Replication Timing

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The replication-timing program regulates when the replication origins are activated and when different parts of the genome are replicated during the S-phase. This program also participates to the genome organization.

Keywords: replication timing ; replication stress ; genomic instability ; cancers

1. Introduction

The process of DNA replication enables the faithful and complete duplication of the cell genome in order to transmit a copy in its entirety to daughter cells after cell division. This molecular process must be finely orchestrated and coordinated with other molecular processes in the nucleus to avoid defects in DNA replication, which can be deleterious to the cell. Although the process of DNA replication takes place during the S-phase of the cell cycle, the regulatory mechanisms, including checkpoints, occur during the G2-phase of the previous cell cycle and throughout the G1-phase [1]. These regulatory mechanisms not only determine the replication starting points throughout the genome, but also define when each part of the genome is replicated during the S-phase. This temporal program defines the replication timing (RT) and seems to play a major role in the organization of eukaryotic genomes. With improvements in high-throughput genomic approaches, several correlations have been demonstrated between replication timing and chromatin stability [2]. It is now well known that the deregulation of replication timing and changes in chromatin can lead to the emergence of cancer cells. However, a key question remains unanswered: What is the first event in the process that ultimately leads to genome instability?

2. Characteristics of the Replication-Timing Program

For many years, it has been well known that different parts of the metazoan genome are replicated at different time points during the S-phase. Genome replication does not occur randomly and is defined by a very precise sequence of events called the RT program [3]. Given the magnitude of this task, this program is not initiated the moment before it occurs, but at the beginning of the G1-phase with the timing decision point (TDP [1]). Notably, the program is implemented before the onset of the spatial program of replication with the origin decision point (ODP, which defines the position of replication origins along the genome [1]), perhaps indicating the preponderance of the temporal program compared to its spatial counterpart. Moreover, if the implementation of the RT program is disrupted, then the replication of the genome during the S-phase is completely disturbed, with consequences for the stability of the genome itself [1][4]. This sequential nature of replication is important for the cell because it enables coordination with the different factors involved such as nucleotides [5]. It is also crucial for its coordination with different DNA repair systems and its adaptive response to replication stress via translesional polymerases [6]. The temporal program of replication is therefore a coordinator that ensures the complete and perfect duplication of the genome in the allotted time. Another factor that suggests the importance of RT is its robustness. Indeed, RT variations between cells of the same tissue are infinitesimal within an organism and close to 1–2% for the same cell type between different individuals. However, RT can vary by about 50% during differentiation or between different cell types [7][8]. Thus, RT can be regarded as an epigenetic mark [9]. Based on when they are replicated, genome segments can be classified into two types: (i) regions that follow the same global replication timing, which are called CTRs (constant timing regions), and (ii) transition regions called TTRs (timing transition region), which are located between two CTRs. CTRs fall into two categories: (i) early CTRs are molecularly characterized by pronounced GC enrichment and a high gene density, and are globally associated with open chromatin and high transcriptional activity; and (ii) late CTRs are associated with AT-rich, gene-poor regions that are enriched with repeated elements, and they colocalize with closed chromatin structures at the periphery of the nuclear membrane [7].

To date, few molecular elements that regulate RT have been identified. Gene invalidation approaches (by knock-out (KO) or knock-in (KI)) have been used to study the effects of these elements, and it appears that the measured percentage of genome-wide RT changes never exceeds a rather low limit. For instance, depletions of some RT regulators such as RIF1,

SUV4-20H, and DNA polymerase θ (Polθ) have been shown to induce around 16%, 15%, and 5% of genome-wide RT changes, respectively [10][11][12]. This indicates that either compensatory mechanisms exist to maintain this crucial program or a large number of regulatory factors cannot be identified because the induced RT changes are too drastic for the cell to survive. This could explain why laboratories studying RT have never observed changes in values beyond a limited threshold and why the detection of RT molecular regulators remains challenging. Despite these difficulties, several *cis* and *trans* classes of RT regulatory factors have been described. First, a strong origin associated with an active promoter in a given region enables its earlier replication in the S-phase [13]. Then, the ERCEs (early replication control elements) associated with certain open epigenetic marks and some other factors allow for the early replication of the replication domains connected to them [14]. Knowing the strong correlation between RT and 3D genome interactions, we can reasonably question whether this effect may be directly caused solely by the destabilization of these interactions. Epigenetic mechanisms may also be associated with RT regulation, as demonstrated by several findings such as the inactivation of the X chromosome, whereby replication of one allele occurs early and that of the other occurs late in the S-phase in female cells [15]. In addition, the H4K20Me3 mark is associated with the control of late-origin activation [11]. These examples are not exhaustive (reviewed in [16]). Finally, the category of factors acting in *trans* also regulates origin activation at a specific time point during the S-phase. The best known thus far is RIF1, whose role in the control of RT is conserved from yeast to humans [10][17][18][19][20]. Pol θ also plays a role in the domains replicated early in the S-phase [12]. It is possible that the helicase domain of this translesional polymerase destabilizes G-quadruplexes (G4), an essential element for the functionality of replication origins [21]. Finally, in yeast, the dimerization of the transcription factors FKH1 and 2 allows them to cluster several early origins for mutual activation [22].

In the last few years, chromatin conformation capture approaches and RT profile studies have shown that there is a strong link between RT, 3D interactions, and the organization of the genome [16][23]. CTRs consist of one or several replication domains (RDs), which replicate at similar times. They are separated into two groups corresponding to the A compartment, which is the active form and localizes with early replication domains, and the B compartment, which represents the inactive form and is associated with late replication domains. A and B compartments likely represent euchromatin and heterochromatin compartments, respectively [16][23][24][25]. RDs are composed of one or several topologically associated domains (TADs). Some of them are located near compartment boundaries, corresponding to RT switching [23][26]. Thus, TADs and RDs, which seem to be intimately linked, can be considered arbitrary units of the organization and structure of a genome [23]. This link appears to be very strong: for example, during cellular differentiation, several studies have shown that the interactions of TADs co-evolve with RT in parallel with associations with the transcriptional program [16][23][26]. Other evidence clearly demonstrates this intimacy between the 3D genome organization and RT [27]. Moreover, there is an interesting parallel between the TDP and the organization of chromatin domains. Interactions between different domains that disappear during mitosis have been observed to re-establish during the same TDP time window [27]. Although these observations do not clarify whether the 3D organization of the genome controls the establishment of RT or vice versa, the same study showed that inter-TAD interactions were not necessary for the establishment of the RT program. Therefore, these two processes are regulated in parallel by common molecular elements such as RIF1 [20].

The organization and structure of the genome are known to be crucial for the identity and fate of the cell. This is similarly the case for RT, as studies using abortive approaches of different factors that regulate it and treatments with different drugs have never shown more than a limited percentage of RT changes [10][11][12][28][29][30]. One possible explanation is that a degree of change beyond this limit is so high that it is catastrophic for the cell, as it must significantly disturb other molecular processes in the nucleus. This hypothesis is supported by studies that have detected a correlation between the disruption of RT and several diseases such as Fragile X syndrome and certain cancers [31][32]. Therefore, RT is now considered one of the “primary functions” of the nucleus. This program is so important that it seems to have been selected during evolution. Studies in yeast of the genus *Saccharomyces* have shown that the RT program is fairly well conserved between different species [33]. Furthermore, a very thorough study was carried out on 10 species of the genus *Lachancea*, covering the continuous evolution of their genomes [34]. The results suggest that the RT program evolves at the same rate as protein-coding sequences and the genome structure [34]. However, the evolution of these coding sequences does not dictate the evolution of the RT program. Indeed, for this genus, the disappearance of some origins of replication and the appearance of new ones have modified RT in different species. However, why and how these origins appear and disappear during evolution is still unknown. Two studies that compared the RT program in human and mouse cells showed very strong evolutionary conservation [24][25]. As previously indicated, there is a strong correlation between the replication timing of different domains and the GC content. However, these studies show that the evolution of RT is independent of the evolution of the GC content. Moreover, the RT is conserved despite different chromosomal rearrangements between these two mammalian genomes. Thus, there is a mechanism selected during evolution that has resulted in the conservation of RT between different mammalian species. Once again, these results show the importance of RT and its key role in structuring the genome.

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