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Visions & Reflections

To open or not to open that is the question: the replication of an entire chromosome

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Initiation of chromosomal replication plays a crucial role in cell growth and differentiation. In the normal cell cycle, chromosomes are replicated only once in S phase in response to various growth stimuli. In differentiating cells, chromosomes either undergo replication unique to each differentiated cell cycle, as in the meiotic cell cycle [1], or become dormant. In all cases, the mode of chromosomal replication is regulated at the initiation step. Furthermore, the regulation of initiation plays an important role in cell surveillance of S phase progression. Understanding the molecular mechanism of initiation at replication origins is therefore essential to understanding the progress and completion of whole-chromosome replication during the S phase in each cell cycle.

Cis-acting components involved in initiation and its regulation

Initiation of replication is unwinding of double-stranded DNA followed by assembly of the replication machinery. The initial step of initiation (unwinding) is performed by interaction between initiator(s) and the replicator, as proposed in the 1963 replicon hypothesis [2]. Specific protein-DNA complexes are formed and then activated in order to assemble the replication machinery at the origin of replication [3]. Components involved in these initiation processes consist of replicators, initiators constituting the core of the pre-replication complex (pre-RC), and regulators that modulate activity of all or individual origins. We were surprised to find that eubacteria share the same initiator, DnaA protein, and replicator containing various numbers of the DnaA-box, a 9-bp DnaA-binding sequence [4]. The number of DnaA-box-containing regions and the number of DnaA-boxes in each region varies widely among replicators. This means that the DnaA-box functions as a modulator that determines the characteristics of the bacterial origin of replication. In contrast, replicators of *Saccharomyces cerevisiae* (usually called ARS) are small and consist of a single sequence-specific binding site, ACS. Variations in the ACS sequence may result in changes in affinity of the ARS with the initiator, ORC. In contrast, ACS-like sequences seem to function as modulators in *Schizosaccharomyces pombe,* where many regions, each containing a multiple number of ACS-like sequences, are present in larger replicators of about 1 kb [5]. Studies on the two different types of ARS in the two yeast species may shed light on the more complex structures of metazoan replicators that shares similar structural elements in the replicator and similar initiators with yeast. At least 23 specific origins have now been mapped in flies, frogs, and mammals [6, 7]. Evidence accumulating is also to suggest that metazoan replicators also contain a multiple number of yeast ACS-like modulators. The replication origin of Archaea is not well-characterized, although a bacterial-type single origin has been suggested recently for one of the Archaea sub-families [8].

Trans-acting components involved in initiation and its regulation

Components that construct the core of the pre-RC are common from bacteria to Metazoa. The essential process of initiation consists of, (i) recognition by and binding of initiator(s) to the replicator, (ii) association of helicase

with the aid of a recruiter, and (iii) association of a protein that recruits the replication machinery. Initiators, DnaA in bacterial, Cdc6 in Archaea, and Orc1 in Eukarya (the largest subunit of the ORC), are homologs belonging to a large ATPase family [9]. At each step of pre-RC formation, CDK kinase(s) is required in Eukarya through which initiation is linked to the progression of the cell cycle and is secured for firing once in a cell cycle. Regulation of pre-RC formation varies in the two bacteria, *Escherichia coli* and *Bacillus subtilis*. DnaA function is repressed by SeqA and DNA polymerase in *E. coli* [10], while in *B. subtilis,* it is modulated by DnaB and DnaD, whose functions have yet to be identified [11]. As described before, the number and location of DnaA-boxes modulate drastically the structure of the pre-RC of the two bacteria. The pre-RC of *E. coli* is similar to the eukarotic nucleosome consisting of about 25 DnaAs and 250 bp DNA, while that of *B. subtilis* forms a loop structure that spans more than 2 kb [11]. Comparative studies of the regulation of initiation of these two bacterial origins may help us to understand the complexity of Eukarya origins that vary in size and the number of ORCs bound to replicators. Pre-RC formation is a pre-requisite step for initiation in Eukarya and it must be activated once in a cell cycle at the specific time allotted for each origin. Components for activation of regulation of the pre-RC may be classified into two, one being required ubiquitously for all origins and the other specifically for individual origins. Cdc7/Dbf4 is the key kinase required for activation of all pre-RCs [12, 13]. Cdc7/Dbf4 itself is regulated by various kinases through which the timing of initiation of individual origins may be determined. Recently, Rad53, a check-point regulatory kinase that determines the timing and efficiency of late-firing origins [13–15], was found to phosphorylate Cdc7/Dbf4 [16]. Many proteins have now been discovered that are involved in the regulation of individual origins. Many of these are involved in remodeling of chromatin during the G2-M phase that may determine the fate of individual origins in the subsequent cell cycle. Geminin (*Xenopus*) is involved in MCM loading [17], and E2F (*Drosophila*) [18], DUP (*Drosophila*) [19], and a histone acetyltrasferase, Sas2p (yeast) [20]/HBO1 (Metazoa) [21], seem to be involved in ORC loading. A remodeling factor SWI/SNF (yeast) [22] may be involved in the regulation of a specific origin. Recently, SIR3, a silent chromatin component, was found to be involved in determining the timing of the telomere-proximal ARS in S. cerevisiae [23].

Replication of a single chromosome as a model of initiation of multiple origins

With these components in hand, we may now reconstruct the initiation of replication of a single chromosome. Chromosome VI of *S. cerevisiae* is the only chromosome for which all the replication origins have been identified, and their timing and efficiency of firing determined [24, 25]. This small chromosome of 280 kb is composed of two replication domains, one being the arm left of the centromere containing five origins and the other the right arm containing four origins. The replication of the left arm starts from the origin nearest the centromere and proceeds sequentially toward the left telomere. Right-arm replication is initiated from the origin near the center of the arm and proceeds bidirectionally toward the centromere and the right telomere. The nine origins on the chromosome are classified as early, middle, and late replicating, depending on when they are fired during the cell cycle. The early origins and some middle origins are used efficiently once every cell cycle, while the others are less efficient and are used only once in every two to ten cell cycles. Except for the first initiating origins in each arm, initiation timing of the other origins roughly coincides with the time when replication forks from the preceding origins approach the adjacent origins, strongly suggesting that succeeding origins are activated by incoming fork movement. Consequently, the overall replication of each domain takes place sequentially (see fig. 1 for the right half of the chromsome).

Firing power and timing devices

How is firing of origins regulated and what are the roles of middle and late origins that are apparently unnecessary to carry out sequential replication? As is known for most yeast origins, the primary structure of the nine origins is similar and their ARS activity in a plasmid construct is equally efficient [26]. Therefore, the difference must reside in higher-order chromatin structures that include a larger DNA area near the ARS. A genome-wide analysis of transcription revealed that all nine origin regions of chromosome VI are characteristically of low or silent in transcription during exponential growth [27]. In particular, the silent region spans about 30 kb around the first replicating origin. Therefore, I suggest that the first replicating (or early) origin can form a specific chromatin structure that binds to a nuclear structure (a putative attachment site) to become a core of replication foci. Formation of initiation foci may facilitate the formation of the pre-RC around the pre-existing ORC (or reconstituted ORC) by concentrating Cdc6, MCM, and CDK kinases around the origins. Transportation of Cdc6 into the nucleus and its concentration have been suggested to be rate-limiting steps of replication initiation. Initiation foci then turn into replication foci through activation by CDK and Cdc7/Dbf4 kinases and assembly of replication machinery aided by Cdc45. When replication proceeds bidirectionally, replicated DNA may be pulled out of the

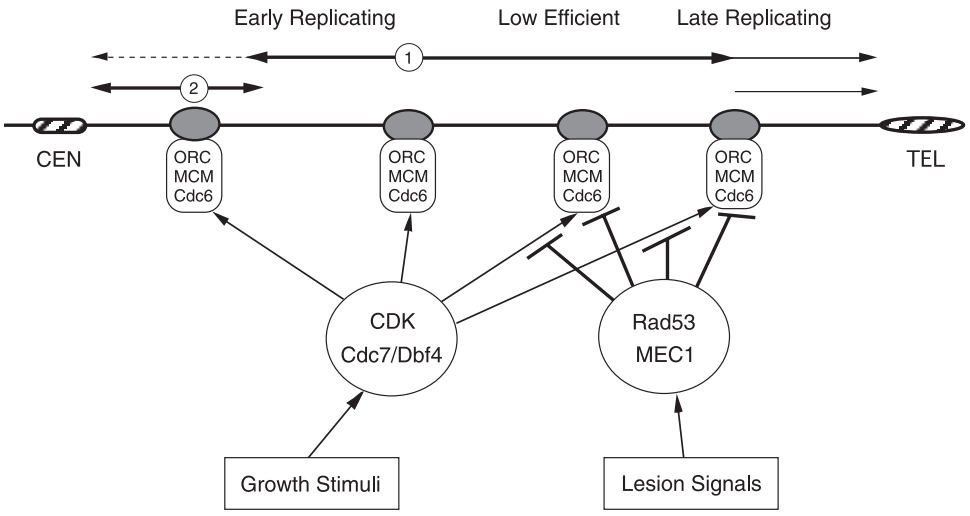

Figure 1. Replication of the right half of chromosome VI of *Saccharomyces cerevisae.* The mode of replication and its regulation through the initiation complex are shown schematically. The order of firing of two early origins are numbered as 1 and 2. The right arm from the centromere (CEN) to the telomere (TEL) with five origin-ORC/MCM/Cde6 complexes is illustrated. Above the chromosome: direction and efficiency of replication from early and late origins are shown by arrows of different thickness (100%, 70%, and 30%) and a dotted line (< 10%). Initiation from the low-efficiency origin is less than 5%. Below the chromosome: activation by CDK kinase and Cdc7/Dbf4 and repression by MEC1/Rad53 (see text for more details).

foci as succeeding origins approach the attachment site and form new initiation foci. If approaching origins enter into the preceding replication foci before forming new initiation-foci, the origin becomes dormant and is replicated passively [28]. If this model is correct, there must be mechanisms by which the formation of initiation foci of middle or late origins is prevented. A search for regulatory factors specific for middle or late origins led us to discover that the Mec1/Rad53 signal pathway determines the time of firing and efficiency of late origins [14]. Rad53 kinase may either modify the MCM-ORC complex directly to make it incompetent for activation by kinases or indirectly through inactivation of Cdc7/Dbf4 kinase (see fig. 1). The discovery of regulation by Rad53 provides an answer to the second question, namely the role of middle or late origins. As described before, replication domains of some 100–150 kb are replicated sequentially, implying that they can be easily replicated by initiation from a single origin during S phase. A question then arises as to why there are as many as one origin every 20–40 kb. The answer is that the late firing-origins play a role in check-point regulation for S phase progression in response to the Mec1/Rad53 signal. The last part of the chromosome to be replicated is the telomere region. There seem to be ARS-like elements associated with the telomere and its adjacent regions, but little is known about how this region is replicated, actively from origins within or passively from outside. Replication of the telomere is vital for completion of chromosome replication and cell surveillance for S phase progression. In recent findings of a role for cohesin [29] in replication and SIR3 for telomere-proximal origins [23] may be related to this last step of chromosome replication.

From yeast to metazoan chromosomes

The application of this model system to large chromosomes of two yeast species and Metazoa requires considerations on how chromosomes are organized in terms of replication domains and replicon clusters [30]. I propose that the smallest domain of replication is about 100–150 kb and composed of multiple replicons that are replicated sequentially from early to late origins. DNA integrity of each domian is checked for each domain by a Mec1/Rad53 negative control on middle/late origins of each domain. In larger chromosomes that contain many domains, the timing of initiation of each domain may vary within the cell cycle. In other words, the early origin in each domain may be fired at various times during the cell cycle. So-called late origins of *S. cerevisiae*, e.g., ARS501 [31], may be early (master) origins of the telomeric domain of chromosome V. We must therefore consider two types of timing device for chromosomal replication. One determines the timing of initiation of domain replication (firing of the first, master origin of each domain), while the second determines the timing of origins within domains. Analysis of chromosome VI of *S. cerevisiae* has revealed how the timing of origins within one domain is regulated. The next problem is to analyze replication of larger chromosomes of more than 1 Mb in *S. cerevisiae* to make a bridge to *S. pombe* and metazoan chromosomes.

Conclusion

Cell growth and division are sequential and cyclic processes, and cells must determine whether or not and when to start the vital events or this process. Initiation of replication is one such vital event, and to initiate (to open) or not to initiate (not to open) is equally important. It follows, therefore that cells are equipped with fine molecular mechanisms to control the dual functions of the replication origins. The single origin-initiator complex performs the dual functions in bacterial chromosomes, while each of the two functions is allotted respectively, to early and late origins of chromosomes of yeast and, possibly, of higher organisms.

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