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CHROMATIN REMODELING AND INITIATION OF DNA REPLICATION

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1. ABSTRACT

While much has been learned in recent years about the process of chromatin remodeling and its role in activation of transcription, relatively little has been reported on the role of chromatin remodeling in DNA replication. However, it is well established that transcription factors and chromatin structure play an important role in replication origin usage. Recent work has begun to indicate that chromatin remodeling factors are likely to play an important role in the regulation of replication origin usage. The results to date are most consistent with the role for chromatin remodeling factors in DNA replication as being indirect, and very similar to their role in transcription. The current evidence suggests that transcription factors bind to auxiliary sequences adjacent to replication origins and recruit chromatin remodeling factors to create either nucleosome-free regions or regions of specifically spaced nucleosomes. This results in activation of the nearby origin, presumably by making the origin region more accessible to replication factors.

Until recently, there has been very little evidence of direct interactions between chromatin remodeling factors and the DNA replication machinery. Recent studies have provided data indicating that direct interactions may exist between chromatin remodeling factors and two cellular replication factors, the Origin Recognition Complex and Proliferating Cell Nuclear Antigen. However, since these replication factors are also involved in other nuclear processes, such as transcriptional silencing and DNA repair, respectively, further study is necessary to establish whether these direct interactions are also important for DNA replication.

2. INTRODUCTION

Chromatin structure imposes a major impediment to all chromosomal events in eukaryotic cells, including transcription, DNA replication, repair, and recombination. Research in the past decade or so has shed significant insight into the molecular mechanism by which the increase of chromatin accessibility leads to enhanced transcription initiation. In particular, numerous biochemical and genetic studies point to two groups of protein complexes as the pivotal players in transcription-related chromatin modification: those that covalently modify the histone tails such as acetylation; and those that reconfigure chromatin structure in an ATP-dependent manner (1, 2). Compared to the explosive developments in our understanding of the biochemical basis of chromatin modification during transcriptional activation, less is known about chromatin structural changes accompanying other nuclear events. However, emerging evidence has suggested that the mechanisms underlying chromatin modification in the initiation of DNA replication may be similar to those in transcriptional activation. In some cases, the very same chromatin modifying factors may be involved in regulating both transcription and replication. In this review, we will discuss recent findings of chromatin remodeling during the initiation of DNA replication and implications for the coordination of DNA replication with other nuclear processes.

3. ACTIVATION OF VIRAL AND CELLULAR DNA REPLICATION BY TRANSCRIPTION FACTORS

Most eukaryotic origins of replication characterized to date contain a core sequence that usually defines the specific initiation site, and nearby cis-acting auxiliary elements that, albeit not essential for DNA replication, stimulate initiation efficiency (3-5). The core sequence serves as the binding site for an initiator protein, which nucleates the assembly of a large initiation complex. For example, the large T antigen of SV40 binds to the corresponding sites in the SV40 ori and subsequently recruits other cellular replication proteins. Similar to viral origins of replication, the core element of the autonomously

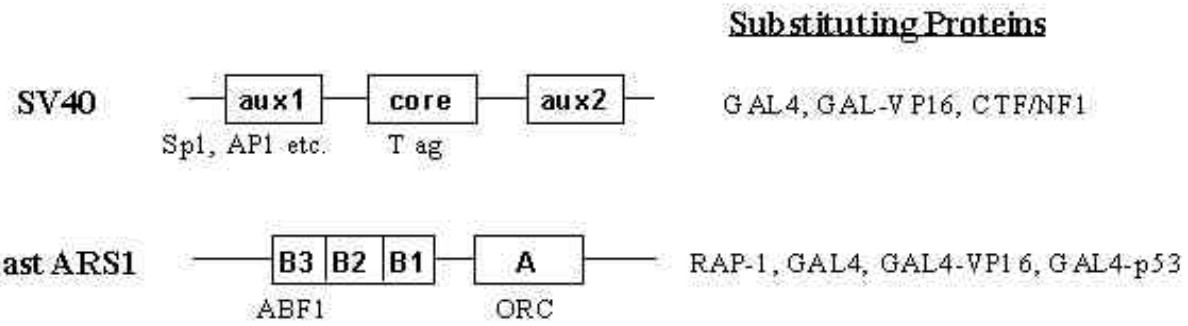

Figure 1. Diagram of the SV40 origin and yeast *ARS1*. Native binding sites for transcription factors and the core origins are depicted. Also listed are heterologous activators that can substitute for the native transcription factors when tethered to the origins

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replicating sequences (ARS's) in *Saccharomyces cerevisiae* is recognized by the origin recognition complex (ORC) (6). ORC interacts with the ARS consensus sequences (ACS) present in each origin of replication in the yeast genome and serves as a landing pad for the assembly of the pre-replicative complex (pre-RC) during the G1 phase of the cell cycle (7, 8).

The auxiliary elements in the replication origin, on the other hand, usually contain binding sites for transcription factors (3, 9-11). The current understanding of the role of transcription factors in replication largely comes from studies of DNA tumor viruses. The fact that adjacent transcription factor binding sites act in cis to stimulate viral DNA replication was observed with polyomavirus, SV40, papillomavirus, adenovirus, and Epstein-Barr virus (12-18). In the case of the SV40 origin, the flanking auxiliary sequences, which are located next to the T antigen-binding core region, contain additional binding sites for T antigen and sites for several cellular transcription factors such as Sp1 and AP1 (Figure 1). Analogous to transcriptional activation, these cis-elements act to increase the frequency of initiation. Furthermore, their function in activation of replication can be substituted by binding sites for heterologous transcription factors (19-22) (also see Figure 1).

Transcription factors have also been implicated in activation of cellular DNA replication. In budding yeast, the sequence of the auxiliary element (B element) is not well conserved among different ARS sequences, but it often contains binding sites for transcription factors. For example, the *ARS1* B element (Figure 1) consists of a binding site for the yeast transcription factor Abf1p (B3) and two other cis-elements (B1 and B2), all of which are collectively important for origin function (23). Abf1p is a multi-functional protein involved in replication, transcriptional activation, mating type silencing, and nucleotide excision repair (24-27). Abf1p binding sites have also been found in several other ARS sequences. In the case of *ARS121*, the Abf1p binding site can function as far as 1.2 kb away from the A element (26, 28). Abf1p does not appear to have a unique role in activating *ARS1* replication, as it can be replaced by a variety of heterologous transcription factors, including mammalian transcription factors that activate viral DNA replication, such as Sp1 and CTF1 (23, 29). These findings strongly suggest that the mechanisms used by transcription factors to activate viral DNA replication in mammalian cells and chromosomal replication in yeast are functionally conserved.

The role of transcription factors in activation of chromosomal replication in higher eukaryotes remains to be established. However, in cases where the origins have been mapped with some precision, the DNA sequence shows numerous transcription factor-binding sites in the vicinity. For example, the locus controlling region (LCR) and the promoter region for the human beta-globin gene are required for origin function at the beta-globin locus (30, 31). Likewise, the promoter region for the DHFR gene in Chinese hamster cells (CHO) appears to be important for initiation of replication from the DHFR locus (Joyce Hamlin, personal communication). Thus, it is likely that transcription factors activate chromosomal replication in higher eukaryotes in a manner similar to that in yeast and viral systems. Given the genomic complexity and higher-order chromosomal organization in multi-cellular organisms, it is tempting to speculate that transcription factors may play an even more prominent role in determining the permissiveness of a specific genomic region for initiation of metazoan replication than they do in viral and yeast replication.

4. CHROMATIN DYNAMICS AROUND ORIGINS OF REPLICATION

Numerous studies have shown that nucleosome structure around an origin of replication has a profound impact on the efficiency of initiation. The mini-chromosomes of DNA tumor viruses such as SV40 and papillomaviruses are packaged into ordered nucleosomes within the nucleus of host cells. However, compared with naked DNA, mini-chromosomes isolated from virally-infected cells or reconstituted into chromatin *in vitro* are very poor templates for viral DNA replication *in vitro*, suggesting that nucleosome structure has a strong inhibitory effect on DNA replication. Binding of transcription factors to their binding sites at the replication origin prior to nucleosome assembly creates a nucleosome-free region around the origin, which in turn increases chromatin accessibility for the initiation proteins and enhances efficiency of initiation of DNA replication (17,

18, 32). In addition to their role in overcoming the nucleosomal repression, transcription factors may utilize additional mechanisms to stimulate viral DNA replication, such as through direct interactions with the replication machinery (22, 33, 34). These functions of transcription factors in activation of viral DNA replication are reminiscent of their roles in transcriptional activation.

Investigation of replication origins in *S. cerevisiae* also demonstrates an intimate link between chromatin structure and chromosomal replication. Nuclease sensitivity analysis of origins of replication in budding yeast shows that the DNA sequences surrounding the cis-acting elements are free of ordered nucleosomes, in contrast to regions outside the origins where DNA is packaged into clearly positioned nucleosomes (35, 36). Mutations that abolish Abf1p binding to the ARS1 origin of replication allow encroachment of nucleosomes into the origin region and severely impair origin function (36, 37). On the other hand, the nucleosome restructuring effect of Abf1p at the ARS1 origin can be functionally replaced by heterologous proteins such as the GAL4 DNA binding domain fused with the activation domains of various transcription factors (37, 38). Furthermore, mutations in the activation domains that abolish the nucleosome restructuring ability also severely impair their ability to stimulate DNA replication and transcription (37, 38) (Miyake, T. and R.L., manuscript in preparation). Therefore, reconfiguration of chromatin structure induced by transcription factors may be a common step in activation of multiple nuclear processes in eukaryotes.

Although nucleosome structure is generally viewed as a road block to initiation of DNA replication, a properly positioned nucleosome at the origin of replication may actually play a positive role in regulating replication initiation. Recent work from Steve Bell's laboratory shows that an ORC-dependent nucleosome configuration at ARS1 of *S. cerevisiae* is required for efficient assembly of the pre-RC and subsequent origin firing (39). Disruption of the ORC-mediated nucleosomal arrangement impairs pre-RC formation and chromosomal initiation. Therefore, while transcription factors at the replication origins may increase chromatin accessibility for binding of replication factors such as ORC, subsequent nucleosome positioning by the origin-bound ORC may help recruit or stabilize additional replication initiation proteins.

5. POSSIBLE MECHANISMS OF CHROMATIN REMODELING AT REPLICATION ORIGINS

Mutational studies of multiple trans-activation domains have shown that activation of transcription and replication are mediated by a common set of amino acid residues (22, 34, 37, 38) (Miyake, T. and R.L., manuscript in preparation). This strongly suggests that the mechanisms used by transcription factors to overcome nucleosomal repression during transcription and DNA replication, are one and the same. While one activation domain could recruit different co-activators to activate the two distinct nuclear processes, it is more likely that the common need for nucleosome-free chromatin structure around active transcriptional promoters and origins of DNA replication results in recruitment of the same chromatin remodeling factors.

Based upon the current understanding of transcription-related chromatin restructuring, at least two types of chromatin modifying enzymes are responsible for antagonizing nucleosomal inhibition of transcriptional initiation: ATP-dependent chromatin remodeling complexes (e.g. the SWI/SNF complex) (2) and histone acetyltransferases (HATs) (1). In principle, both types of chromatin modifying enzymes could be recruited to origins of replication and confer greater chromatin accessibility to the replication machinery. In support of a possible involvement of HATs in DNA replication, it has been shown that one of the human ORC components, ORC1, is associated with a histone acetyltransferase HBO1 (40). Similar types of HATs in budding yeast (i.e. SAS proteins) also display genetic interactions with yeast ORC (41). It has recently been shown that p300, a mammalian HAT known to be involved in transcriptional activation, binds to the replication/repair factor, Proliferating Cell Nuclear Antigen (PCNA) (42). Although this interaction appears to be primarily involved in the DNA repair function of PCNA, one cannot exclude the possibility that this interaction may also play a role in recruiting p300 to sites of DNA replication.

Despite the interaction between various HATs and replication factors, it is not known whether these HATs play any direct role in modifying either histones or the ORC proteins themselves, to facilitate replication initiation at replication origins. It is well established that following DNA replication, newly deposited histones are hyperacetylated (43); however, there is no evidence that histone hyperacetylation is involved in the initiation of replication. In fact, SV40 minichromosomes fail to replicate in the *in vitro* replication system whether assembled with hyperacetylated histones or histones without N-terminal tails (44, 45). These findings suggest that, unlike the circumstances at transcription promoters, post-translational modification of the histone tails may not be sufficient for activation of replication initiation from a nucleosome-embedded origin. Rather, more dramatic alterations of the chromatin structure, such as either nucleosome repositioning or removal, may be required for the assembly of pre-RCs at origin sequences.

Several lines of evidence support the notion that chromatin remodeling is involved in activation of replication initiation. First, nuclease sensitivity assays have shown that transcription factors that bind to replication origins and activate origin function induce a nuclease digestion pattern that is similar to that of naked DNA (37, 38). Such a transcription factor-dependent change in nucleosome structure appears to be a cause, rather than a result, of enhanced replication initiation. Second, several known chromatin remodeling complexes have been implicated in activating replication origins *in vitro* and *in vivo*. For example, one of the components of the human SWI/SNF complex, hSNF5, interacts with the viral replication initiator E1 of human papillomavirus and is required for efficient viral DNA replication *in vivo* (46).

The SWI/SNF complex in *S. cerevisiae* has also been shown to mediate the stimulatory effect of GAL4 on the function of a replication origin that contains GAL4 binding sites (47). Using SV40 chromatin templates reconstituted in Drosophila embryo extracts, Alexiadis *et al*. demonstrated that the chromatin accessibility complex (CHRAC) from Drosophila is capable of remodeling the chromatin structure around the viral replication origin *in vitro*, and that this change results in increased chromatin accessibility of the origin and efficient initiation of replication (48). Taken together, these findings strongly suggest that dramatic changes of the chromatin structure mediated by the ATPase-dependent chromatin remodeling machines may be an important mechanism for overcoming nucleosomal repression of replication initiation.

6. PERSPECTIVES

Studies of both viral and yeast DNA replication have clearly demonstrated the importance of transcription factors in activation of DNA replication. Furthermore, emerging evidence also shows that significant changes in chromatin structure around replication origins may be required to activate replication initiation from nucleosome-covered replication origins. However, compared with the extensive studies of transcriptional activation, the biochemical basis for chromatin remodeling at the origins of replication remains to be elucidated. While certain chromatin remodeling complexes have been implicated in activation of viral DNA replication *in vitro*, further *in vivo* studies are needed to ascertain their physiological role in activation of cellular chromosomal replication. It will also be of interest to determine whether the action of the replication-related chromatin remodeling complexes is origin-specific. If a remodeling complex is recruited to the origin via specific transcription factors, the origins that the particular remodeling complex could act upon would likely be determined by the presence of the binding sites for those specific transcription factors. This model could explain the origin-specificity of the SWI/SNF results (47). On the other hand, if the remodeling machinery is tethered to the origin via its association with general replication initiation factors such as ORC, the remodeling complex would be expected to be present at most, if not all, replication origins. It is also possible that post-translational modification of the components of the pre-RC, such as ORC, may facilitate reconfiguration of the local nucleosome structure.

Another important question concerns the impact of the transcription factor-mediated chromatin remodeling on the cell cycle control of chromosomal DNA replication. Studies of several cellular systems demonstrate that the cell cycle control of chromosomal DNA replication requires an ordered assembly of many proteins at replication origins (7, 8, 49). The origin recognition complex (ORC) is bound to replication origins throughout most, if not all, of the cell cycle. The ordered assembly of pre-RCs involves sequential loading of Cdc6p, MCM, Cdc45p, and other components of the replication machinery. *In vivo* footprint analysis has shown that Abf1p is also bound to *ARS1* throughout the cell cycle (50). Consistently, transcription factor-dependent chromatin remodeling of ARS1 also occurs throughout the cell cycle and is independent of a functional ORC binding site (37, 38). Thus, transcription factors themselves may not be directly involved in restricting replication to once per cell cycle. However, they play a pivotal role in controlling the chromatin accessibility to multiple replication proteins, which in turn limit replication to once per cell cycle. Although transcription factors are likely to be bound to their sites at origins of replication throughout the cell cycle, it remains possible that they execute their function in replication at a fixed stage of the cell cycle. In such an event, missing the "window of opportunity" would result in a lack of origin activity during the following S phase. On the other hand, once transcription factors accomplish their task during the execution period, their continuous presence at the replication origin would not be obligatory.

Increased chromatin accessibility at replication origins may be critical for cell proliferation under different physiological conditions. For example, it may help retain replication competence of replication–origins in cycling cells, and reestablish replication competence of dormant origins in cells reentering the cell cycle from a quiescent state. A change in chromatin structure around replication origins may also be important for controlling initiation of replication through metazoan development, during which there are profound changes in origin utilization (51, 52). Changes in chromatin structure may also influence the timing of replication in different regions of the genome in S phase (53). A combination of biochemical and genetic approaches are needed to shed more light on these important mechanistic questions regarding chromatin accessibility and regulation of eukaryotic DNA replication.

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