From the Replicon to Replication Programs in Space and Time: Regulation of DNA Replication and Implications for Genomic Instability

From the Replicon to Replication Programs in Space and Time: Regulation of DNA Replication and Implications for Genomic Instability

This year marks the 50th anniversary of the presentation of the replicon theory at a Cold Spring Harbor Symposium by François Jacob, Sydney Brenner, and François Cuzin proposing a model to explain the regulation of DNA synthesis in bacteria and its coordination with the cell cycle and cell division at a time where there were still little experimental information on this mechanism [1]. The model proposed that DNA in bacteria is organized into autonomous units of replication each harboring a *cis*-acting element (the replicator) and a *trans*-acting element (the structural gene for the initiator), whose interaction triggers replication initiation at the replicator (now called replication origin) and its collinear sequences that constitute the replicon. In this model, the *initiator* binds the *replicator* to promote DNA unwinding, which allows the recruitment of the replication machinery. Replication forks progress bi-directionally from the *replicators* until replication of the circular bacterial chromosomes is complete. In the following 20 years or so, the replicon model was validated more or less precisely as Jacob *et al.* imagined to describe the replication of bacterial chromosomes, as well as phages, plasmids, and viruses. The concept has also reliably served as a framework to investigate the regulation of DNA replication in eukaryotic cells and has shaped our views about how cells manage to faithfully duplicate their genetic material. Understanding organization of budding yeast replication suggested that the replicon model might apply universally to all organisms, although larger genomes would require additional replicators and much more complex regulations to orchestrate their proper firing. In this issue, Yoshida *et al.* present evidences supporting the view that the replicon theory is applicable to eukaryotes, even though prokaryotic and eukaryotic replicons differ in many ways [2].

Proper elongation from each fired origin is ensured by protein complexes termed replisomes. In *Escherichia coli,* the replisome includes a ring-shaped sliding clamp that facilitates the replication of chromosomal DNA by DNA polymerase III. The heptameric clamp loader loads the β_2 ring of DNA polymerase III onto DNA in an ATP-driven process. One of the subunit mediates interaction between the clamp loader and the tetrameric ssDNA (single-stranded *DNA*) binding protein SSB_4. This interaction stabilizes the clamp loader–SSB_4 complex and the polymerase–template–primer interactions during replication. The primary role of SSB_4 is to protect ssDNA from being degraded and to maintain cooperation with DNA binding proteins. Upon DNA binding, a switch between active and inactive states of SSB_4 occurs, which further enhances the activity of the clamp loader. Although some X-ray crystal structures are available for some of these components, the structures for SSB_4 with and without DNA and with the clamp loader have not been reported. In this issue, Carol V. Robinson and colleagues use mass spectrometry to define multiple subcomplexes and to construct an assembly pathway of the full clamp loader bound to SSB_4, in the presence or absence of ssDNA. They also investigate structural features of the 11-subunit clamp loader bound to SSB_4 and conformational changes induced upon binding of ssDNA [3]. Also published in this issue is the work of Timothy Lohman and colleagues, indicating that a single SSB tetramer must interact simultaneously with multiple protein partners during molecular events that are essential in genome maintenance [4].

In Archaea, the DNA polymerase holoenzyme complex synthesizes DNA distributively and with low processivity, unlike most other well-characterized DNA polymerase holoenzyme complexes. Here, Michael Trakselis and colleagues reveal kinetic mechanisms underlying the assembly of clamp loading and holoenzyme in Archaea. This work unveils a novel mode for dynamic processivity that occurs by a polymerase exchange mechanism. This work also suggests a potential mechanism for the switching of DNA polymerase to bypass DNA lesions during repair [5].

In eukaryotes, the sliding clamp protein, termed PCNA (*p*roliferating *c*ell *n*uclear *a*ntigen), acts as an interaction scaffold for numerous replication and repair factors and coordinates DNA transactions ranging from maturation of Okazaki fragment (short, newly synthesized DNA fragments formed upon replication of the lagging strand) to chromatin assembly and mismatch repair. How PCNA is loaded onto DNA has been studied in detail. Until recently, however, it was unclear how PCNA is

removed from DNA upon completion of DNA synthesis. In this issue, Helle Ulrich discusses studies that implicate a replication factor C-like complex in the unloading of PCNA during replication in yeast and human cells and unveil mechanisms involving this complex in maintaining genome stability. Accurate control over PCNA's residence on chromatin, maintained by a balance of loading and unloading, therefore appears to be crucial for its proper function [6].

While bacterial chromosomes consist of single replicons, the chromosomes of eukarya and some archaea are multi-replicon structures. Multi-replicon archaeal chromosomes are mosaics of distinct replicator/initiator systems that fire fairly efficiently in each cell cycle. Such highly efficient initiation correlates with a high specificity of replicator–initiator interactions. The specificity of these interactions generally decreases with the increase in genome complexity, which probably contributes to more plastic and adaptive regulation of initiation events in higher eukaryotes. The firing may occur stochastically, with firing probability of each origin being affected by genetic/epigenetic environment or physiological conditions, namely subjected to developmental changes. While origin selection in eukaryotes appears to be under plastic regulation, inhibition of re-replication appears to be of central importance in both prokaryotes and eukaryotes, and multiple mechanisms operate to ensure that re-replication is avoided. These issues are discussed here by Hisao Masai, Philippe Pasero, Olivier Hyrien, and their colleagues in different review articles [2,7,8].

Although many protein components of initiators have been shown to be conserved in different eukaryotes during evolution, DNA sequences at replication origins have diverged. In this issue, Francisco Antequera and colleagues review recent comparative genomic analyses in yeasts including fission yeasts (*Schizosaccharomyces pombe*) that contribute to our understanding of how the specification of replication origins has evolved along with yeast evolution [9]. To explain why there are so many different types of replicons, it was suggested that cellular DNA replication mechanisms first originated and diversified from the large diversity of mobile elements of viral origin, as reviewed in this issue by Patrick Forterre [10].

A large number of reports have now established that origins in eukaryotes are not determined solely by DNA sequence and that only a fraction of initiator-bound origins actually initiate replication in a given cell cycle. Helping to make sense of the structure and regulation of eukaryotic replicons, studies of DNA replication timing, a unique feature of eukaryotes, have provided insight into hierarchical levels of large-scale chromosome organization as Hisao Masai, Philippe Pasero, and their colleagues discussed here in two different review articles [2,7].

Indeed, metazoan chromosomes display a patchwork of segments replicating in a defined temporal sequence. These segments are far too large to be accounted for by a single replicon, and they instead result from concomitant initiation of clusters of individual origins. Additional layers of regulation are now emerging from studies demonstrating that the organization of chromosomes into tissue-specific domains underlies segmental replication, as discussed here by David Gilbert and colleagues [11]. Until recently, the structures of metazoans replicators have been rather elusive due to the lack of a simple consensus sequence and to the lack of a convenient system to assay origin function. Recent technological advances for mapping replication origins or initiator binding sites genome wide have made it possible to catalogue all the potential replicators and deduce some common features. Notably, 30–50% of the origins display sequences having the potential to form G-quadruplexes, as discussed during the meeting "Celebrating the 50th anniversary of the Replicon Theory" (Pasteur Institute, Paris, France) by several speakers (Marcel Méchali, Marie-Noelle Priolleau, Benoît Miotto, and so on). In addition, Benoît Le Tallec and colleagues present in this issue the technique of molecular combing to assess the dynamics of DNA replication at the genome-scale level from the cumulative analysis of single DNA fibers. This technique enables measurement of replication fork speed and fork asymmetry and distances separating initiation and termination events. The authors evaluate requirements critical to accurate measurement of replication parameters by molecular combing [12].

Protecting genomic integrity is essential to guarantee the faithful transmission of genetic information through cell generations, thus to avoid the occurrence of genetic diseases, including cancers. This is highlighted by the importance of the DDR (*D*NA *d*amage *r*esponse), a complex network of surveillance mechanisms aiming to safeguard genome integrity all along the cell cycle and, notably, in S phase. Indeed, genomic DNA becomes very vulnerable during the course of DNA replication if replication fork progression is disturbed by internal or external causes, including reduced nucleotide supply, collisions with proteins tightly bound to the template or with R-loops (DNA–RNA hybrids generated by ongoing transcription), unusual DNA structures, and agents affecting the functioning of the replication machinery, DNA damage, and so on. Stalled replication forks can turn into catastrophic DNA lesions if not correctly detected and protected.

Classical DNA polymerases halt when they encounter DNA sequences capable of adopting non-B DNA structures, which impacts genome stability and, in some instances, play a causal role in disease development. Indeed, cruciforms, hairpins, H DNA, Z DNA, and G-quadruplexes may form

in the genome at specific DNA repetitive sequences. Along with dedicated DNA helicases, the specialized DNA polymerases emerge as major actors performing DNA synthesis through these secondary structures, as described in this issue by Jean-Sebastien Hoffmann and colleagues [13]. In addition, Madalena Tarsounas and colleagues focus their discussion on one of these non-classical DNA structures, the G-quadruplexes that form at some guanine-rich sequences. These structures are intriguing since they can be useful or harmful to cell physiology depending of the genomic context. For example, they can not only promote transcription and replication but also act as replication barriers and interfere with inheritance of epigenetic marks. At the telomeres, G-quadruplexes stall replication fork progression but also confer end protection [14].

Another major drawback to DNA replication is the transcription process. To avoid interference between the two processes, prokaryotic cells have chosen strategies that involve genomic organization in which they have placed highly expressed genes in the leading strand, thus preventing head-on collisions between DNA and RNA polymerases. The presence of additional genetic elements or the spatial and temporal coordination of the two processes has also been exploited in eukaryotic cells to minimize the risk of collision. There are special circumstances in which cells in S phase are subjected to major changes in their transcriptional capacity. For instance, in response to environmental stresses, cells dramatically change their pattern of gene expression to maximize cell survival in the new conditions. This transcriptional outburst considerably increases the risk of collision between the replication and transcription machineries. To coordinate both processes, cells have evolved a dedicated checkpoint that delays S phase progression while permitting proper transcription of stress-responsive genes. Cells have also evolved an independent pathway, taking place during environmental stress, to protect DNA from external insults during replication. Whether similar mechanisms operate in other situations that involve an outburst of transcription remains to be assessed. These questions are discussed by Francesc Posas and colleagues in this issue [15].

Replication stress has emerged as a significant source of genome instability during the early stages of carcinogenesis. Replication-based mechanisms have been also proposed to underlie genome rearrangements in genomic disorders. To overcome fork obstacles, cells have evolved multiple strategies that fall in three categories: (i) preventing the activity of fork barriers, (ii) stabilizing the halted replisome to allow it to resume progression, and (iii) when the replisome cannot resume, a new replisome can be rebuilt in order to restart the fork. For example, fork restart by the DNA primosome PriA is crucial in prokaryotes, and stabilization and protection of stalled forks by replication fork auxiliary factors may prevent fork collapse or fork inactivation in eukaryotes [7]. In this issue, Masamichi Kohiyama and colleagues analyzed mechanisms during processing of arrested replication fork in *E. coli*. These authors reveal the existence of a cellular mechanism that neutralizes genotoxicity of ssDNA [16].

Among the fork stabilization and fork restart pathways, HR (*h*omologous *r*ecombination) is a pivotal mechanism ensuring the progression of replication forks. This function has been mainly deciphered in *E. coli*, aided by the specific replication dynamic of the bacterial chromosome. In eukaryotes, the mechanisms by which HR promotes replisome protection and restart or rebuilding of replication forks have only recently started to emerge and the function of each HR proteins still needs to be documented. Because HR uses a homologous sequence to repair broken DNA, this mechanism has generally been considered as a faithful pathway, contributing to the maintenance of genome stability. HR is now emerging as a pathway that ensures the robustness of DNA replication in eukaryotes, and this can clearly occur, at least, in some instances, by a mechanism independent of a double-strand break. However, fork restart by HR has also detrimental consequences. In yeast models, recent investigations have established that HR-dependent fork restart is a source of genetic instability mediated by both homology and microhomology, suggesting that replication-induced genome instability stems, in part, directly from the ability of HR to restart replication forks. In this issue, Anthony Carr and Sarah Lambert assess the mechanisms by which HR contributes to the robustness of DNA replication and focus on the induction of genome modifications that might fuel cancer progression [17].

The eukaryotic cell cycle comprises a series of events, whose proper ordering depends on the oscillating activity of Cdks (*c*yclin-*d*ependent *ki*nases), which safeguard timely duplication and segregation of the genome. Cell division is intimately connected to the evolutionarily conserved DDR, which involves DNA repair pathways that reverse DNA lesions, as well as checkpoint pathways that inhibit cell cycle progression while repair occurs. There is increasing evidence that Cdks are involved in the DDR, in particular, in DNA repair by HR and in activation of the checkpoint response. However, Cdks have to be carefully regulated because even an excess of their activity can affect genome stability. Here, Maria Pia Longhese and colleagues consider the physiological role of Cdks in the DDR [18].

We would like to dedicate this special issue to Francois Jacob, one of the fathers of the replicon model. Francois Jacob passed away this year, on April 19th 2013, a few weeks after a symposium was held at the Pasteur Institute (Paris, France) to celebrate the 50th anniversary of the "replicon" theory. Francois Jacob did not attend the symposium, but the organizers Benoit Arcangioli (Pasteur

Institute, Paris, France), Michelle Debatisse (Curie Institute, Paris), and Masamichi Kohiyama (University Paris Diderot) visited him at this occasion. We brought him, at his request, the abstract book of the symposium and he actively discussed with us the themes and content of the different sessions. It is with a profound emotion that we remember how he was enthusiastic about this anniversary and how he was still fascinated by ongoing science.

It has been a pleasure to assemble a special issue that recognizes the advance made in the field of the regulation of DNA replication since its inception. We are looking forward to progresses in the years to come as new molecular and genetic approaches are now undertaken to unveil mechanistic insights on regulation of replication programs and their contribution to genomic stability, protecting cells against cancer and developmental disorders. Many of the authors who kindly contributed to this special issue attended the symposium in Paris. We are obliged to the authors and reviewers for their timely contributions and their positive support. We are grateful to Max Gottesman and Moshe Yaniv, Editorial Board Members at the *Journal of Molecular Biology*, for kindly providing constructive critics and/or taking on some of the articles published in this issue.

References

[1] Jacob F, Brenner S, Cuzin F. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp Quant Biol 1964;288:329–48.

[2] Yoshida K, Poveda A, Pasero P. Time to be versatile: regulation of the replication timing program in budding yeast. J Mol Biol 2013;425:4695–704.

[3] Politis A, et al. Integrative modelling coupled with ion mobility mass spectrometry reveals structural features of the clamp loader in complex with single-stranded DNA binding protein. J Mol Biol 2013;425:4789–800.

[4] Antony E, Weiland E, Yuan Q, Manhart CM, Nguyen B, Kozlov AG, et al. Multiple C-terminal tails within a single *E. coli* SSB homotetramer coordinate DNA replication and repair. J Mol Biol 2013;425:4801–18.

[5] Bauer RJ, Wolff ID, Zuo X, Lin H-K, Trakselis MA. Assembly and distributive action of an archaeal DNA polymerase holoenzyme. J Mol Biol 2013;425:4819–35.

[6] Ulrich HD. New insights into replication clamp unloading. J Mol Biol 2013;425:4726–31.

[7] Masai H. A personal reflection on the replicon theory: from R1 plasmid to replication timing regulation in human cells. J Mol Biol 2013;425:4662–71.

[8] Hyrien O, Rappailles A, Guilbaud G, Baker A, Chen C-L, Goldar A, et al. From simple bacterial and archaeal replicons to replication N/U-domains. J Mol Biol 2013;425:4672–88.

[9] Mojard'ın L, V'azquez E, Antequera F. Specification of DNA replication origins and genomic base composition in fission yeasts. J Mol Biol 2013;425:4705–12.

[10] Forterre P. Why so many and diverse replication machineries? J Mol Biol 2013;425:4713–25.

[11] Pope BD, Gilbert DM. The replication domain model: regulating replicon firing in the context of large-scale chromosome architecture. J Mol Biol 2013;425:4689–94.

[12] Técher H, et al. Replication dynamics: biases and robustness of DNA fiber analysis. J Mol Biol 2013;425:4844–54.

[13] Boyer A-S, Grgurevic S, Cazaux C, Hoffmann J-S. The human specialized DNA polymerases and non-B DNA: vital relationships to preserve genome integrity. J Mol Biol 2013;425:4766–80.

[14] Tarsounas M, Tijsterman M. Genomes and G quadruplexes: for better or for worse. J Mol Biol 2013;425:4781–8.

[15] Duch A, de Nadal E, Posas F. Dealing with transcriptional outbursts during S phase to protect genomic integrity. J Mol Biol 2013;425:4744–54.

[16] Kohiyama M, et al. Trashing of single-stranded DNA generated during processing of arrested replication fork in *E. coli*. J Mol Biol 2013;425:4836–43.

[17] Carr AM, Lambert S. Replication stress-induced genome instability: the dark side of replication maintenance by homologous recombination. J Mol Biol 2013;425:4732–43.

[18] Trovesi C, et al. Regulation of the DNA damage response by cyclin-dependent kinases. J Mol Biol 2013;425:4755–65.

Marina Ostankovitch[1]
1 - *Journal of Molecular Biology, Elsevier Inc., 600 Technology Square, Cambridge, MA 02139, USA*
E-mail addresses: m.ostankovitch@elsevier.com.

Michelle Debatisse[2]
2 - *Institut Curie, 26 Rue d'Ulm, 75005 Paris, France*
E-mail addresses: michelle.debatisse@curie.fr.