear what the mechanisms orchestrating this coordinated process are. Recent studies have identified factors that regulate the timing of origin activation, including Rif1 which plays crucial roles in the regulation of the replication timing program in yeast as well as in higher eukaryotes. In mammalian cells, Rif1 appears to regulate the structures of replication timing domains through its ability to organize chromatin loop structures. Regulation of chromatin architecture by Rif1 may be linked to other chromosome transactions including recombination, repair, or transcription. This review summarizes recent progress in the effort to elucidate the regulatory mechanisms of replication timing of eukaryotic replicons.

Origins of DNA replication

Marking and activation

DNA replication is initiated at defined loci known as replication origins. In prokaryotic replicons, replication is initiated from a single locus in most cases, and the sequence specificity of origin activation is very high; generally one base substitution within an origin leads to loss of initiation [1]. By contrast, replication initiates at multiple loci on eukaryotic genomes [2]. Although initiation occurs within specific loci on each genome at specific times during S phase, the sequence specificity can be significantly relaxed compared to bacteria. It appears that cells prepare many potential origins for possible uses during S phase, but only a subset of these are utilized during the normal course of S phase. Other origins may be used at later stages of S phase or may not be used at all (dormant origins [3]).

Preparation for DNA replication starts as early as late M or early G1 with assembly of pre-RCs (pre-replicative complexes) at selected locations on chromosomes. This step, also called origin licensing, proceeds through the stepwise assembly of Orc, Cdc6, and Cdt1–Mcm, resulting in the loading of Mcm onto the chromatin (helicase loading). The selected pre-RCs are activated by the actions of Cdc7 kinase and Cdk when cells enter S phase [2,4]. Once in S phase, origin licensing is strictly inhibited by layers of mechanisms that prevent rereplication [5]. These mechanisms are largely conserved from yeasts to human.

Regulation of origin firing during S phase

Once S phase is initiated, origins are fired (i.e., pre-RCs are activated by a series of phosphorylation events to generate active replication forks) in a coordinated and regulated manner, until the entire genome is replicated. There are origins every 50–150 kb; about 300 in budding yeast, about 1100 in fission yeast, and more than 20000 in human (Figure 1). Yeasts (budding and fission yeasts) have served as excellent model organisms for the study of regulation of origin firing due to their small genome sizes and ease of genetic manipulation. Thus, the precise locations of all the origins and the order in which they are fired have been established (Figure 1). In metazoans, firing of origins appears to be regulated on a domain basis [6,7] – that is, clusters of nearby origins present in the same domain may be spatially and temporally coregulated [8,9]. Recent genomics studies demonstrated the presence of cell type-specific 'replication domains' that define the segments of the chromosomes containing the coregulated origins (ranging in size from several hundred kb to 1 Mb) [10]. How these replication domains are generated and regulated in different cell types remains an open question [10,11]. Rif1, originally identified in budding yeast as a Rap1-interacting factor 1 involved in telomere length regulation [12], has recently come into the spotlight because of the unexpected discovery of its participation in origin regulation.

Here, we summarize various factors and conditions that regulate the origin firing/replication timing program in various eukaryotes (Table 1; for factor nomenclature in different species see Table S1 in the supplementary material online; also Box 1). We then discuss Rif1, which may be a global regulator of replication timing domains in metazoans.

Regulation of the origin firing/replication timing program

Possible mechanisms

For simplicity, let us assume that there are two classes of replication origins; early- and late-firing. There are two

Corresponding author: Masai, H. (masai-hs@igakuken.or.jp).

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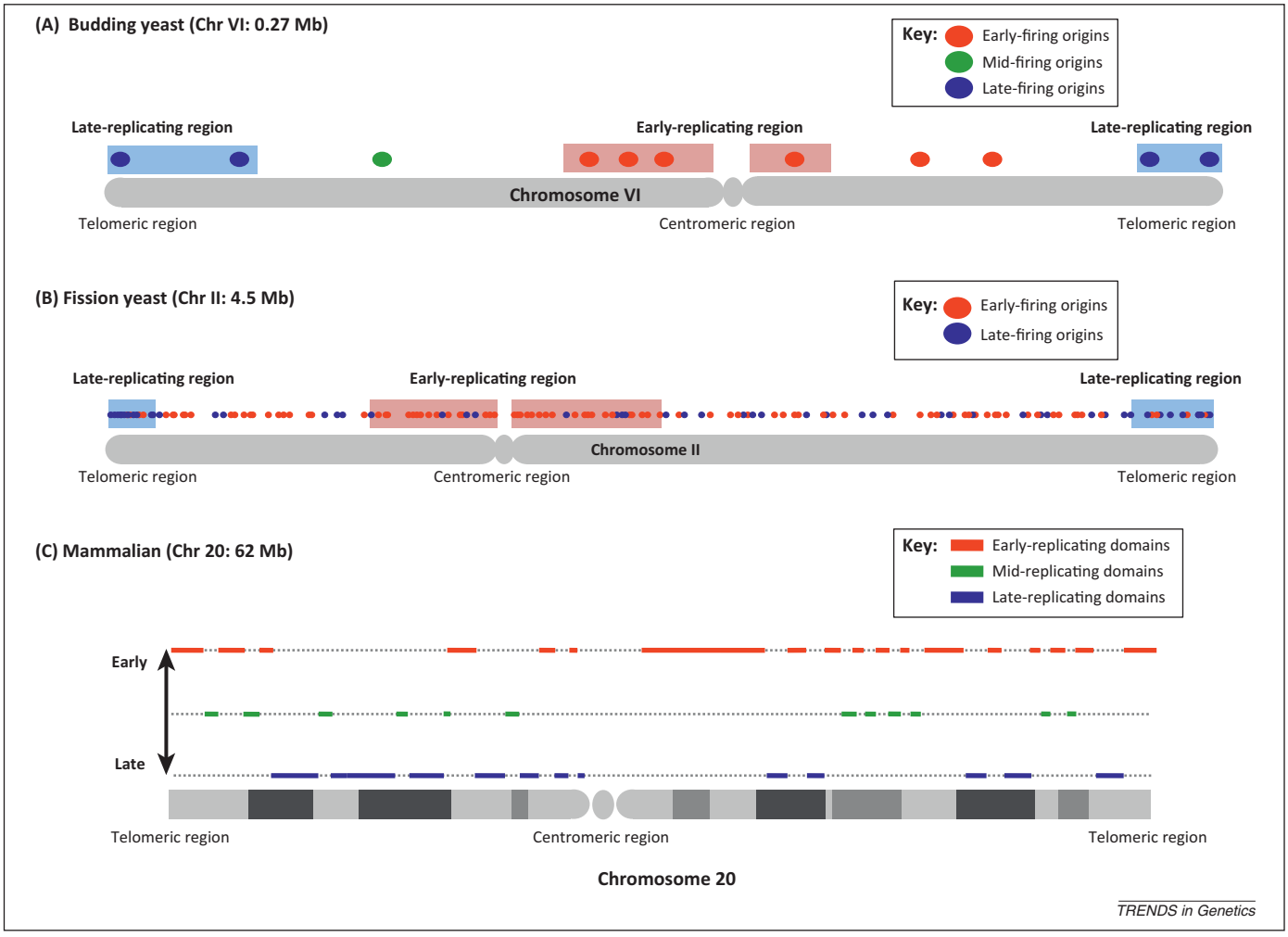

Figure 1. Replication programs of budding yeast, fission yeast, and human chromosomes. **(A)** The locations of replication origins on chromosome (Chr) VI (0.27 Mb) of budding yeast. Red, early-firing origins; green, mid-firing origins; blue, late-firing origins [80]. **(B)** The locations of replication origins on the chromosome II (4.5 Mb) of fission yeast. Red early-firing origins (origins firing in the presence of HU); blue late-firing or dormant origins [53]. **(C)** Replication timing profile of the human chromosome 20 (62 Mb) in K562 cells. Early-, mid-, and late-replicating domains are deduced from data in [11] and are shown by red, green, and blue horizontal bars, respectively. For simplicity, subtle timing differences within the early or late domains are not reflected in this drawing.

possible mechanisms by which the cell could distinguish between early- and late-firing origins. The first is to mark the early-firing origins. The chromatin structures in which the origins are embedded could dictate early-firing. Another way to mark early-firing origins could be through covalent modification of the pre-RC components or another factor(s) that selectively associates with specific origins before initiation (Figure 2A). The late origins might be differentially marked or marked later in S phase for initiation. The second possibility is to assume that all the origins are ready to fire at the onset of the S phase (the default state), but late origins are somehow actively prevented from firing by being sequestered from interacting with replication initiation factors (Figure 2B). At later S phase, this constraint is released and late origins are fired. Combinations of these two mechanisms are also certainly possible, and data supporting both mechanisms have recently been reported.

Temporal and spatial consideration

The replication timing program is established at a discrete point during the early G1 phase [13]. At this point, termed the TDP (timing decision point), major chromatin repositioning takes place, relocating chromatin to its respective subnuclear positions [14].

In mammals, it is well known that chromatin in the nuclear interior is replicated in early S phase whereas chromatin at the nuclear periphery is preferentially replicated during late S phase. For example, the late-replicating inactive X chromosome allele is associated with the nuclear periphery whereas the early-replicating active chromosome is located in the interior of the nuclei [15]. Thus, the spatial arrangement of chromosomes may play an important role in the origin firing program. In budding yeast, forced cell cycle-specific dissociation of telomeres from the origins demonstrated that the decision for late activation is made between mitosis and START (corresponding to the restriction point in mammalian cells) in the subsequent G1 phase. It is also interesting to note that, once established, late origin activation can be enforced even if telomeres are released from the target origin [16,17]. Furthermore, late origins associate with the nuclear envelope during G1 phase whereas early origins are randomly localized within the nucleus throughout the cell