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Initiation of DNA Replication: a New Hint from *Archaea*

In this issue, Robinson and coworkers provide new insights into the mechanisms of initiation of chromosome replication in *Archea*. This and other studies, focused on model organisms, will certainly help to understand how the replication process has evolved in Eukaryotes.

Chromosome replication is a complex process that requires the coordinated action of several factors and the harmonization with cell cycle progression, checkpoints, DNA repair, and DNA recombination, which contribute to control of and assist the correct duplication of the genome. As often happens for important biological processes, the initial step is usually the critical one. And that is certainly the case for chromosome replication as the cell has to decide when, where, and how to assemble the machinery that will replicate the genome.

In the original replicon model proposed 40 years ago, Jacob and colleagues postulated the existence of two important elements that would be required for replication initiation, the replicator and the initiator (Jacob, 1963). The replicator is where replication initiates and the initiator is the positive *trans*-acting factor implicated in recognizing a specific sequence in the genome that overlaps with the replicator. We later learned that the timing of replication is also crucial as the genome can be duplicated once and only once per cell cycle and initiation of DNA synthesis has to be highly coordinated with cell growth.

It is now clear that the eukaryotic chromosome contains multiple replicators. Replication starts at many sites, called origins of replication, which are distributed throughout the chromosomes (Newlon, 1997). In the yeast *Saccharomyces cerevisiae* perhaps the best characterized eukaryotic organism at the level of chromosome replication, 16 chromosomes are replicated using 332 origins (Raghuraman et al., 2001). Remarkably, these origins are activated continuously throughout S phase. However, in yeast cells, the number of potential origins is certainly higher as many origins are normally not fired and remain in a dormant state. The frequency of initiation at replication origins can be influenced by the local chromatin structure, by the distance and timing of activation of neighboring origins and by the activation of specialized surveillance mechanisms called checkpoints (Bell, 2002; Donaldson and Blow, 2001). However, how origins are chosen for activation and how timing is determined remain open questions.

The situation is clearly more complex in multicellular organisms where the average frequency of initiation events seems to be regulated even during development. In *Drosophila* and *Xenopus* at early embryonic stages, S phase is very short and replication initiates randomly at sites that are spaced close to each other, however, later at the midblastula transition, specific initiation sites are selected. In human somatic cells, replication initiates from 10,000–100,000 origins but, again, primary and secondary origins exist, where some origins are fired and others are not, and this seems to be influenced by the nucleotide pools and by interorigin spacing (Anglana et al., 2003).

Extensive work from different laboratories has contributed to the identification of the eukaryotic initiator, the origin recognition complex called ORC (Bell, 2002; Diffley, 2001). ORC is a six polypeptide complex essential for initiation of replication and appears to represent the analog of *E. coli* DnaA. ORC remains bound to origins during most or all the cell cycle and likely represents a landing pad for recruiting other replication proteins involved in the initial step of DNA synthesis. ORC is a DNA binding protein, but it is unlikely that the sequence specificity of ORC is sufficient for localization to origin sequences. Another protein, Cdc6, which shares some similarity with Orc1, seems to mediate the specificity of ORC association with DNA. Cdc6 is a highly regulated protein that cooperates with ORC to load other initiation factors.

The mechanisms by which ORC and Cdc6 are localized to origins of replication remains incompletely understood and further work will be required to fully elucidate the details of the initiation reaction.

An important contribution to the understanding of the molecular events occurring at the origins of replication will certainly come from in vitro systems reconstructed from purified components. Today, nearly every cellular process, including DNA replication, DNA recombination, and even membrane vesicle transport are studied using in vitro reconstituted reactions. However, the large number of factors involved in these cellular processes makes such experimental approaches difficult. From this arose the need for simple model systems, possibly representing the core version of higher eukaryotic organisms, which could help to recapitulate complex cellular pathways.

Steve D. Bell's lab went back in evolution to study *Archaea* (Robinson et al., 2004 [this issue of *Cell*]). *Archaea* were for a long time considered to be close to *Bacteria*, but taxonomic analysis based on genome sequencing has firmly established that, in fact, they constitute a distinct domain of life, representing an interesting mixture of bacterial, eukaryotic, and unique features. Hence, it is not entirely a surprise that archaeal proteins involved in DNA replication are more similar in sequence to those of *Eukarya* than to those of *Bacteria*. Since *Archaea* possess a minimal replication apparatus, it is now evident that they are one of the best model organisms for studying DNA replication and that will certainly provide relevant details on what is happening in eukaryotes.

Robinson and colleagues (2004 [this issue of *Cell*]) have found that chromosomal duplication in *Sulfolobus solfataricus* is initiated from two distinct origins of replication. This is, so far, the first documented example in *Archaea* of a two-replicator mechanism of initiation. In *Sulfolobus*, there are three initiator factors that resemble

the eukaryotic Cdc6 and Orc1 proteins and, therefore, they have been named Cdc6 1-3. Remarkably, the two origins of replication map very close to the Cdc6-1 and Cdc6-3 loci, although, this genomic colocalization of replicators and initiator genes is not unusual in *Archea* (Robinson et al., 2004 [this issue of *Cell*] and references therein). Intriguingly, they also found that origin identity seems to be mediated by a subset of Cdc6 proteins that exhibit selective specificity for binding to origin sequences. The three Cdc6/Orc1-like proteins show a dramatic difference in the expression profile: two of them peak in G1 and S phase while Cdc6-2 accumulates specifically in G2, raising the interesting possibility that these proteins, collectively, might contribute to positively and negatively regulating origin firing. Hence, this *archaeon* recapitulates the eukaryotic situation in which multiple replicators are used to initiate DNA synthesis and provides a powerful tool to address the mechanism of origin selection and cell cycle control of replication, that are still, in eukaryotes, not completely understood.

An important question to answer is why this organism has selected a mechanism of replication based on two replicators? Is it just to speed up the replication process, or does firing of multiple replicators have implications for cellular process other than replication efficiency? In this respect, another interesting finding from Bell's lab is that, in *Sulfolobus*, origin firing results in the accumulation of sister chromatid junctions that resemble the ones described also in eukaryotes (Benard et al., 2001). Although the nature of these structures is still elusive, they might have relevant implications for sister chromatid cohesion and for the quality control of the replication process. Interestingly, ORC has been suggested to play a role in coupling replication to cohesion (Bell, 2002). With this in mind, we should consider the possibility that *Archaea* will help us to define not only what happens during the replication process but might also provide important clues for those cellular processes tightly connected with chromosome replication, such as recombination, sister chromatid cohesion, cell cycle regulation, and perhaps even checkpoints.

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A Common Switch used by Plants and Animals

Comparisons of plant and animal development usually highlight their differences. The discovery that a kinase of the MAPKK class plays a key role in cell specification at the first division of the *Arabidopsis* embryo suggests that there may be similarities based on a common logic.

If one compares a traditional Japanese farmhouse to its counterpart in England, one is first struck by the differences: paper versus plaster walls, tile versus thatch roofs, sliding versus hinged doors, tatami mats versus stone floors. The differences stem from the near total independence of their designs. And yet at closer inspection one sees that many of the basic materials are the same: wooden beams, stone hearths, metal pipes. In fact the fundamental design is identical—a rectangular structure providing shelter from the elements, with entranceways and means of internal heating. The similarities originate in the shared purposes and needs of the builders and reveal a consistent logic.

Comparisons of animal and plant embryogenesis usually focus on their differences: massive cell movements versus cells constrained by walls, organs developed in utero versus continuous organogenesis, minimal versus dramatic responses to environmental signals. The differences are readily traced to the fact that the common ancestor of plants and animals was unicellular and each evolved a developmental program independently. And for a time, it appeared that plants and animals had dipped into their common genetic toolbox and come up with very different solutions for orchestrating embryonic development. This seemed to be the conclusion drawn from a saturated screen for mutations in plant embryonic development carried out by Gerd Jürgens and colleagues who identified several lines with phenotypes that initially appeared similar to *Drosophila* gap mutants (Mayer et al., 1991). However, when the affected genes were isolated, they encoded components of the secretory machinery or were involved in cytokinesis (Shevell et al., 1994; Lukowitz et al., 1996). The recent discovery that the secretory machinery regulates the internal trafficking of the plant hormone auxin (Geldner et al., 2003), whose localization correlates with the emergence of embryonic organs (Friml et al., 2003), only served to reinforce the impression that plants use novel strategies to define embryonic fates.

In this issue of *Cell*, Lukowitz and colleagues (2004) describe the discovery of a gene that points in the other direction, hinting at intriguing similarities between plant