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Review

Progress in understanding DNA replication control

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ABSTRACT

Completion of genome duplication during the S-phase of the cell cycle is crucial for the maintenance of genomic integrity. In eukaryotes, chromosomal DNA replication is accomplished by the activity of multiple origins of DNA replication scattered across the genome. Origin specification, selection and activity as well as the availability of replication factors and the regulation of DNA replication licensing, have unique and common features among eukaryotes. Although the initial studies on the semiconservative nature of chromosome duplication were carried out in the mid 1950s in *Vicia faba*, since then plant DNA replication studies have been scarce. However, they have received an unprecedented drive in the last decade after the completion of sequencing the *Arabidopsis thaliana* genome, and more recently of other plant genomes. In particular, the past year has witnessed major advances with the use of genomic approaches to study chromosomal replication timing, DNA replication origins and licensing control mechanisms. In this minireview article we discuss these recent discoveries in plants in the context of what is known at the genomic level in other eukaryotes. These studies constitute the basis for addressing in the future key questions about replication origin specification and function that will be of relevance not only for plants but also for the rest of multicellular organisms.

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1. Introduction

The whole series of unidirectional processes that occur in a highly regulated manner, spanning from the birth of a cell until it divides, defines the cell division cycle. Among the several events that occur during the cell cycle one that is particularly crucial is DNA replication during S-phase. This process results in the duplication of the entire genome, which needs to be faithful to avoid problems in gene expression, chromatid cohesion and maintenance of epigenetic features.

Considering the specific features of plant development, genome duplication and maintenance of genome integrity become of special relevance. In animals, organs develop at an embryonic stage but, contrary to this determinate situation, organogenesis in plants is a postembryonic process that occurs continuously during the entire life of the organism, contributing to generate an indeterminate plant body. The continuous growth of plants requires new cells to be supplied from divisions of stem cells localized in the main meristems [1], a process that may last for hundreds of years in certain cases. Furthermore, organogenesis depends on the balance between pools of proliferating cells, cells that exit the cell cycle and cells undergoing endoreplication, the process where nuclear ploidy increases as a consequence of repeated rounds of full genome duplication in the absence of cell division [2–4].

The basic strategy for copying the genome accurately is largely conserved across all kingdoms [5]. However, the mechanisms regulating the various steps involved, particularly initiation of DNA

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replication, vary in different organisms and are still poorly understood [6,7]. Most of our current understanding of DNA replication control in eukaryotes comes from studies in budding (*Saccharomyces cerevisiae*) and fission (*Schizosaccharomyces pombe*) yeasts, mammalian cells in culture, and to a lesser extent, in the frog *Xenopus laevis* and the fruitfly *Drosophila melanogaster*. Comparatively, work in plants has received much less attention.

The increase in genome size and the existence of different cell types with specific and highly regulated transcriptional programs impose a number of restrictions on genome duplication. The first of these is that the activity of a single origin of DNA replication, as it occurs in bacteria, is insufficient to duplicate the entire genome in the relatively short period of a few hours that span the S-phase. Chromosomal DNA replication in eukaryotes occurs from multiple origins distributed across the genome. Replication units (replicons) cover the entire genome and they replicate at different times throughout the S-phase. That is, replication timing is one of the distinctive features of different genomic regions in terms of DNA replication. A key question is to identify the sites where DNA replication starts, that is, the origins of DNA replication and the molecular characteristics that define them. Finally, it is estimated that ~50,000 active origins may exist in a mammalian cell [8], but not all of them fire in each cell cycle. Therefore, it is necessary to define the mechanisms leading to different origin usage. The "when", "where", "which" and "how" constitute the basic questions in the DNA replication field [9].

2. Replication timing

Since the pioneering work demonstrating the semiconservative nature of chromosomal DNA replication [10], very few reports have appeared trying to answer these questions in plants, for which only the topic of replication timing and the overall replicon organization have received the interest of the research community. Early studies using DNA fiber autoradiography revealed that the *Arabidopsis thaliana* genome was organized in two large replicon families (Fig. 1) [11]. The apparent replication order observed strongly suggested that a temporal control of DNA replication occurs. Similar studies in *Pisum sativum* [12] and in mammalian cells [13] supported a generalization of the concept that temporal regulation of replicon firing occurs during S-phase and that the genome is basically organized in early and late replication families. This has been fully confirmed in plants [14,15] using more sophisticated approaches based on the use of fluorescent, instead of radioactive, labeling of newly synthesized DNA and chromatin spread, the so-called DNA combing technique [16].

Genomic approaches, facilitated by the availability of the full genome sequence of *A. thaliana*, have been instrumental in recent studies that have significantly upgraded our knowledge on DNA replication in plants. In one of these studies, the DNA replication temporal profile of *Arabidopsis* chromosome 4 was assessed [17], combining cell sorting of early, mid, and late S phase nuclei of 5-bromodeoxyuridine-labeled cells in culture with tiling microarray hybridization. The data obtained clearly indicated a biphasic mode of replication, with the majority of the euchromatin replicating in early/mid S phase and the heterochromatin and remaining euchromatin replicating late (Fig. 1). The early/mid replicating domains are characterized as regions of open chromatin state enriched in actively transcribed genes and in acetylated histone H3 at lysine 56 (H3K56ac), and depleted of 5-methyl-cytosine (5mC) and dimethylated histone H3 at lysine 9 (H3K9me2) (Table 1). Conversely, the late replicating domains are enriched in transposon elements and displayed repressive epigenetic marks characteristic of heterochromatin, i.e. H3K9me2 and 5mC. These observations are in strong agreement with the existing reports for animal systems, such as *Drosophila* [18] and mammals [19]. It is well established that the transcriptional and chromatin status of a cell influences the DNA replication program in terms of replication origin usage [20,21]. DNA replication timing has been studied in plants using DNA fiber autoradiography (see above), but the availability of new high-throughput genomic tools, such us chromatin immunoprecipitation followed by full-genome microarray hybridization and/or next generation DNA sequencing, should be very helpful in future studies. Furthermore, the postembryonic pattern of organogenesis makes plants excellent models to assess the spatial and temporal dynamics of DNA replication at different developmental stages.

One of the newest observations on replication timing control was the implication of H3K56ac in DNA replication (Table 1). H3K56ac has a fundamental role in nucleosome reassembly following DNA replication and repair [22–24] and also in replication-independent histone turnover at promoter regions [25]. At a global level, and independently of its role in gene transcription, the H3K56ac epigenetic mark is highly associated with early replication and with initiation zones of late replicating regions in *Arabidopsis*. Preliminary observations suggest that H3K56ac level could peak at the replicon midpoints, but no direct link with origin activation has been provided to date. It remains to be addressed whether this histone modification involved in DNA transcription, replication, and repair is merely a signal of newly synthesized H3 needed for replication- or repair-coupled nucleosome incorporation and for nucleosome exchange at regulatory regions, or is indeed also part of the molecular signature of DNA replication origins.

3. Replication origins

A major issue in the field is defining where DNA replication starts in each replicon and the molecular nature of origins. The reason is the large diversity of origin features in various model systems, making it very difficult to establish the basic rules governing origin activity. The situation in *S. cerevisiae*, where origins are strictly dependent on the presence of the autonomously replicating consensus sequences, seems to be a unique situation in eukaryotes [6]. In *S. pombe*, strict sequence dependence does not exist, although origins are characterized by A+T richness [26,27], likely due to the unique presence of AT-hooks in the *S. pombe* Orc4p subunit [28].

In multicellular eukaryotes, the lack of sequence specificity and the increase in genome size impose additional layers of complexity to origin specification and function. Genomic analyses of DNA replication have been carried out in *Drosophila* [18,29] and mammalian cells [30,31]. However, the identification of DNA replication origins in plants has been elusive. A first approach consisted of scanning the ribosomal DNA region of cultured root meristem pea cells to find out whether the non-transcribed spacer contained an origin, as it occurs in other eukaryotes [5]. Indeed, a replication origin was identified within a 1.5 kb of the non-transcribed spacer, as well as a replication termination site [32,33]. These data were further confirmed by 2D-gel electrophoresis [34] whereas sequencing of the region revealed the presence of a high AT-rich domain as well as, interestingly, of several good matches to the yeast ARS consensus sequence [35].

The activation of DNA replication origins depends on the assembly of pre-replication complexes (Fig. 1) [36]. The six-subunit origin recognition complex (ORC) is the first to bind to potential replication origins and then other proteins including CDC6, CDT1 and the MCM complex are sequentially recruited [36]. All pre-replication components have been also identified in plants [37–48]. Unlike other organisms, the *Arabidopsis* genome contains two paralogs of each ORC1, CDC6 and CDT1 genes, which have distinct expression patterns, suggesting different roles in development [42,44]. *Ara-*