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# The Replication Domain Model: regulating replicon firing in the context of large-scale chromosome architecture

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## Abstract

The “Replicon Theory” of Jacob, Brenner and Cuzin has reliably served as the paradigm for regulating the sites where individual replicons initiate replication. Concurrent with the replicon model was Taylor’s demonstration that plant and animal chromosomes replicate segmentally in a defined temporal sequence, via cytologically defined units too large to be accounted for by a single replicon. Instead, there seemed to be a program to choreograph when chromosome units replicate during S phase, executed by initiation at clusters of individual replicons within each segment. Here, we summarize recent molecular evidence for the existence of such units, now known as “replication domains”, and discuss how the organization of large chromosomes into structural units has added additional layers of regulation to the original replicon model.

## Introduction

In their celebrated theory, Jacob, Brenner and Cuzin hypothesized that the DNA of *Escherichia coli* was organized as “replicons”, with each replicon consisting of a replicator sequence element and a structural gene encoding an initiator protein that activated DNA replication through interaction with the replicator [1]. Within approximately twenty years of the theory’s introduction, prokaryotic replicons were characterized more or less precisely as Jacob *et al.* imagined [2]. Isolation of budding yeast replicons [3] suggested the theory might apply universally to all organisms, with the caveat that larger genomes require additional replicators. However, ensuing research indicated that replicators in other eukaryotes are not determined solely by DNA sequence and that only a fraction of initiator-bound replicators 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. In this perspective we will discuss how various levels of mammalian chromosome organization are superimposed on the simple structure of replicons Jacob *et al.* proposed for prokaryotes.

## Individual replicons versus replication domains

Replication domains were initially observed by cytological means and described as adjacent chromosome segments that incorporated thymidine-$H^3$ asynchronously during the S phase of cells from smooth hawksbeard root [4] and Chinese hamster [5]. Similarly, metaphase chromosomes from cells pulse labeled with 5-bromo-2′-deoxyuridine exhibited an oscillating incorporation pattern corresponding to Giemsa-stained chromomeric bands [6,7]. More recently, profiling of replication timing in mammals has allowed clear segmentation of chromosomes into replication domains with defined genomic sizes and locations [8–17]. Two

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general models have been proposed to explain the appearance of domains. On the one hand, domain-like patterns of replication could emerge fortuitously from the relative firing time and distribution of individual replicons [18–20]. In this case, local contexts would influence each replicon independently [21,22] and domain "boundaries" would simply appear at the edges of the earliest firing replicon clusters in a given region. On the other hand, each domain could be a unit of regulation, with physical characteristics and size independent of replicon distribution, influencing when the replicons within its boundaries could fire [15,23–26]. We refer to this latter model as "The Replication Domain Model".

## Sub-nuclear replication compartments and replication foci

Early autoradiography experiments indicated chromatin dispersed throughout the nuclear interior was replicated simultaneously at the onset of S phase [27,28], while replication at later time points was confined to sites along the nuclear periphery [29–33]. Subsequent experiments demonstrated that the sub-nuclear positions of synchronously firing replicons were maintained throughout interphase and were consistently re-established in daughter cells [34,35] even after 15 generations [36]. Consistent with a direct link between the spatial organization and regulation of replicons, a discrete point during G1 phase was discovered at which the replication-timing program is established each cell cycle (the Timing Decision Point; TDP), which coincides with the anchorage of chromatin to its respective sub-nuclear positions following mitosis [37]. Finally, maps of chromatin-interaction [38], which align with replication-timing profiles more closely than any other chromatin property mapped to date [15,23–26,39], have confirmed the spatial compartmentalization of replicons with distinct temporal regulation and provided independent evidence for the existence of structural chromosome units on the scale of replication domains.

Detailed cytological analysis revealed that individual sites of active replication, called "replication foci" [36,40,41], correspond to clusters of synchronously firing replicons visualized along the length of isolated DNA fibers [42]. Foci are abundant (~10,000 during the S phase of mouse 3T3 fibroblasts) and, although they vary in size [43], are estimated to encompass approximately 1 Mbp of DNA [40], similar to the unit size of developmental replication-timing regulation (400–800kb, see below) later defined by genomics studies [11,14,15,17]. The number of simultaneously replicating foci, and hence the rate of DNA synthesis during S phase, was shown to be controlled by cyclin-dependent kinase activity and intra-S-phase checkpoints independently from the regulation of individual initiation events within the foci [44,45]. Collectively, these data argue that replication foci are the equivalents of replication units defined by genome-wide replication timing and chromatin interaction maps.

## Units of replication-timing regulation

If the replication-timing program were truly related to chromatin structure and function, one would expect changes in replication timing to accompany cell differentiation during the development of multicellular organisms. Detailed analysis of the replication timing of individual regions in different cell types suggested replication timing could be cell-type specific [46–50]. Subsequent genome-wide experiments revealed that programmed developmental changes in replication timing involve at least half the genome in mammals and these changes primarily occur in 400–800 kb units [11,14,15,17]. The discrete size of developmental changes in replication timing suggests replication domains comprise multiple, independent units of regulation.

The replication domain model was recently put to the test by analyzing the replication timing of a *trans*-chromosomic mouse carrying a heavily rearranged and freely segregating Human Chromosome 21 [51]. In two distinct mouse tissues, the *trans*-chromosome generally exhibited normal, human-specific replication timing [52]. However, in cases where

rearrangements juxtaposed chromosome fragments that normally replicated at different times, the replication timing of one fragment appeared to spread across the breakpoint into the other fragment. By comparing the replication-timing shifts at these rearrangements to control replication profiles from both matching and non-matching human cell types, it was discovered that timing shifts extended up to the nearest replication boundary, even if that boundary was not normally detected in the matching cell type. This apparent insulating effect observed at the positions of replication boundaries detected in non-matching cell types suggested that static structural boundaries delineate independent units of replication-timing regulation. Consistent with this result, in a study where genome-wide replication-timing profiles were generated from 17 patient leukemia samples, many replication-timing aberrations were observed, which shared the sizes and boundaries of developmental changes in replication timing, again suggesting that developmentally regulated replication-timing units have static structural boundaries [53]. Intriguingly, average "signatures" of DNaseI hypersensitivity [54], the CCCTC-binding factor [26], and a combination of histone modifications (H3K4me1/2/3, H3K36me3, H3K27ac enrichment and H3K27me3, H3K9me2/3 depletion) [15] near the boundaries of replication domains were reported previously. The extent to which these "signatures" or other insulating features define unit boundaries throughout development remains an interesting question for future research. Altogether, these results strongly suggest that replication boundaries coincide with static insulating elements that facilitate independent regulation of neighboring units, even when those units replicate at the same time and thus cannot be distinguished by replication-timing analysis.

## Dynamic regulation of stable replication units

To directly assess the stability of replication units and their boundaries during developmental replication-timing changes, another recent study examined the dynamics of two replication units that change replication timing during differentiation [25]. Using fluorescence in situ hybridization (FISH) probes evenly spaced across each unit and the surrounding regions, it was observed that early replication was associated with a dramatic increase in the volume of chromatin confined within the replication boundaries of the switching units. Chromatin conformation mapping of these same regions revealed that each replication unit was flanked by interaction boundaries (sharp trough in the frequency of chromatin interactions) at the same positions in both the distended early-replicating and more compact late-replicating states. However, interactions between each unit and the surrounding regions did change during differentiation, with both units preferentially interacting with other early regions when the units were early-replicating and other late regions when the units were late-replicating, even more so than with *cis*-linked neighboring regions that replicated at a different time. Hence, replication units also switch their sub-nuclear spatial compartment when they change replication timing.

Surprisingly, despite the increased volume of chromatin observed within these two units when they switched from late to early replication, the sensitivity of the units to nuclease attack did not change [25]. In fact, genome-wide analysis revealed that units that switch replication timing harbor some of the least sensitive chromatin in the genome and maintained low nuclease sensitivity when both late- and early-replicating [55]. Although nuclease sensitivity is not coordinately regulated with developmental replication-timing changes, some histone modifications do change with replication timing [11,15]. Hence, some physical properties are associated with the stable structural features of replication domains, while others are dynamically associated with the replication time of the domain.

## Conclusion

The evidence is now compelling that mammalian DNA replication is regulated at levels beyond individual replicons. In mammals, replication is coordinated across large units of chromosomes, or replication domains, whose structural boundaries are stable during the cell cycle and development. The temporal order in which these units replicate, however, is cell-type specific and is closely associated with the sub-nuclear compartmentalization of units, manifest by the preferential interaction of units that replicate at the same time. Initiator-replicator binding, determination of replication timing, and selection of which replicators will fire during S phase each occur independently at distinct times during G1 phase [37,56]. The mechanisms coordinating these different layers of regulation are only now being worked out, with the first proteins to regulate the replication-timing program globally in both mammals [57,58] and yeast [59,60] only identified in the last year. Moreover, there are likely to be additional levels of regulation. For example, disruption of lncRNAs such as Xist or ASAR6 in mammalian fibroblasts appears to influence replication timing throughout their respective chromosomes [61–64]. Although these various levels of regulation may act to a greater or lesser extent in different organisms, they ultimately converge on a common replicon structure to initiate the DNA replication program.

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- DNA replication in mammals must accommodate large-scale chromosome architecture
- Dynamics of DNA replication timing reveal various levels of chromosome organization
- Replication boundaries mark static insulators between independent regulatory units
- Regulation varies in different organisms but converges on common replicon structure