Chromatin Structure and Replication Origins: Determinants Of Chromosome Replication And Nuclear Organization

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Abstract

The DNA replication program is, in part, determined by the epigenetic landscape that governs local chromosome architecture and directs chromosome duplication. Replication must coordinate with other biochemical processes occurring concomitantly on chromatin, such as transcription and remodeling, to insure accurate duplication of both genetic and epigenetic features and to preserve genomic stability. The importance of genome architecture and chromatin looping in coordinating cellular processes on chromatin is illustrated by two recent sets of discoveries. First, chromatin-associated proteins that are not part of the core replication machinery were shown to affect the timing of DNA replication. These chromatin-associated proteins could be working in concert, or perhaps in competition, with the transcriptional machinery and with chromatin modifiers to determine the spatial and temporal organization of replication initiation events. Second, epigenetic interactions are mediated by DNA sequences that determine chromosomal replication. In this review we summarize recent findings and current models linking spatial and temporal regulation of the replication program with epigenetic signaling. We discuss these issues in the context of the genome's three-dimensional structure with an emphasis on events occurring during the initiation of DNA replication.

A. Introduction

Accurate genome duplication, which is necessary to prevent the accumulation of mutations and disease, is in part determined by precise control of the replication program. Initiation of DNA replication is a carefully coordinated process that adheres to an organized spatial and temporal progression and follows tissue-specific and developmental cues. [1–5] Although local chromatin factors and site-specific nuclear protein interactions influence the replication and transcriptional activity of certain genomic regions, mounting evidence suggests that these factors are constrained by stable large-scale chromatin structure. [6; 7] Both the transcription and replication machineries create stable chromatin loops where nuclear components can aggregate and modulate three-dimensional organization of chromosomes in the

nucleus. [3; 4; 8] Many recent reviews have extensively described the implications of specific histone modifications on chromatin structure and the initiation of DNA replication. [1; 9–14] In this review we will discuss possible other epigenetic determinants of chromosomal replication, starting from global determinants of nuclear structure and zooming inside the nucleus to modulators of local chromatin loops and interactions at replication initiation sites (Figure 1). We concentrate on two sets of molecular interactions affecting nuclear organization of DNA replication events: structural nuclear components and non-histone proteins affecting nuclear organization, and epigenetic interactions anchored on *cis*-acting DNA elements (replicators) that can dictate the location and the timing of chromosomal replication.

B. Background: Organization of chromosomal replication events

Chromosomal DNA replication occurs during the S-phase of the mitotic cell cycle and exhibits a carefully orchestrated program with several redundant mechanisms to ensure faithful duplication of both genetic material and associated epigenetic modifications. Replication is a key event in the mitotic cell cycle, which is regulated by the activity of cyclin-dependent protein kinases (CDKs) and their molecular modulators, including activators, inhibitors, and ubiquitin ligases that periodically target cell cycle components for degradation.[15] The balance between CDKs and their activating partners, the cyclins, controls the temporal replication program by regulating local and global replication initiation and stabilizing replication forks in response to DNA damage.[16; 17]

Chromosomal replication starts from discrete locations termed DNA replication origins. Replication origins often cluster together in replication initiation regions. Genetic elements containing DNA sequences that genetically determine the ability to start replication are called replicators. Replicators often, but not always, colocalize to active replication origins.[5; 9] Replication origins initiate replication in two consecutive steps. First, pre replication complexes (Pre-RCs) are formed at potential replication origins in an ATP dependent process[18], termed origin licensing. Second, select Pre-RCs are activated by kinases (CDKs and Dbf4-dependent kinases, DDKs) to initiate DNA replication. Pre-RCs form only during the G1 period of the interphase, which begins right after cell division and is characterized by suppressed CDK activity.[15] Once CDKs are activated, those kinases act locally at replication initiation sites to activate helicases[19] and to recruit DNA polymerases and accessory factors.[15] Replication starts only from Pre-RCs that formed during the G1 period because a variety of mechanisms restrict the assembly of new Pre-RC once CDKs are activated during S-phase.

Each chromosomal region that is replicated by a single origin is termed a replicon. In metazoans, there are more replication origins than active replicons because the number of Pre-RCs assembled is larger than the number that actually initiate replication. This discrepancy suggests that each replicon might contain several potential origins that can be activated alternatively each cell cycle.[1; 5; 20; 21] Activating only one of multiple origins in each replicon allows for flexibility in origin use that can coordinate replication with transcription or other nuclear processes.[22] For example, if a replication origin is rendered inactive by transcription interference or a replication fork stalls due to DNA damage,

replication could initiate from adjacent sites within the same replicon, providing initiation flexibility among cell type (or chromosome homologs in the case of monoallelic transcription). The flexibility achieved by an excess of potential origins creates a "safety net" to ensure complete replication if some replication forks fail.[20; 21]

Replication in human cells exhibits punctate spatial patterns, termed replication foci, which appear when labeling active replication with nucleotide analogs. The distribution of the foci changes over the course of the S-phase, observed by a dramatic shift in foci pattern during mid S-Phase concomitant with replication of peripheral heterochromatin.[4; 23] Replication foci represent cytological units, presumably composed of several replicons that are activated together. Patterns of replication foci are highly sensitive to CDK levels, suggesting that CDKs control replication initiation at a larger scale, in addition to activating individual replication forks.[24] Several studies comparing replication timing and genome topology suggest that replicons are clustered into large (~1Mb) chromatin units, close to the estimated size of replication foci, called replication domains, which are located at distinct areas of the nucleus during G1 and replicate concomitantly. [23;25–28]

Replication domains are created by topological reorganization in nuclear space and define regulatory function related to gene expression and genome maintenance.[7; 29] In metazoans, the association of particular replication domains with subnuclear compartments which determine that region's replication timing occurs at a distinct time during the G1 phase of the cell cycle, termed the timing decision point (TDP).[30] These observations are consistent with data in yeast suggesting that the timing of replication of a late origin requires its association with telomeres early during G1 of the same cell cycle.[31] Early and late replicating loci occupy distinct nuclear regions in yeast[32], and metazoan replication domains exhibit strong concordance with high-order chromatin domains revealed using Hi-C, a technique that measures interactions between distal chromatin regions on a genome-wide scale *in vivo*.[26; 33] Confirming this observation, a modified version of Hi-C suggests that loci brought together by chromatin architecture exhibit similar replication timing.[34] Prior to TDP, chromatin lacks the determinants of replication timing but maintains the spatial organization of chromatin domains.[35] The association between replication timing domain organization and the spatial arrangement of nuclear chromatin[36] prompted the Replication Domain Model, proposed by Pope and Gilbert[37], suggesting that replication domains are stable in each cell type but can exhibit cell-type and development associated relocations that alter replication timing.

The size and composition of replication domains are altered during differentiation[38], but the impact of nuclear localization on replication timing and on gene expression remains unclear. Differentiation of embryonic stem cells can promote the spatial reorganization of an originally late-replicating, lineage-specific gene away from the nuclear periphery, accompanied by a transition to early replication and acquisition of activating transcription marks at the promoter of the gene.[39] During differentiation, neighboring loci can also relocate from the nuclear periphery, hence acting as "passengers ". Such passengers exhibit earlier replication but are not transcriptionally active, suggesting that relocation is insufficient for regional gene activation but sufficient for advanced replication. However, replication delay can also occur without an accompanying relocation towards the nuclear

periphery.[40] This evidence suggests that an association between late replication and location near the nuclear periphery is not obligatory.[30; 38] In budding yeast, tethering an origin to the nuclear periphery does not delay replication despite nuclear relocalization.[41] This finding might imply that in yeast, DNA sequence is a larger determinant of replication timing than nuclear localization, consistent with the observation that yeast have more defined sequences for replication origins than metazoans. The following sections discuss the balance between structural components and specific DNA sequences in enabling replication competency within the three-dimensional organization of the nucleus.

C. Structural determinants facilitating proper chromosomal organization and replication

1) The nuclear lamina

The nuclear lamina is composed of intermediate filament proteins that create a lattice within the inner nuclear membrane. This lattice structurally anchors chromatin to the nuclear envelope.[42] Chromatin attaches to the nuclear matrix through Scaffold Attachment Regions (SARs)[42] or Matrix Attachment Regions (MARs)[43], and scaffold structural proteins assist in forming chromatin loops.[6] The internal nuclear matrix maintains chromosomes within their respective territories[44], and has been implicated in replication foci formation.[43] Given that replication factories locate internally within the nucleus and are only observed at the nuclear periphery in late S-phase, the relevance of the nuclear lamina in facilitating the formation of all replication complexes may not be obvious.[4; 45; 46] Experimental evidence suggests that stable anchorage points formed by lamina proteins found at the periphery are necessary for the replication program to proceed. In *Xenopus* egg extracts, immunodepletion of the lamina proteins lamin LIII and lamin B3 prevent initiation of DNA replication without affecting the import of replication proteins and without affecting the formation of either the nuclear envelope or the nuclear pore complexes.[47; 48] These observations led to a model proposing that the association between the nucleoskeleton and the nuclear lamina facilitates the loading of replication proteins onto chromatin loops.[42]

Supporting a role of lamina proteins in chromatin organization, the lamin B receptor (LBR) has been found to directly associate with the heterochromatin protein HP1.[49] This interaction is managed by mitotic phosphorylation of LBR, which abrogates binding with HP1 until interphase when chromatin structure is reconstituted in anticipation of the next round of DNA replication. The interaction of a heterochromatin structural protein with peripheral lamina is consistent with the known late replication of heterochromatin domains that can exhibit distinct localization to the edge of the nucleus. This evidence suggests a role for lamin-associated proteins in organizing chromatin by sequestering heterochromatin to the nuclear periphery, potentially influencing the replication-timing program.

The role of the nuclear lamina in Pre-RC assembly and chromatin loop organization implicates the lamina as one of the primary determinants of higher order chromatin organization in the nucleus. The specific nature of molecular interactions that modulate replication remains to be elucidated. One potential regulatory interaction was revealed in experiments conducted in cell free *Xenopus* egg extract, in which Pre-RC assembly and

initiation of DNA replication were stimulated by Lamina-Associated-Polypeptide-2 (LAP2), a resident protein of the nuclear lamina[50], and by an interaction between an isoform of this protein, LAP2β, and one of its binding partners, HA95.[51] HA95 also binds the Pre-RC component Cdc6[51], suggesting a direct role of nuclear lamina interaction in replication origin licensing. Further studies into lamin associated chromatin regions can indicate if and how this molecular interaction and interactions with other structural components of the nuclear lamina organize chromatin and assist in replication initiation.

2) Global chromatin organizers: CTCF and cohesins

Besides the nuclear lamina, several other DNA-protein complexes might provide external anchor points to create a chromatin environment composed of functional replication domains. The CCCTC-binding factor (CTCF) is a chromatin-associated protein that has diverse roles in maintaining chromatin architecture. CTCF acts as a transcriptional repressor that maintains barriers between active and repressed chromatin[52] and anchors DNA loops on the nuclear matrix.[53] By associating with the nuclear matrix, CTCF can assist in creating functional domains that provide additional chromatin structure supported by nuclear scaffolding. In a study examining CTCF binding sites genome wide among 56 human cell lines, CTCF was found to work with the cohesin complex to maintain common topological domains that were constitutive to all cell lines.[54] The role of CTCF in creating chromatin loops has been most carefully explored in the context of transcriptional silencing, but given the importance of DNA looping and the maintenance of chromatin domains to DNA replication it may be possible that CTCF plays some role in regulating DNA replication.

Cohesin, a ring-shaped protein complex, may function with CTCF to delineate replication domains. Cohesin is involved in sister chromatid cohesion and rearrangements during compaction to the mitotic chromosome.[55] Cohesin may also be involved in managing specific DNA loops. Cohesin has been observed to cluster with established classes of transcription factors and to remain bound to chromatin through mitosis when other transcription factors are removed.[56] The retention of cohesin through the cell cycle suggests a role in the memory of both transcription and replication programs. Genome-wide studies show that CTCF and Cohesin are found at similar genomic regions, suggesting a shared function in genome organization. For example, the cohesin subunit SMC1A shares 65% of its DNA binding sites with identified CTCF binding regions.[57] In another example, 89% of the DNA binding sites of a different cohesion subunit, SCC1, colocalize with CTCF binding sites.[58] The common functions of CTCF and cohesin seem to involve establishing chromatin loops that define transcriptional activity and chromatin domain boundaries.

Comparative genome-wide analyses of cohesin binding and depletion experiments that investigated chromatin looping and DNA replication established a functional relationship between cohesin binding and DNA replication. Cohesin was shown to bind Mcm2–7 proteins, which form the Pre-RC and the replicative helicase, and the SCC1 subunit of cohesin was enriched in a subset of human replication origins[59]. In concordance, cohesin interacts with ORC proteins, members of Pre-RCs, in *Drosophila*[60] and *Xenopus* egg extract[61]. *Xenopus* cohesin also associates with the Pre-RC activating kinase, DDK, which is required to tether cohesin to Pre-RCs.[62] Depletion of cohesin in human cells decreased in

the number of active origins, accompanied by a larger inter-origin distance, but did not affect fork velocity.[59] This finding is consistent with observations in other model organisms and implicates cohesin in Pre-RC assembly and origin activation, but not active replication.

Several studies suggest that chromatin loop sizes and origin activities are coordinated and regulated by cohesin complexes. Fluorescent DNA halo assays, which measure global chromatin loops by the radial extension of chromatin fibers from the core of the nucleus, observed enlarged loops during interphase in cohesin-depleted cells.[59] Replication factories have been observed to localize to the anchor of DNA loops.[63] The increase in loop size under conditions of cohesin depletion could cause disorganized replication domains reflected in the reduced intensity of replication foci. Cohesin, perhaps in concert with CTCF or other chromatin structural proteins, may therefore create favorable chromatin architecture that promotes replication in a domain-defined manner. Lack of structure provided by these proteins can cause dysregulation of the DNA replication program due to incomplete Pre-RC loading during interphase.

3) Selective chromatin organizers: Rif1

While cohesin and CTCF globally modify chromatin organization and replication domains, other chromatin regulators might modulate chromatin structure at more discrete loci and with more defined roles in the organization of DNA replication. Rif1 is a conserved telomere-binding factor in fission yeast that interacts with the Hsk1/Cdc7 (DDK) kinase.[64] Rif1 binds to yeast chromosomes during the M-to-G1 transition and is essential for normal replication timing. In humans, Rif1 localizes to mid S-phase replication foci and depletion of Rif1 results in the loss of those replication foci, indicating premature initiation of DNA replication at mid-S-phase.[65] The change in replication timing in Rif1 depleted cells suggests that Rif1 helps form chromatin loops, perhaps facilitated by the interaction of Rif1 with nuclear-insoluble structures (such as the nuclear matrix, described above). Changes in replication timing are also seen in Rif1 depleted mice[66], indicating a high level of functional conservation for this protein. Rif1 might prevent initiation from a fraction of replication origins by excluding mid S-phase replication domains from the activating kinase DDK. In budding yeast, Rif1 interacts with the phosphatase PP1 to counteract the phosphorylation of Mcm4 by DDK that leads to replication initiation.[67] These findings suggest that Rif1-mediated phosphatase activity occurs in G1 to prevent unwanted origin activation. In addition, Rif1 might also play an S-phase specific role in preventing mid-S-phase origins from firing prematurely. In aggregate, these observations suggest that Rif1 differentially affects chromatin architecture in discrete timing domains as it facilitates initiation of DNA replication from early S-phase origins while preventing initiation from later origins.

4) Nucleosome binding proteins: HMGN family

The HMGN protein family is a member of the HMG superfamily that interacts with chromatin by binding the core nucleosome particle and influencing gene expression profiles. The five members of the HMGN protein family, only found in vertebrates, contain conserved N-terminal nuclear localization signals and nucleosome-binding domains with divergent C-terminal domains. HMGN proteins recognize the H2A and H2B dimer when arranged with DNA into the nucleosome core particle. The members in this family influence

gene expression in a variant-specific manner that affects the transcriptional program, which designates cell-type.[68] HMGN1 is subject to post-translational modifications by enzymes that also modify histone H3,[69] perhaps creating competition for these enzymes. Binding of HMGN proteins influences chromatin structure by modulating levels of histone modification, the activity of ATP-dependent chromatin remodeling complexes, and the availability of chromatin to transcriptional machinery.[69; 70]

Members of the HMGN family have been implicated in the regulation of chromosomal DNA replication. The HMGN1 protein interacts with the DNA polymerase sliding clamp, PCNA, by assisting in its steady state binding to linker DNA adjacent to nucleosomes.[71] PCNA is a factor involved in DNA replication and DNA damage responses that travels with the replisome. HMGN1 depletion reduces DNA damage repair, but the interaction with PCNA occurs independent of DNA damage response, perhaps implying a role for HMGN1 in the assembly of active replisomes. Studies of another member of the HMGN family, HMGN2/HMG-17, indicate a function of this protein in replicating minichromosomes containing an SV40 origin of replication by unfolding chromatin structure to increase accessibility.[72] These proteins provide further evidence for the importance of nuclear non-histone proteins in facilitating the DNA replication program through modulation of chromatin organization.

5) Local chromatin interactions

5a) Transcriptional regulators and chromatin modifiers—Transcription factors and other nuclear proteins can contribute to the replication profile by binding to specific genomic loci and helping to recruit replication factors[73; 74] or by facilitating the formation of structural features such as local chromatin loops. For example, the transcription factor Myc forms a functional chromatin domain[75] in *Drosophila,* and Myc binding sites which retain Myc through mitosis colocalize with insulator proteins, implying a structural function. The framework created by these elements contains the chromosomes within the nucleus in a manner that allows for genetic information to be accessed. In another example, the Forkhead transcription factors contribute to origin activation in budding yeast by clustering origins through local *cis* interactions and recruiting replication factors.[76] Forkhead transcription factors contribute to the expression of cell cycle regulated genes in yeast and humans[77], and bind to a subset of early replicating yeast origins in a cell cycle dependent manner.[78] Similarly the RIP60 zinc-finger containing protein that binds the Chinese hamster *DHFR* replication origin helps to form a small DNA loop *in vitro*, possibly to facilitate replication origin activation.[79]

Chromatin remodeling complexes are required for transcriptional activity by sliding and removing nucleosomes, but are also linked to DNA replication and chromatin structure. These complexes share core enzymatic subunits but display disparate roles *in vivo* that are important for defining chromatin states within higher order structures.[80] A study of SWI/SNF chromatin remodeling complex subunits supports the role of this complex in domain formation and DNA replication. SWI/SNF subunits were found to colocalize with regions that define chromatin domains (CTCF and lamins), as well as with replication origins. This complex was also found to associate with regions of chromatin that display

transcriptional activity, but there was no preference for high or low gene expression levels.[81] Further evidence comes from a study investigating the catalytic subunit of SWI/SNF-related complexes, Brg1.[82] By visualizing extended chromatin fibers marked for Pre-RC components and for active replication by a thymidine analog, Brg1 was found to localize with replication machinery, including ORC, GINS, and PCNA. The authors suggest a role in origin firing given the observation of Brg1 interaction with the replication activator TopBP1. However, the observation of Brg1 colocalization with PCNA suggests an accompanying, rather than a determinant function in DNA replication by removing and reconstituting nucleosomes during active replication.

Chromatin remodeling complexes associate specifically with the replication machinery, and have also been implicated in controlling chromatin domains during interphase. Depletion of the SWI/SNF subunit, BAF53, led to an expansion of chromosome territories, as well as a reduction in chromatin compaction in mouse cells. This depletion caused a cell cycle defect that was not related to a change in transcriptional activity, indicating the importance of BAF53 in chromatin domain formation.[83] Interestingly depletion of core SWI/SNF subunits, including Brg1, did not result in defects of chromatin compaction. These studies support the role of chromatin remodeling complexes in DNA replication and domain formation, and implicate the diversity of composition within these complexes in specifying function.

5b) Histone modifications—The relative contribution of genetic and epigenetic determinants to replication initiation is subject to intense study. Whole genome mapping of replication origins recently concluded that the two major determinants of replication initiation are large order chromatin organization and local epigenetic factors.[84; 85] In one study[81], early origins represented 32% of all origins and were localized in 1 Mb domains, supporting the presence of replication timing domains. A second wave of origin activation was observed in late S-phase, corresponding to CDK-cyclin activation times.[15; 16] As previously observed[85], early replication origins were associated with DNase I hypersensitivity, and only moderately with active transcription. These studies support the concept that local epigenetic markers contribute to origin placement by associating with Pre-RC factors. For example, methylation of histone H4 at lysine 20 by the histone methyltransferase PR-Set7 has been implicated in origin recognition complex (ORC) recruitment.[9] Other proteins, such as the ORC associated protein, ORCA, assist in Pre-RC formation by closely associating with Pre-RC components.[86] Pairing studies that identify origins with other genome-wide datasets can reveal local chromatin features that assist in determining the location of replication initiation.

In Drosophila, a computational model was able to accurately predict replication timing across cell types using pairwise combinations of histone modifications.[87] However, in vertebrate, adjacent replication initiation sites can be found within both hypoacetylated and hyperacetylated chromatin.[88] This evidence for a limited or indirect effect of histone modifications on replication timing is consistent with a recent model that was able to accurately predict replication timing across human cell types using DNase-hypersensitive sites without considering histone modifications.[89]

Nucleosome positioning is closely controlled to allow for the origin recognition complex (ORC) to bind.[60; 90–92] Management of nucleosome composition and histone tail modification is closely organized with active replication and needed to retain epigenetic memory.[10; 93] In *Drosophila*, combined modENCODE datasets revealed that activating histone modifications were enriched at early origins.[94] Histone modifications have been observed to not only dictate where replication initiates, but also to act in other functions related to DNA replication. In human cells, an expansion of dimethylation on histone H3 lysine 79 that occurs during S-Phase has been implicated in preventing re-replication.[95] In *Arabidopsis* mutations in histone 3 lysine 27 (H3K27) monomethyltransferases resulted in re-replication of heterochromatin regions in a process that is also modulated directly by DNA methylation.[96; 97] This observation suggests that in some genomic loci particular chromatin modifiers play a defined role in regulating replication through histone modification. Together these results illustrate the complexity and dynamic nature of epigenetic factors that determine local replication initiation. Revealing the histone reader factors that detect modified histone tails may provide insight to the role of these additions in determining replication origins.

D. Replicators: DNA sequences as chromatin organizers?

Several studies have identified key genetic elements that are required for replication initiation.[98–103] These observations are consistent with the replicon model proposed by Jacob, Brenner, and Cuzin in 1963, hypothesizing that DNA replication initiates via the interaction between DNA elements, termed replicators, and *trans* acting proteins that activate replication, termed initiators.[104] Studies that dissect replicators that had been moved from their original locations to ectopic sites for the ability to initiate replication indicate that recruitment of initiator proteins by replicators is important for replication initiation, but also show that sequence structure may be crucial for origin functionality. These studies suggest that despite the importance of chromatin structure and epigenetic factors in determining replication initiation, the underlying genetic sequence also conveys some information to dictate where and when replication will occur.

Binding of specific DNA sequences by nuclear components can facilitate chromatin loop formation and might affect DNA replication as observed in the well-characterized locus control region (LCR) of the human beta-globin locus, which assists in replication and transcriptional activity.[105] This interaction is mediated by two protein complexes in erythroid cells. The LCR-associated remodeling complex (LARC) is responsible for preventing gene silencing.[106] The DNA-associated replication and transcription complex (DAART) is a chromatin modifying complex implicated in both transcription and replication.[107] These complexes bridge the DNA elements at the beta-globin locus, suggesting the importance of DNA-protein interactions in determining chromatin structure. Distal interactions that activate DNA replication are also known to occur at the promoter of the Chinese hamster *Dhfr* locus [108], and an enhancer of the *Th2* locus [109].

Replicator regions exhibit flexibility in replication initiation that is coordinated with transcriptional activity. As was discussed in detail in recent reviews, this flexible activation of replication origins can provide backup to insure complete replication in cases of

replication fork stalling and can account for evidence from DNA fiber analyses suggesting that cell populations might contain chromosomes that vary in replication origin utilization. [1; 5; 21] Although the initiation of replication and transcription do not seem to colocalize, replication initiation often occurs adjacent to transcription initiation events [22; 85] and replicator elements can contribute to transcriptional activation. For example, the introduction of an active replicator to transcriptionally silent transgene cassettes prevented gene silencing, caused early replication at that region, and conferred euchromatic histone acetylation.[110] Similarly, a DNA unwinding element is required for initiation at the *c-Myc* origin, concomitant with acetylation of histone H4 and recruitment of discrete protein complexes that play a role in initiation (including E2F1, Pre-RC and a site-specific binding protein, DUE-B, which is necessary but not sufficient for initiation).[103; 111] In agreement, an artificial construct containing a USF transcription factor binding region flanking the beta-globin replicator on either side was able to change replication timing, suggesting that USF recruitment influenced local histone modifications that favor early replication timing.[112] Finally, insertion of an active replication origin, but neither insertion of active transcriptional elements nor recruitment of histone modifiers, could activate replication when inserted into a mouse region that separates early and late replication domains (a temporal transition region - TTR) which is naturally devoid of active origins. [113]

Consistent with the above, active replicators are able to initiate replication at ectopic locations despite chromatin state.[102; 110] Similarly, heterochromatin formation due to X chromosome inactivation does not alter the location of replication initiation events and affects only the timing of initiation.[114] While these data suggest that replicator function is independent of chromatin state, it is important to note that replication timing might also be determined by the primary DNA sequence. Heterochromatin formation and replication delay can be regulated by distinct DNA sequences at distal loci, possibly operated *via* non-coding RNAs such as XIST for the X chromosome[115; 116] and ASAR6 for the autosomal chromosome 6 in cancer cells.[117; 118] These long non-coding RNA are are implicated in the control of mono-allelic expression, but may more broadly be involved in chromosomal maintenance throughout the cell cycle.[118] Such loci can mediate long-range chromatin effects via RNAi, analogous to similar mechanisms shown for heterochromatin spread in S. pombe.[119] The relationship between replication timing and specific DNA loci with accompanying non-coding RNA, demands further investigation, as the extent of these mechanisms across all mammalian chromosomes is unknown.

In aggregate, these studies suggest that the activity and timing of both transcription and replication origin activation may restrict and define each other. These studies further imply that replicators determine replication initiation through two mechanisms: 1) containing singular DNA sequences, which are more easily unwound, might orchestrate unique chromatin conformations (e.g. create nucleosome-free regions or participate in particular distal interactions) and therefore facilitate initiation events; 2) recruiting sequence-specific proteins that facilitate origin activation and possibly modify local and distal interactions on chromatin. Understanding the epigenetic determinants of replication domains, replicators, and patterns of local origin activation can reveal mechanisms that control DNA replication initiation in metazoans.

E. Concluding remarks

Chromatin architecture, which defines the replication profile, is established during chromosome decompaction following mitosis. The scaffold of structural proteins that inhabit nuclear space intrinsically assist in the looping of chromatin that is required for replication competency. The studies outlined above highlight the importance of chromatin-associated proteins in regulating global and local chromatin structure. These studies suggest that genome topology and chromatin looping are functionally critical for facilitating chromatin-templated processes, including DNA replication. In addition, replicator sequences are implicated in organizing chromatin and orchestrating replication initiation events. The emerging picture suggests that the spatial and temporal organization of DNA replication reflects many levels of nuclear architecture, from the structural components that shape the nuclear matrix to molecular interactions dictated by single replicators. The molecular determinants that affect each level of regulation are just beginning to be elucidated. Clearly, future studies will reveal many more proteins with unique properties that affect each regulatory level. It would be especially intriguing to elucidate if and how replication profiles are affected by the activity of large clusters of transcriptional enhancers (super enhancers)[120], given the clustering of active replication into observable replication foci and the associations between cell-identity and replication timing domains. Higher-order chromatin structure, along with replication timing, have been suggested to affect the single nucleotide substitution mutation frequency in both normal and cancer genomes[121], which may contribute to disease progression. Understanding the common and unique features of replication profiles in a variety of cells can provide insights into the replication process and uncover mechanisms that regulate DNA replication in health and disease.

Acknowledgments

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Highlights

- DNA replication initiation is regulated by genetic and epigenetic factors

- Non-histone structural proteins facilitate and modulate DNA replication

- DNA elements that mediate cis interactions organize DNA replication

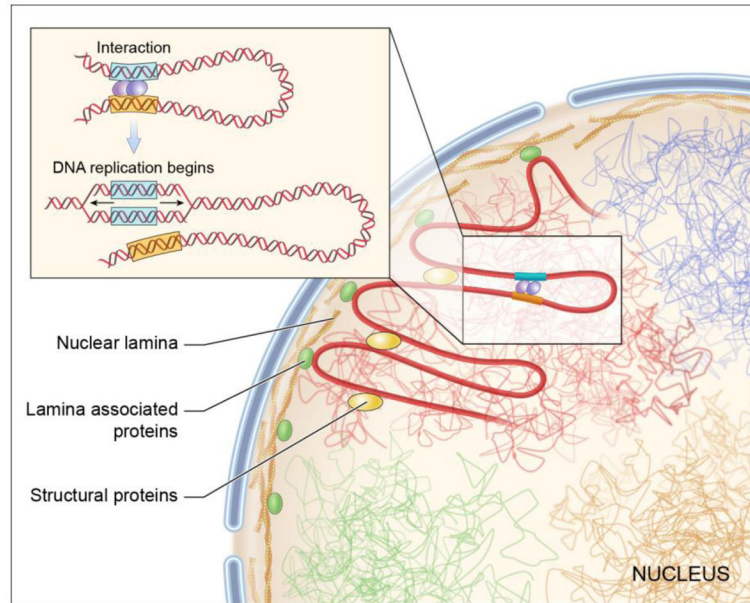- Conversely, replicator sequences can modify chromatin structure

Figure 1. Chromatin modifiers mediate interactions at replication origins
During interphase, chromatin in the nucleus is packed in chromosome territories (represented by distinct colors). Chromosomes are anchored by the nuclear lamina (orange fibers) with particular chromosomal regions interacting with lamina-associated (green) and other structural proteins (yellow). These interactions anchor intra-chromosomal loops held together by replication-associated protein complexes (purple) that could facilitate distal interactions among replication origins and regulatory regions (orange and blue lines - inset) to initiate DNA replication.