The activities of eukaryotic replication origins in chromatin

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Abstract

DNA replication initiates at chromosomal positions called replication origins. This review will focus on the activity, regulation and roles of replication origins in \textit{Saccharomyces cerevisiae}. All eukaryotic cells, including \textit{S. cerevisiae}, depend on the initiation (activity) of hundreds of replication origins during a single cell cycle for the duplication of their genomes. However, not all origins are identical. For example, there is a temporal order to origin activation with some origins firing early during the S-phase and some origins firing later. Recent studies provide evidence that posttranslational chromatin modifications, heterochromatin-binding proteins and nucleosome positioning can control the efficiency and/or timing of chromosomal origin activity in yeast. Many more origins exist than are necessary for efficient replication. The availability of excess replication origins leaves individual origins free to evolve distinct forms of regulation and/or roles in chromosomes beyond their fundamental role in DNA synthesis. We propose that some origins have acquired roles in controlling chromatin structure and/or gene expression. These roles are not linked obligatorily to replication origin activity per se, but instead exploit multi-subunit replication proteins with the potential to form context-dependent protein–protein interactions.

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1. Eukaryotic replication origins: overview and background

The goal of this review is to summarize selected experiments that provide evidence for roles of chromosome context and chromatin structure in regulating the activity of eukaryotic replication origins. The DNA sequences required for replication origin activity have been extensively defined and characterized in only one eukaryotic organism, the budding yeast \textit{Saccharomyces cerevisiae}. In addition, two recent independent and complementary whole-genome studies of DNA replication in vivo have identified virtually every potential and active chromosomal replication origin in this organism \cite{1,2}. For these reasons, and the tractability of \textit{S. cerevisiae} as a genetic and biochemical model for DNA replication, many of the mechanistic ideas we have about chromatin structure and its impact on the activity of replication origins have come from studies in budding yeast. Therefore, this review will focus primarily on experimental observations made in \textit{S. cerevisiae}, but where appropriate, such observations will be related to other organisms.

This section provides a background on yeast replication origin activity and organization (Sections 1.1 and 1.2) and the steps in replication initiation that could be affected by chromatin structure (Section 1.3). Section 1.4 considers the potential impact of origin activity and the organization and regulation of origins within the genome on both chromosome replication and other chromosomal functions.

1.1. Replication of the eukaryotic genome uses many individual replication origins

Extensive mechanistic information is available concerning the steps of DNA replication in the model prokaryote \textit{E. coli}, and many studies indicate that several aspects of \textit{E. coli} DNA replication serve as paradigms for eukaryotic DNA replication. For example, the replicon model for DNA replication postulated the existence of “initiator proteins”
that interacted with “replicator” DNA elements to direct duplication of the prokaryotic genome. Compelling support for this model came from elegant studies of the DNA sequences and proteins that controlled the initiation of DNA replication of the *E. coli* genome [3]. These classic studies provided powerful guides for identifying the DNA sequence elements and proteins critical to replication initiation in eukaryotes [4,5]. It is now well established that both prokaryotic and eukaryotic cells depend on specific DNA binding proteins to mark particular genomic regions as sites to initiate DNA replication.

However, significant differences in both the mechanisms and regulation of genome replication in prokaryotes and eukaryotes exist, and one of the most obvious is the number of replication origins that are used to duplicate the genetic material. *E. coli* uses a single replication origin to duplicate its entire 4.6-Mb genome. In contrast, eukaryotic cells initiate DNA synthesis from hundreds to thousands of replication origins distributed over the multiple chromosomes that constitute the genome. For example, in budding yeast, chromosome III (0.32 Mb) uses 11 origins for its duplication while chromosome X (0.75 Mb) uses about 20 [1,2,6]. Therefore, multiple replication origins per chromosome is a fundamental and defining feature of the eukaryotic genome (Fig. 1).

1.2. Individual eukaryotic origins are functionally distinct

The *S. cerevisiae* genome contains approximately 400 replication origins, and each individual origin is distinct in terms of efficiency and activation time during the DNA synthesis phase (S-phase) of the cell cycle. Replication origin efficiency refers to the frequency at which an origin initiates DNA replication (fires) within a population of cells. In yeast, many origins are efficient, firing in virtually every dividing cell within a population. However, some replication origins are less efficient, initiating in fewer than half the dividing cells within a population. Thus, for some chromosomal fragments, the direction of fork migration during DNA synthesis may change from one cell division to the next (Fig. 2).

Individual replication origins fire at characteristic and reproducible times during S-phase. For example, the yeast origins *ARS305* and *ARS607* fire shortly after cells have entered S-phase and are considered “early” origins, whereas the yeast origin *ARS1* fires during the first half of S-phase but several minutes after *ARS305* [1]. Replication origins such as *ARS501* or the cluster of origins on chromosome XIV (*ARS1411, 1412, 1413 and 1414*) fire near the end of S-phase and are considered “late” origins [7,8]. A recently constructed temporal replication map for the yeast genome

![Diagram](image)

Fig. 1. The diagram depicts the temporal replication pattern of yeast chromosome VI showing many but not all of the origins on this chromosome. Origins near the centromere fire earlier than those near the ends of this chromosome. For a more complete description of the replication of this and other yeast chromosomes that focuses on distilling information from the genome wide studies [1,2], see the short review by Newlon and Theis [70].
indicates that in general, most early origins are positioned toward the central portion of yeast chromosomes, while late firing origins are positioned nearer the telomeres such that the central portion of chromosomes replicate before the ends [1] (Fig. 1).

There is not a consistent relationship between replication origin efficiency and the time at which an origin fires during S-phase—some late firing origins are efficient and others are not [9,10]. Some late origins are inefficient because they are located near another earlier firing origin. The earlier origin establishes a replication fork that replicates the later origin before it has a chance to fire [11]. Presumably, this inactivates the competent initiation complex assembled at the late origin and the mechanisms that prevent re-replication of DNA during a single eukaryotic cell cycle prevent the late origin from acquiring the capacity to fire until the next S-phase (Fig. 2A and Ref. [12]). Direct experiments support a role for such an “origin interference” mechanism in origin efficiency [13–15]. Specifically, if an earlier firing neighbor origin is inactivated, the normally inefficient origin initiates more frequently during S-phase, but the time at which it fires does not change (Fig. 2B). Other origins, regardless of the time during S-phase when they fire, may be inherently inefficient because they fail to assemble the multi-protein complex necessary to mark them as replication origins [2]. Several independent mechanisms may affect origin efficiency or timing including DNA sequence features intrinsic to the particular origin. For many origins it is clear that chromosome context and/or specialized chromatin structures play a role (Section 2).

1.3. Mechanism of replication initiation

1.3.1. Sequence requirements of replication origins in S. cerevisiae

The first yeast origins were identified as small chromosomal regions (DNA elements of a few hundred base pairs) that gave extra-chromosomal circles (plasmids) the ability to replicate autonomously [16]. Later experiments using molecular techniques for direct origin mapping [17] indicated that these autonomous replicating sequences (ARS) coincided with bona fide replication origins in their native chromosomal contexts. Because of this history, origins in yeast are named ARS followed by a number that most often designates their chromosomal position.

There are only a few individual ARS elements among the ~400 that have been characterized systematically by comprehensive linker-scan mutational analysis, so it is not possible to describe a single common organizational structure for a yeast origin. However, the analyses that have been completed suggest that many origins may share some common features, including a conserved A-element and B1-element that form the Origin Recognition Complex (ORC) binding site (Fig. 3A). ARS1 is ~150 bp and consists of four functionally defined modular elements: an A-element found in most yeast origins, including ARS305 and ARS307 [20–22], and a series of B-elements, B1, B2 and B3 that lie 5’ of the A-rich strand of the A-element [18]. Mutation of the A-element in any of the ARSs shown in Fig. 3A abolishes origin activity. However, the A-element
is not sufficient; each of these origins also requires at least one other B-element for origin activity. However, in contrast to the A-element, the B-elements vary considerably between origins in terms of their sequences, number and distance from the A-element (Fig. 3A). Studies of ARS1 indicate that ORC contacts both the A- and B1-elements and it is probable that an A- and B1-element together form a bipartite recognition site for ORC in most origins [23–25]. The ARS1 B2-element may contribute to the loading of the six-subunit MCM (mini-chromosome maintenance) helicase. In ARS1, the B3-element contains a binding site for the transcription factor Abf1p; many origins do not contain Abf1p binding sites, including ARS305 and ARS307, but may contain binding sites for other as yet unidentified factors [20–22]. Finally, although the A-element is conserved in most replication origins, some origins contain multiple near matches to the A-element that must be collectively mutated to abolish origin activity [26]. These “compound” origins have not been characterized as systematically as the ARS elements pictured in Fig. 3A and so their organization is less clear. However, their existence indicates that not all origins in yeast necessarily share the structure suggested by the ARSs in Fig. 3A.

The sequences required for replication initiation are termed replicators whereas the positions at which replication initiates (origin unwinding and priming of DNA synthesis) are termed origins. A PCR-based technique called Replication Initiation Point (RIP) mapping can map the position of the start of leading strand synthesis to a single nucleotide [27]. RIP mapping experiments of ARS1 suggest that in yeast, replicators and origins are essentially coincident. For ARS1, leading strand synthesis begins at a single nucleotide near the binding site for that organism’s ORC [29]. Thus, ORC may mark the site of origin unwinding and the start of leading strand synthesis in all eukaryotic organisms. However, it is important to note that only a limited number of origins have been examined and the sequences required for full replicator function may lie some distance from the origin site for some origins [30–33].
1.3.2. Proteins and steps required for replication initiation

A current model for replication initiation at a yeast origin is shown (Fig. 3B). Experiments performed in a variety of organisms indicate that the proteins and steps required for initiation are largely conserved in eukaryotes even though the sequence elements required for origin activity are not. (For comprehensive reviews see Refs. [4,34].)

The replication initiation cycle can be divided into at least four phases. The first is formation of an origin DNA–ORC complex [19]. ORC binds to an origin via direct protein–DNA contacts between at least four of its six different subunits and nucleotides present in both the A- and B1-elements [24]. ORC binds ATP and is an ATPase, and origin binding by ORC requires ATP binding but not hydrolysis [19,35]. In vivo footprinting and chromatin binding experiments indicate that ORC is bound to its target sites throughout most if not all of the cell cycle [36,37]. Thus, one of ORC’s major roles is to mark origins as the sites of replication initiation. ORC also recruits additional initiation proteins during G1 that together form the pre-replicative complex (pre-RC), a multiprotein DNA complex necessary for origin firing. ORC may have roles in the subsequent events of replication initiation [38,39] including later steps that contribute to elongation and genome integrity [40].

In the second phase, ORC promotes assembly of a multiprotein complex called the pre-replicative complex (pre-RC) at origins. The pre-RC was defined operationally as the change in the in vivo footprint that can be detected at origins during the G1 phase [36]. Formation of the pre-RC requires minimally ORC, Cdc6p, Cdt1 and the MCM complex [4]. Cdc6p directly interacts with ORC and is required together with Cdt1p to “load” MCM, the putative replicative helicase, at the origin in a reaction that requires ATP [41, 42].

In the third phase, additional proteins join the pre-RC immediately prior to replication initiation. These include Cdc45p, the recently described four-subunit GINS complex and the replicative DNA polymerases [4,43]. Cyclin-dependent kinase (CDK) activity is required for Cdc45p to associate with the pre-RC [44], and the association of Cdc45p with origins coincides with their time of activation—late origins recruit Cdc45p later in S-phase than earlier origins [45]. The Cdc7-Dbf4p kinase also associates with chromatin just prior to S-phase and interacts with ORC [46,47]. Cdc7-Dbf4p may activate the MCM helicase or the Polo-primase at origins. These sequential steps involve at least 20 different polypeptides that must assemble coordinately within a chromatin context at hundreds of replication origins throughout the genome for S-phase to commence.

The fourth phase, origin firing, signals the beginning of S-phase, and involves localized DNA unwinding at the origin and the start of DNA polymerization. The mechanism of origin unwinding and the initiation of DNA synthesis are not understood at eukaryotic origins and may occur simultaneously with the association of some of the factors outlined in phase three. For instance, phosphorylation catalyzed by the Cdc7-Dbf4 kinase may promote conformational and/or polypeptide changes within the multiprotein complex bound to origins that are necessary for DNA unwinding and the start of DNA polymerization. Finally, CDK activity, which remains high during the S- and G2/M-phases, negatively regulates ORC, MCM and Cdc6p to prevent the assembly of new pre-RCs [12,48,49,50]. Therefore, CDK activity is required both to activate the pre-RC’s by promoting the binding of Cdc45p (third phase) and simultaneously prevent new pre-RC assembly (and re-replication within a single S-phase) until the next G1 phase.

In summary, replication initiation at a given origin is a complex process requiring multiple proteins binding in several steps. Each individual step could conceivably be enhanced or inhibited by particular chromatin environments.

1.4. Why so many replication origins?

The number of replication origins per chromosome is not merely a consequence of the larger genomes in eukaryotes. S. cerevisiae has a 12-Mb genome distributed over 16 chromosomes, and therefore each single yeast chromosome is considerably smaller than the 4.6-Mb E. coli genome. Yet yeast replication origins occur on average every 20 to 40 kb, a hundred times more densely distributed that one would predict by comparison to the E. coli genome. The vast difference in fork migration rates between eukaryotes and prokaryotes may explain in part the need for multiple replication origins per eukaryotic chromosome. DNA replication forks migrate at rates about 30 times slower (fork migration rates of ~ 3 kb/min [1,50] in yeast compared to E. coli (fork migration rates of ~ 100 kb/min [51]), yet under optimal growth conditions DNA synthesis periods are comparable (~ 40 min) and yeast cells divide only four to five times more slowly than E. coli. Eukaryotic chromatin is often invoked as an explanation for the slow migration rate of DNA replication forks, but a direct experimental test of this idea is not yet possible. In particular, chromatin templates that recapitulate the complexity of biological chromatin are still in early stages of development and structural characterization [52,53]. In addition, the bacterial chromosomes are also packaged with and condensed by various proteins. Although these proteins differ from the highly conserved and well-described eukaryotic histone octamer, it is difficult to imagine that the condensation of the prokaryotic genome might not present a similar challenge to migration of a replication fork. Thus, it is not clearly established whether or how chromatin structure itself may slow replication fork migration in eukaryotes. Regardless, the use of multiple initiation events per chromosome probably compensates for slower fork migration rates in maintaining an efficient rate of genome duplication and S-phase progression in eukaryotic cells.

Despite the contribution that multiple origins per chromosome may make to efficient genome duplication in S.
cerevisiae, there are still many more replication origins than necessary. For example, based on the values discussed above, S. cerevisiae would need about 100 replication origins to duplicate its genome at a rate sufficient to accommodate its S-phase, about four times less than the current estimates for origin numbers in this organism [1,2]. In addition, direct experimental evidence indicates that yeast chromosomes have more origins than they need for their timely replication during the S-phase; several origins on chromosome III can be deleted without substantially affecting the ability to inherit faithfully this chromosome during cell division [54]. Therefore, for the purpose of genome duplication, yeast replication origins are redundant. Such redundancy might permit individual origins to evolve more specialized functions within chromosomes that are distinct from a dedicated role in DNA synthesis.

Although there is no compelling evidence that eukaryotic replication origins have any direct role beyond initiating DNA synthesis, it is clear from studies in several organisms that one of the central and conserved protein complexes required for origin activity, ORC, has evolved to perform functions distinct from its role in DNA synthesis [38]. For example, in budding yeast, ORC has an important role in establishing chromosomal domains of repressive (silent) chromatin at the cryptic mating-type loci [55]. ORC interacts with Heterochromatin Protein 1 (HP1) in both Drosophila and Xenopus and is concentrated within defined heterochromatin regions in the Drosophila genome [56]. The human Orc6p subunit is localized to kinetochores and the cleavage furrow during M-phase and so may have roles distinct from its role in replication in human cells [57]. Thus ORC, and perhaps other origin proteins, have non-DNA synthesis roles in regulating chromatin structure in yeast and metazoans.

Replication origins may also have important albeit indirect non-DNA synthesis roles in the genome by virtue of their central role in establishing the temporal and physical genome replication maps. In particular, studies of S. cerevisiae indicate that the temporal and physical pattern of chromosome replication is established primarily by the activity of replication origins [1], indicating the potential to alter a chromosome’s replication map by controlling origin activity. In terms of the temporal map, the time during S-phase when a chromosomal region is replicated correlates strikingly with chromatin structure and gene activity in many organisms [58]. Transcriptionally inactive genes and/or heterochromatin are often the last portions of the genome to be duplicated. This is perhaps because origin activation within heterochromatin is delayed and therefore a fork that came from a distant origin replicates heterochromatin. Even in budding yeast the rare portions of the genome controlled by chromatin structures analogous to multicellular heterochromatin are extremely late replicating [1]. A recent chromosome-scale study of replication timing in Drosophila melanogaster Kc cells reveals a significant correlation between late replication and transcriptional quiescence [59]. However, this correlation is not absolute either in Drosophila or other organisms [59–61]. Further examinations of the relationship between origins, replication timing and chromatin structure will clarify the view of the mechanisms of and purpose for a defined temporal map of genome replication in both microbes and metazoans.

Initiation at an individual origin establishes a defined asymmetric physical pattern of replication to flanking chromosomal regions because of the inherent 5' to 3' polarity of DNA synthesis. A fragment replicated by a fork moving from a given origin gives rise to one daughter molecule as a result of lagging-strand synthesis and another daughter molecule as a result of leading-strand synthesis. Thus, the two daughter molecules, although identical in sequence, have a different molecular history (Fig. 2). This naturally arising asymmetry between daughter molecules is implicated in regulating mating-type switching in S. pombe [62,63] and contributes to strand bias in mutagenesis in S. cerevisiae[64]. Regulating the activity of individual origins is an obvious mechanism to change the direction from which a particular chromosomal region is duplicated, and the different physical replication patterns that would result could affect gene expression and differentiation through chromatin structure if particular chromatin assembly or modification pathways were differentially partial to DNA arising from either leading- or lagging-strand synthesis [63,65,66].

Another simple consequence of having multiple origins is the ability to control the rate of the cell division cycle by controlling the number of active replication origins. This could be important in multicellular organisms. In particular, during embryogenesis of multicellular organisms, the earliest cell cycles are rapid and may depend in part on the ability to use many sequences as replication origins [67–69]. By reducing the number of active origins per chromosome later during development, the S-phase and hence the entire cell division cycle could be reduced to a rate more compatible with cell differentiation that requires the synthesis of more specialized and complex cellular structures. The possible relationship between reducing the number of origins and developmental fates remains to be established.

2. Chromosomal context and chromatin structure modulate origin activity

This portion of the review is divided into four subsections, each of which focuses on different evidence supporting roles for chromosome context or chromatin structure in the activity of budding yeast replication origins. In Section 2.1 we will review the evidence for chromosomal position effects on the activation time and efficiency of yeast replication origins. In Section 2.2 we will summarize recent studies that implicate specialized chromatin proteins in controlling a subset of origin position effects. In Section 2.3 we will discuss a recent study that provides evidence that the acetylation state of nucle-
osomes surrounding an origin can regulate origin activation time. In Section 2.4 we will present selected studies that provide evidence for nucleosome positioning in controlling cellular origin activity.

2.1. Chromosome position influences the efficiency and timing of origin activation

A number of observations indicate that origin activity in yeast is subject to a position effect that resembles classic chromatin-mediated position effects on gene transcription. An early indication of this phenomenon was that some sequences that provide ARS activity to plasmids (and thus function well as replication origins in this context) fail to function as chromosomal origins in their native context [11,70,71]. Thus, a DNA sequence element with the inherent capacity to function as an origin can be completely inhibited in particular chromosomal environments. An example of a chromosomal region capable of exerting such a position effect is the cryptic mating-type locus HML—a normally active origin (ARS305) placed within HML becomes inactive [72].

Position effect phenomena can also account for the activation time of efficient but late firing origins. For example, ARS501 is an efficient late origin in its chromosomal context but when it and the surrounding ~ 15 kb of chromosomal sequence are moved to a plasmid, the ARS501 origin fires early [7]. Thus, sequences many kilobases distal from ARS501 are somehow influencing the time during S-phase at which this origin fires. Significantly, moving an earlier firing origin, ARS1, near ARS501 on the chromosome causes ARS1 to fire later and at a time similar to origin activation for ARS501. The position of ARS501 relative to the telomere (27 kb from the right telomere of chromosome V) is important, indicating that telomeres can exert fairly long-range position effects on origins [73]. As stated earlier, based on whole-genome replication timing analysis in yeast, origins located proximal to telomere ends (~ 40 kb or closer) are often activated later in S-phase [1]. Recent studies have identified proteins required for the telomere-position effect (TPE) on replication origins (Section 2.2).

ARS501 is a late replication origin because of its proximity to telomeres, but the cluster of late origins on chromosome XIV (ARS1411, 1412, 1413 and 1414) are over ~ 150 kb from the telomere. Also in contrast to ARS501, the late activation time for at least two of these origins, ARS1412 and ARS1413, can be recapitated in a plasmid context [8], supporting the idea that their late activation is telomere-independent. However, in the case of both ARS1412 and ARS1413, considerable amounts DNA sequence flanking the intrinsic origin sequences are required to fully recapitate the late origin activation time, and it appears that there is not an easily identifiable discrete sequence element that determines their late activation. Thus, for both a telomere-proximal late origin and internal late origins, large chromosomal regions distinct from the intrinsic ARS elements are required for determining their late S-phase activation.

Although most of the existing data for inefficient and/or late activating origins support a role for large regions external to the origin in controlling the origin’s distinguishing activation properties, one recent exception is worth noting. In particular, studies of one extremely late activating origin, ARS301, suggest that this origin is inefficient on the chromosome regardless of its position [72]. ARS301 is one of the few origins in yeast closely associated with elements called silencers present at the yeast cryptic mating-type loci HMR and HML and necessary for assembling the heterochromatic-like state at these loci (reviewed in Ref. [74]). In its normal location at HML, ARS301 is inactive in part because it is programmed to fire so late that it is replicated by a replication fork from a more distant origin before it ever has a chance to fire [75]. However, ARS301 functions as an origin in a plasmid context, although it is important to note that even in this context it is inefficient. HML can exert a position effect on an origin—substituting ARS301 with the active and early firing ARS305 at HML causes ARS305 to become chromosomally inactive. And yet, surprisingly this position effect cannot explain fully the late and inefficient activity of ARS301. Specifically, replacing the active and early firing ARS305 with ARS301 does not lead to early and/or efficient activation of ARS301. Rather, ARS301 remains inactive or, in the appropriate mutant backgrounds that allow extremely late origins to fire [76], extremely late firing. Thus, for some origins intrinsic sequences may also contribute to their efficiencies or activation times [72].

Even if intrinsic sequences contribute some of the distinctive features to individual origins, the significant effect of chromosomal context on origin activation is well established. The phenomenon is so reminiscent of classic chromatin-mediated position effect on transcription that it is not surprising that some of the proteins required for position effects on transcription in yeast also have effects on origin activation (Section 2.2).

2.2. “Specialized” chromatin structures and origin activation

2.2.1. The Ku proteins are required for late activation of ARS501 and other telomere-proximal origins

A recent study provides evidence that the telomere-binding Ku protein complex (Ku) controls the late activation of the telomere-proximal ARS501 origin and probably other telomere-proximal origins [77]. Ku has critical functions in the repair of double-stranded DNA breaks and also binds and functions at telomeres, the defined ends of linear eukaryotic chromosomes [78,79]. Significantly, Ku, consisting of the yeast Ku proteins yKu70 and yKu80, also contributes to the specialized chromatin structure that forms at telomeres and causes a TPE on gene transcription [80–82]. TPE describes the phenomenon whereby RNA polymerase II genes placed within several kilobases of telomeres are repressed by a
mating-type loci repressing copies of mating-type genes present at the silent (Sir2p, Sir3p and Sir4p) initially identified for their roles in three of the silent information regulator (SIR) proteins [83]. TPE requires a number of different proteins including the mechanism akin to position-effect variegation in Drosophila [83]. TPE requires a number of different proteins including those of the silent information regulator (SIR) proteins (Sir2p, Sir3p and Sir4p) initially identified for their roles in repressing copies of mating-type genes present at the silent mating-type loci HMR and HML [84]. Ku is required for TPE because it helps recruit the Sir proteins that function together to assemble a heterochromatin-like structure near the ends of chromosomes [82,85]—the SIR-dependent heterochromatic-state nucleates at telomeres and “spreads” into the chromosome up to 10 kb [86,87]. ARS501, about 27 kb from the telomere is beyond the limit of the SIR-dependent TPE and yet its late activation is telomere position-dependent.

In a yeast strain containing a deletion of the yKu70 gene the origin activation time for ARS501 shifts to a significantly earlier time in S-phase [77]. This deletion also advances the replication times for other regions near telomeres but not the internally positioned chromosome XIV ARS cluster, consistent with a role for Ku in controlling the late activation time of telomere-proximal origins. One postulate is that Ku70/Ku80 contributes to a specialized chromatin environment near chromosome ends that extends into the chromosome 30–40 kb from the telomere that may require association with the nuclear periphery, another property of telomeres that requires the Ku complex [80]. And yet this chromatin structure is distinct from the SIR-dependent chromatin structure at the ends of telomeres that also requires the Ku complex. Specifically, a deletion of SIR3 has little effect on the origin activation time of ARS501 even though it abolishes TPE and does contribute to the activation of origins within the zone that is subject to SIR-dependent TPE [88]. Thus, the Ku-mediated position effect on late origin activation is exerted over a large distance from telomeres (~35 kb) and in this regard is distinct from the SIR-dependent chromatin-mediated TPE that is exerted over a shorter distance (~10 kb) from telomeres.

2.2.2. SIR effects on origin activation

As stated above, the Sir proteins function in the assembly of specialized chromatin structures analogous to heterochromatin. Several recent studies indicate that SIR-dependent chromatin can affect the activity of replication origins. Before discussing these studies, we present a brief summary of SIR-dependent chromatin formation. Several recent reviews offer more detailed and comprehensive information on this subject [89–91].

SIR-dependent chromatin structures, also referred to as silent chromatin, repress the transcription of fully intact RNA polymerase II transcribed genes. Silent chromatin forms at three different regions in the yeast genome (reviewed in Ref. [91]): (1) the ends of telomeres, (2) the nucleolar rDNA locus and (3) the cryptic (silent) mating-type loci HMR and HML. All three types of silent chromatin require the Sir2p, the founding member of a conserved family of NAD-dependent protein deacetylases [92]. Current models propose that Sir2p deacetylates nucleosomes within all three loci and that this modification contributes directly to the formation of a heterochromatin-like structure. All three forms of SIR-dependent silent chromatin require a nucleation event whereby specific multi-protein–DNA complexes recruit the SIR protein(s) to the locus. The recruitment proteins and precise interactions necessary for nucleation differ at the three different regions [93]. After the nucleation step, the silent state physically “spreads” from the nucleation site to assemble a larger domain of silent chromatin encompassing an array of nucleosomes. At telomeres and the HM loci, the spreading mechanism appears similar—current models posit that Sir2p deacetylates N-terminal histone tails within a nucleosome near the nucleation site, Sir3p then binds the deacetylated nucleosome [94] and recruits an additional complex of Sir2p and Sir4p and so on until an entire array of nucleosomes is encompassed into the silent state [91,95]. At rDNA, Sir3p and Sir4p are not required for the silent chromatin-state but Sir2p certainly is. Remarkably, the directional movement of the RNA polymerase I machine that transcribes the rDNA in this locus is also required for the SIR2-dependent spreading of the silent state [96]. Regardless of the precise mechanisms, all three silent regions require the deacetylase activity of Sir2p and additional proteins that assemble a larger domain of higher-order repressive chromatin.

Strong evidence for a SIR-chromatin effect on origin activity of ARS elements associated with telomere-repeat sequences comes from a study focused on understanding the late replication time of telomeres using the right telomere of chromosome V (ARS501, discussed above, is ~35 kb from this telomere) [88]. Telomeres consist of highly repetitive sequences (telomere repeat sequences) at their extreme ends—these are the sites of SIR-nucleation. Next to these lie sub-telomeric repeats, the X and Y’ elements. Each of these elements contains an ARS but this study demonstrates that the X element ARS on chromosome V is an inactive origin due to the SIR-dependent chromatin state in this region. Because of the repetitive nature of the X and Y’ regions and their presence at most telomeres, it is not simple to examine X or Y’ origin activity directly by 2-D origin mapping. However, the region just next to these repeats is unique and, strikingly, in a strain containing a deletion of SIR3, this region replicates 9 min earlier than in a SIR3+ strain. Several careful experiments provide compelling evidence that this is because the Y’ ARS is activated earlier during S-phase and the X ARS is now an active origin. In contrast, the activation time of ARS501 appears unaffected by a deletion of SIR3. If ARS1 is engineered next to telomeric repeats (essentially replacing the Y’ and X elements) it fires less efficiently and much later than it does at its native position, but again, deletion of SIR3 allows earlier and more efficient activation of ARS1 at this location. Thus the SIR3 effect on origin activity is not origin-specific as expected for a factor that mediates a position effect.
Together the SIR3 and Ku study let us picture two mechanisms that control telomere-associated position effect on origin activity. First, origin activation for origins close to the telomere (within 10 kb) is probably controlled by the same SIR-dependent position effect responsible for TPE. Second, the telomere-end binding protein complex Ku additionally controls the late activation of more distal telomere-associated origins (35–40 kb) by a SIR-independent mechanism that may require chromosome association with the nuclear periphery.

Another recent independent study indicates that the HMR-E silencer, which is the silencer that nucleates the assembly of SIR-dependent silent chromatin at HMR can, when placed in two tandem copies near the active early origin ARS305, cause ARS305 to fire late [97]. The late activation of ARS305 requires the SIR1 gene, the only SIR whose function in silent chromatin is confined to the HMR and HML loci. In addition, the late activation time of ARS305 correlates with the ability of either the two copies of HMR-E or a tethered Gal4-Sir4p to transcriptionally silence an adjacent gene. Thus SIR-dependent chromatin can exert a position-effect on both transcription and origin activation.

The final SIR effect on origin activity we will discuss is the effect of SIR2 on origin activation within the rDNA cluster [98,99]. As discussed above, a type of silent chromatin forms within the rDNA cluster that can repress heterologous RNA polymerase II genes placed between rDNA genes, but this chromatin is distinct from the SIR-dependent chromatin at the HM loci and telomeres in that it requires only one of the SIR genes, SIR2. Nevertheless, SIR2 is required for regulating both the position effect on transcription and origin activation within the rDNA and so these data are most appropriately discussed here. The rDNA locus is located within the nucleolus and contains 100–200 copies of a 9.1-kb repeat that encodes the 5S and 35S rRNA genes that are divergently transcribed by RNA polymerase III and RNA polymerase I, respectively. Each repeat contains an ARS element located between the two rRNA genes and a replication fork barrier is located to the left of the ARS element such that forks can only proceed to an adjacent repeat in the rightward direction. Only 20% of the ARS elements are active in any given cell cycle, indicating that some mechanism is preventing the activation of many origins (reviewed in Ref. [99]).

A recent study that uses the technique of molecular combing to examine replication of individual DNA strands indicates that SIR2 inhibits the activation of many origins within the rDNA repeats [98]. In wild-type (SIR2) cells, only 25% of the origins are active within a single cell cycle as measured by molecular combing. In addition, this single molecule technique indicates no epigenetic control of the origins that fire within successive cell cycles—that is, an origin active in one cell cycle might be inactive in the next and vice versa. Large gaps (>60 kb) are present between actively replicating DNA in early S-phase, indicating that active origins are separated by several inactive origins. In contrast, in the absence of SIR2 (sir2Δ) almost twice as many origins are activated within the array. Thus, although SIR-mediated position effect within rDNA may employ different mechanisms than SIR-mediated silencing at the HM loci and at telomeres, it shares the ability to inhibit the activity of local origins.

Based on the association between SIR-mediated chromatin and the inefficiency or delay in origin activation, it is somewhat surprising that the inactive origins present at the HM loci are not subject to SIR-control. Both HMR and HML contain ARS elements that are closely associated with the silencer elements that nucleate the assembly of SIR-dependent silent chromatin at these loci. As described above, at HML the ARS elements are inactive on the chromosome but are functional as plasmid origins. The HMR silencers are associated with ARS elements that act as extremely inefficient chromosomal replication origins [100,101], and the HMR-E silencer-associated origin fires late in S-phase in the few cells in which it is active [102]. Therefore, both the HML and HMR loci are replicated late during the S-phase, primarily by forks coming from distant origins. Interestingly, however, and in contrast to the effect of SIR-dependent chromatin on the sub-telomeric repeat ARSs, the origin activity at HMR and HML is not affected dramatically by mutations in SIR genes. In a strain lacking SIR4, HML origins remain inactive even though the SIR-dependent chromatin state, as measured by gene activation and chromatin-binding experiments on the other Sir proteins [71], is abolished. Similarly, in a strain lacking SIR2, the HMR-E origin activity is only slightly enhanced and the locus is still replicated late in S-phase [101,102]. Thus, other features at these loci contribute to the inefficiency and late activation of their associated replication origins, possibly even features inherent to the sequences in these regions. Given that these loci are located near the ends of chromosome III but out of the range of the TPE, it is possible the yeast Ku proteins contribute to the late replication of these loci. Regardless, it is noteworthy that SIR-mediated chromatin can affect replication origin activity in some but not all contexts in which it forms.

The mechanisms by which SIR-mediated chromatin can inhibit origin activity are unknown. However, the Sir2p is a protein deacetylase, and recent evidence indicates that histone acetylation can promote origin activity (Section 2.3). Thus, the SIR-mediated effect on origin activity may reflect a more general role for the acetylation state of histones surrounding origins on the efficiency and timing of their activation. Intriguingly, recent studies indicate that the Sir2p deacetylase may inhibit origin activity on a more extensive scale that includes origins outside of the rDNA and telomeric domains, raising the possibility that Sir2p itself might have more global roles in negatively regulating replication initiation (Donald Pappas and M. Weinreich, unpublished observations).
2.3. Histone acetylation advances the activation time of origins

Studies in the past decade have established a role for posttranslational histone modifications in the control of eukaryotic transcription in eukaryotes (discussed in Refs. [103,104]), and a recent study indicates that such modifications will also have critical roles in origin activation. Specifically the acetylation state of nucleosomes surrounding origins affects their activation time during S-phase, with increased levels of acetylation advancing origin activation time [105].

The first clue that the acetylation state of nucleosomes might influence origin activation came from studies of the role that one of the major histone deacetylases, Rpd3p, plays in the histone acetylation state of the yeast genome. Genome-wide studies show that deletion of RPD3 results in global changes in both histone acetylation and the steady-state mRNA levels of over 400 genes, consistent with the now well-established importance of these modifications in transcription [103,104]. Notably, in the absence of RPD3, the acetylation state of many intergenic chromatin regions increases [106]. Since most origins are also intergenic [2,107], it follows that the acetylation state of chromatin surrounding many origins should increase in cells lacking RPD3 and indeed chromatin immunoprecipitation experiments indicate that this is true for several individual origins [105]. Even more intriguingly, the origin activation time for each of these origins advances to an earlier time after the cells lacking RPD3 are released from a G1-cell cycle arrest, and the extent of advancement correlates well with the extent of increased acetylation. For example, in a strain lacking RPD3, the acetylation state of chromatin surrounding both ARS603 and ARS305 increases, but the increase is greater for ARS603. In turn, the activation time for both origins advances relative to the wild-type strain but the activation time for ARS603 advances to a greater degree. Consistent with the idea that Rpd3p deacetylates chromatin surrounding a large number of yeast origins, replication of the entire genome is advanced in a strain lacking RPD3. For example, 20 min after release from G1 arrest, few of the cells from a RPD3+ strain have acquired a 2C DNA content but one third to one half of the cells from a strain lacking RPD3 have. Normally, late replication origins, such as those in the ARS chromosome XIV cluster, may be particularly sensitive to genome acetylation status controlled by RPD3; such late origins behave as bona fide early origins in strains lacking RPD3 because they now escape the checkpoint inhibition of late origins in the presence of replication stress normally exerted by Rad53p [76] (Oscar Aparicio, personal communication).

These observations provide compelling evidence for a role for Rpd3p and the acetylation state of chromatin in controlling origin activation time on a rather global scale, but they do not address whether this control is direct. For example, the mRNA levels for hundreds of genes change in strains lacking RPD3. One might expect a similar effect on global origin activation even if only one of these genes encodes a protein that is limiting for origin activation. In addition, TAP-tag purification experiments suggest that Rpd3p and Cdc45p are in a common protein complex (http://www.yeast.cellzome.com). Since Cdc45p must bind origins prior to firing, one possibility is that Rpd3p directly inhibits Cdc45p and delays its association with origins and consequently origin activation. However, an experiment that targets increased acetylation to a single specific origin argues against these interpretations and for a more direct role of histone acetylation in enhancing origin activation time [105]. Specifically, targeting the GCN5 histone acetyl transferase (HAT) to a region near the late origin ARS1412 increases the level of acetylation of the chromatin surrounding ARS1412. This engineered version of ARS1412 with locally enhanced chromatin acetylation fires up to 20 min earlier than the normal ARS1412 from an otherwise identical yeast strain and, importantly, this earlier firing depends on the presence of GCN5, indicating that it is not a technical consequence of the DNA sequence changes near ARS1412 required to recruit this HAT. Together with the analysis of replication in strains lacking RPD3, this study provides compelling support for the view that the acetylation state of nucleosomes around an origin’s local environment has a direct effect on that origin’s activation time and that the Rpd3p deacetylase is a major negative regulator of origin activation in yeast.

The next level of experiments related to the role of nucleosome modifications in origin activation should delve into the types of chromatin modifications most permissive (or non-permissive) to origin activation and the steps of origin activation that are sensitive to an origin’s chromatin neighborhood. For example, are there specific patterns of histone acetylation that are particularly crucial to origin activation and are such patterns cell cycle-regulated? In terms of steps in origin activation that may be affected by chromatin, one hint may come from analysis of the Cdc45p’s association with origins [45]. As stated in an earlier section, origins recruit the Cdc45p at a time that correlates with their activation. And in strains lacking RPD3, Cdc45p loading is temporally advanced along with origin activation [105] (Oscar Aparicio, personal communication). Thus, Cdc45p loading that is essential for initiation may be a step that is directly enhanced by acetylated nucleosomes, but the mechanism by which this enhancement occurs is unknown. It is possible that acetylated nucleosomes around origins directly enhance the accessibility of an initiation protein, such as MCM, to Cdc45p or that they bind Cdc45p directly. However, it is possible that nucleosome acetylation enhances the binding or modification of another factor that in turn helps recruit Cdc45p. Reconstructing pre-RC assembly in the context of defined nucleosome
arrays may help address this issue, as will continual refinement of our understanding of the factors and modifications leading to changes in the pre-RC that occur just prior to initiation.

2.4. Nucleosome positioning and origin activity

In addition to histone modification, nucleosome movement and positioning, and the ATP-dependent complexes that can move nucleosomes and alter chromatin structure, have emerged as major factors in transcription regulation [108–110]. Several studies indicate that nucleosome positioning may regulate origin activity as well [111–113]. Studies of ARS1 indicate that both the A inconsistently binding site and ORC binding site (A-element) of this origin act as nucleosome boundaries, positioning nucleosomes outside of and flanking this origin. Mutations in the Abf1p nucleosome-free zone of yeast ARS1 origin activity [114]. Similarly, engineering a specific protein-binding site outside of ARS1 that results in positioning a nucleosome over the otherwise functional A-element reduces origin activity of ARS1 [112]. These data indicate that ARS1 is in a nucleosome-free zone and further that nucleosomes formed over essential origin sequences can inhibit origin activity.

A more recent biochemical study of ORC and Abf1p binding to ARS1 assembled into chromatin in an in vitro chromatin assembly extract confirms and extends these findings [113]. In particular, ORC and Abf1p bind to their target sites in ARS1 and precisely position nucleosomes around ARS1 in a pattern that recapitulates the nucleosome positioning observed at the ARS1 origin in vivo. Thus, the nucleosome-free zone of yeast ARS1 is recapitulated in a heterologous chromatin assembly extract (Drosophila S190 extract [115]) in the presence of purified ORC and Abf1p—no other yeast factors are required. In vivo, an intact A-element is required in both ARS1 and another origin, ARS307, to prevent a nucleosome from encroaching on the origin sequences. These in vitro and in vivo data confirm the inhibitory role for nucleosomes in origin activity and indicate roles for both ORC and Abf1p as potent nucleosome boundary or positioning factors.

Additional experiments provide evidence for a positive role for nucleosomes positioned outside but near the A-element of ARS1 by the binding of ORC [113]. Specifically, if a sequence-specific DNA protein-binding site is introduced at the A-rich strand of the A-element of ARS1, the nucleosome normally positioned by ORC adjacent to the A-element is displaced away from the origin in vivo. This displacement correlates with a reduction of ARS1 origin activity as measured by either plasmid stability or direct chromosomal 2-D origin mapping. Notably also, MCM, but not ORC, is significantly impaired in its ability to bind this ARS1 origin DNA in vivo as measured by ChIP experiments. A simple interpretation of these observations is that formation of the pre-RC requires both ORC and a nucleosome positioned by and next to ORC. Since MCM is also required for the elongation phase of DNA replication [116], additional cell-cycle restricted assays for pre-RC assembly are needed to distinguish whether the primary role of the positioned nucleosome is in pre-RC assembly during G1 or stability of MCM with chromatin during S-phase.

If nucleosome positioning can regulate origin activity, a reasonable prediction is that ATP-dependent chromatin remodeling machines that move nucleosomes can modulate the activity of at least some individual origins, particularly those that might lack the inherent nucleosome positioning features of ARS1. Given the observations described above, the ability of these complexes to re-position nucleosomes either over or around origins could conceivably either inhibit or enhance origin activity, respectively. To date, there is a paucity of directed and comprehensive studies examining the role of chromatin remodeling complexes on origin activity in yeast. Perhaps this is due to the difficulty of identifying useful mutant versions of these complexes (since these complexes also have critical roles in transcription) and/or appropriate origins. However, studies of viral mediated replication in vivo and in vivo indicate that chromatin-remodeling complexes can enhance origin activity [117].

In this study, origin activity was assessed in plasmid stability assays and several different ARSs were examined in the context of the same plasmid backbone. ARS1 and ARS307 do not require the SWI/SNF for full plasmid stability, but ARS121 does, even though ARS1 and ARS121 confer similar mitotic stabilities to the plasmid and ARS121 confers greater stability than ARS307. In mutant strains defective in SWI/SNF, ARS121 plasmid stability is reduced several fold whereas the stability of the ARS1 and ARS307 plasmids is unaffected. Interestingly, an ARS1 mutant plasmid containing a defect in this origin’s Abf1p binding site requires SWI/SNF for full stability, suggesting that a sequence-specific replication enhancer and the SWI/SNF complex play redundant roles in origin activity. In addition, a mutation in the B1-element that should reduce ORC’s affinity for ARS1 [23, 24] shows a similar enhanced dependence on SWI/SNF. These observations are consistent with the proposed role of ORC and Abf1p in positioning nucleosomes around but outside of ARS1—when this ability is reduced, ARS1 origin activity relies more heavily on the SWI/SNF chromatin-remodeling machine. A critical test of this idea requires nucleosome-mapping studies coupled with 2-D origin analysis of selected natural and mutant origins in wild type and SWI/SNF defective strains. Additional investigations may reveal a role for other chromatin remodeling proteins in origin activity as well.
3. Roles for ORC in chromatin structure

A role for ORC in establishing domains of specialized chromatin is now well established [38], and several independent studies indicate that this role is distinct from ORC’s role in replication initiation at origins [101,119–123]. A number of the mechanistic studies relevant to this issue have used the yeast silent mating-type locus HMRa and some of the more recent studies will be discussed here.

The elements that nucleate silencing at HMR, the HMR-E and HMR-I silencers, are closely associated with weak but nevertheless detectable origin activity, and an early postulate was that the silencer activity of this element depended on its origin activity [124]. Obviously, both origin and silencer activity require binding of ORC to its target site; mutant silencers or ORC alleles that reduce ORC binding sufficiently reduce both silencer and origin activity at HMRa [55,120,125]. However, it is clear that ORC’s roles as a replication and silencing protein are separable. In particular, it is possible to isolate ORC alleles that show silencing defects but no discernible replication defects. For example, the N-terminal ~230 amino acids of the Orc1p subunit govern a direct and physical interaction between the Sir1p, one of the four Sirs essential for silencing HMR, and ORC and yet this N-terminal region is unnecessary for ORC1’s essential role in replication [123]. Specific alleles of ORC5 can also be isolated that reduce silencing but have no effect on genome replication [121]. Conversely, some alleles of ORC2 and ORC5 can provide silencing to HMRa without providing sufficient replication function to rescue replication defects caused by temperature-sensitive alleles of these genes [119,121]. Thus, the ORC’s role in silencing, postulated to be recruitment of the Sir1p silencing protein for the nucleation of silencing [126–128], is mechanistically distinct from its replication role at origins.

The multi-subunit heteromeric nature of ORC and its potential ability to form different conformations and context-dependent protein recognition surfaces probably contribute to its multi-purpose nature in chromosomes. Two studies indicate that the conformation of yeast ORC can be regulated. In one, ORC exhibits two distinct conformations that can be distinguished by electron microscopy [129]. The presence of origin DNA and ATP stabilizes one conformation. An alternate conformation is observed in the absence of nucleotide and the presence of single-stranded DNA to which ORC can bind in a sequence-non-dependent manner. In another study, the Cdc6p promotes an alternative ORC conformation in vitro [130]. The mechanistic role for these ORC conformations is not yet clear. But the existence of alternate ORC conformations raises the possibility that subtler conformations of ORC may exist, perhaps dependent on ORC’s target site and neighboring sequences or proteins, that could modulate ORC’s roles in chromatin structure or origin activity.

One recent study indicates that ORC’s target site within a silencer is quantitatively and perhaps qualitatively different from its target sites in many non-silencer replication origins [102]. The ORC target site (A/B1-element) within the HMR-E silencer binds ORC with about a 10-fold or even higher affinity than many target sites from active non-silencer replication origins, including ARS1. Based on estimates of the cellular ORC concentration [25,131] and comparisons to other origins, the binding affinity of ORC for its target site in the HMR-E silencer is greater than necessary for full occupancy by ORC. Surprisingly, the high-affinity ORC binding site actually inhibits replication initiation from HMR in a SIR-independent manner, suggesting that ORC’s binding to the silencer is optimized for silencer activity and not compatible with efficient origin activity. Perhaps the target site for ORC present at the HMR-E silencer promotes an ORC conformation optimized for interaction with the Sir1 protein and high-affinity binding is a by-product of that conformation. Alternatively, extremely stable binding by ORC may contribute to a stable chromatin structure that survives the chromosome dynamics of the cell cycle. This level of binding, well beyond what is necessary for efficient occupancy by ORC, may prohibit origin firing, perhaps because it prevents ORC from performing some origin-specific role in replication initiation. Many more target sites must be examined, both in vitro and in vivo, for a clear picture to emerge. However, these data provide a hint that differences in the intrinsic nature of ORC–DNA interactions at origins could influence ORC’s role in chromatin structure.

Other than the silencers, no other ORC binding site in yeast is known to control specialized chromatin domains, but three recent studies indicate that ORC has a flexible ability to adapt new roles in chromatin structure. Two of these studies focus on an unusual form of SIR-independent silencing that can be established at HMRa by a dominant allele of the SUM1 gene, SUM1-I [132,133]. Sum1p normally acts as a direct repressor of meiotic genes [134,135]. Sum1p does not bind to HMRa or function in HMRa silencing. The SUM1-I mutant allele, however, has the amazing capacity to restore silencing in strains lacking any of the SIR genes, and the Sum1-1p binds to HMRa as measured by ChIPs. Sum1-1p mediated silencing is similar to SIR-dependent silencing in that it requires an NAD-dependent deacetylase, but Sum1-1p uses HST1 instead of SIR2 for its NAD-dependent deacetylase. Therefore, Sum1-1p-dependent silencing must be quite different from SIR-dependent silencing.

Remarkably, however, Sum1-1p-dependent silencing still requires ORC; deletion of the Orc1 N-terminus abolishes Sum1-1p dependent silencing [132]. Sum1-1p’s association with HMRa requires ORC because mutations in ORC, such as the orc5-1 allele, reduce a Sum1-1p-HMRa association as measured by ChIPs [132]. In addition, Sum1-1p interacts with Orc5p in a two-hybrid assay and with ORC by co-immunoprecipitation [133]. Thus, an alternative form of ORC-dependent repressive chromatin can be created by a single amino acid change in the Sum1p. ORC is not required...
for Sum1p’s role in meiotic repression, but even wild-type Sum1p has some ability to bind ORC [133]. Perhaps ORC and wild-type Sum1p function together at other as yet unidentified loci. Regardless, it is striking how little genetic change is required to create an alternative form of heterochromatin at HMRa that still requires ORC, and intriguingly the same region(s) of ORC required for SIR-dependent silencing.

Evidence from the third study suggests that other origins, not previously known to possess silencer activity, can provide silencer activity to HMR[136]. Specifically the 2μARS cannot substitute for the HMR-E silencer and establish SIR-dependent silencing at HMR. Although 2μARS-dependent silencing requires ORC and the other SIR genes, it is distinct from HMR-E dependent silencing in that it also requires another NAD-dependent protein deacetylase SIR2 homolog, HST3 and the Mig1p that has a binding site within the 2μARS. Thus, an entirely different origin, distinct from the silencer-associated origin, can establish an ORC-dependent heterochromatin structure. ORC appears readily capable of adapting new roles in chromatin structure by exploiting certain DNA sequences, chromosomal contexts and/or neighboring proteins. This capability suggests that origins may readily acquire alternative non-replication roles (Section 1.4).

ORC has roles in controlling alternative chromatin structures in Drosophila and possibly other organisms as well. Perhaps some ORCs mark positions as replication origins during the rapid divisions of early development in multicellular organisms, and later during development, when the cell division cycle slows and less active origins are required, these same chromosomal positions, through their associated ORCs, may function as specialized chromatin nucleation sites. Interestingly, studies in both Xenopus and Drosophila support the idea that the number of active origins is reduced during development as more defined specialized tissues begin to form [137,138]. If these inactive origins still bind ORC, they may serve roles in tissue-specific chromatin structures and gene expression.

4. Concluding remarks

Many independent studies over the last several years have established a role for chromosome context and chromatin structure in controlling the efficiency and timing of origin activation during S-phase in S. cerevisiae. More recent studies have implicated specialized chromatin-binding proteins, chromatin-modifying enzymes and chromatin-remodeling proteins in controlling origin activity. Future examinations of known and as yet undiscovered chromatin effects on both global and selective origin regulation will continue to flourish in S. cerevisiae because of the combination of whole-genome origin maps and the well-recognized experimental advantages offered by this model organism. The study or metazoan origins and tissue-specific origin regulation will continue to advance as technologies for genome-wide studies of replication proteins in chromatin and replication dynamics improve. It is probable that many of the rules for chromatin effects on and by origins that we learn from S. cerevisiae will be applicable at some level to the tissue-specific regulation of origin activity in metazoans. Even so, a replication map for even a single mammalian chromosome that is analogous to the genome-wide replication maps of yeast will provide tremendous fuel for future mechanistic studies.

References


M. Weinreich, B. Stillman, Cdc7p-Dbf4p kinase binds to chromatin during S phase and is regulated by both the APC and the RAD53 checkpoint pathway, EMBO J. 18 (1999) 5334–5346.


A.S. Ivessa, V.A. Zakian, To fire or not to fire: origin activation in Saccharomyces cerevisiae ribosomal DNA, Genes Dev. 16 (2002) 2459–2464.


