Journal of Cellular Biochemistry 97:684–689 (2006)

Chromatin Remodeling in DNA Replication

Karina B. Falbo and Xuetong Shen*

Department of Carcinogenesis, Science Park Research Division, University of Texas MD Anderson Cancer Center, Smithville, Texas 78957

Abstract Chromatin remodeling complexes have evolved to solve a very basic problem for eukaryotic cells accomodation of the genome to fit the dimensions of the nucleus without loss of access to the DNA molecule. In the nucleus, DNA is wrapped around histones to form nucleosomes and other higher order compact chromatin structures. Chromatin remodeling complexes enable highly regulated access to DNA sequences in the context of chromatin, and it is well known that these complexes are involved in regulation of transcription. However, gene expression is not the only process that occurs in the nucleus. DNA has to be replicated, recombined, and repaired. In this regard, it is notable that the recent discoveries have linked ATP-dependent remodeling complexes to DNA damage repair. These results have raised challenging questions about the possible versatility of chromatin remodeling complexes in other nuclear activities, particularly in DNA replication, since a number of recent studies have suggested a connection between this essential cellular process and chromatin remodeling. However, the chromatin remodeling events regulating DNA replication have not been extensively investigated. The aim of this prospect is to summarize recent studies that implicate chromatin remodeling in DNA replication and to address potential roles of chromatin remodeling at various stages of eukaryotic DNA replication. J. Cell. Biochem. 97: 684–689, 2006.

Key words: ATP-dependent chromatin remodeling; replication; repair

ATP-DEPENDENT CHROMATIN REMODELING

Chromatin is the natural substrate for most nuclear processes. Transactions with chromatin substrates, such as transcription, replication, recombination, and repair have in common that they are initiated and regulated by DNA-binding factors. Interactions of these factors with DNA require partial unraveling of local chromatin structure. Chromatin can be modified and regulated at two main levels. First, post-translational modifications of the histone tails through acetylation, methylation, phosphorylation, and other modifications enable tight regulation of the chromatin structure. Second, ATP-dependent chromatin remodeling factors alter histone–DNA interactions, such that nucleosomal DNA becomes more accessible to interacting proteins. These two major forms of chromatin modifications enable a fluid state of the chromatin in which diverse nuclear processes can occur in an orderly fashion. In particular, ATP-dependent chromatin remodeling complexes can use the energy supplied by ATP hydrolysis to affect nucleosomal organizations by either "sliding" the nucleosomes along the DNA or by displacing or replacing histones within nucleosomes. Therefore, ATP-dependent chromatin remodeling can actively and directly modify chromatin structures, while histone modifications are thought to mainly play signaling roles through the so called "histone code," which defines specific interactions between chromatin and its interacting partners. In some cases, modifications of histones may have direct effect on chromatin folding. There is also growing evidence that the two major types of chromatin modification are interconnected.

ATP-dependent chromatin remodeling is carried out by multi-subunit protein complexes with ATPases of the Swi/Snf family as catalytic centers [Becker and Horz, 2002]. It has been well established that these complexes function in both transcriptional activation and repression. For transcriptional regulation, it is

*Correspondence to: Xuetong Shen, Department of Carcinogenesis, Science Park Research Division, University of Texas MD Anderson Cancer Center, Smithville, TX 78957. E-mail: snowshen@mac.com

Received 1 November 2005; Accepted 7 November 2005

DOI 10.1002/jcb.20752

thought that sequence specific transcription factors are responsible for recruiting these chromatin-remodeling complexes to the promoters of genes, and local remodeling of chromatin around the promoters facilitates either activation or repression of genes. Interestingly, recent findings also suggest direct roles of ATP-dependent chromatin remodeling complexes outside of transcriptional regulation, since they are implicated in DNA repair [Morrison et al., 2004; van Attikum et al., 2004]. Similarly, evidence for a role of chromatin remodeling in DNA replication has also emerged. Together, these studies opened interesting and novel areas in chromatin biology, in which the modifications of chromatin are likely to play important roles in nuclear processes other than transcription. However, it should be cautioned that the potential involvement of chromatin remodeling in processes outside transcription could be indirect. For example, it is possible that chromatin remodeling may affect the transcription of genes that are required for DNA replication and repair, thus indirectly affecting DNA replication and repair. These possibilities will need to be addressed in studies aimed at linking chromatin remodeling to processes other than transcription.

CHROMATIN REMODELING IN THE CONTEXT OF DNA REPLICATION

The heritability of cell-specific gene regulations argues that chromatin structures must be propagated through generations of cell divisions [Annunziato, 2005]. Therefore, not only the high DNA sequence fidelity but also the associated chromatin structure has to pass on to the next generation to ensure that both the genetic and the epigenetic information remain unaltered over generations. Lack of accuracy in this information affects processes such as cell differentiation [McNairn and Gilbert, 2003]. Chromatin remodeling can result in changes in the location of nucleosomes or alterations of histone–DNA interactions [Becker and Horz, 2002], and recent work indicates that chromatin remodeling can also completely remove [Boeger et al., 2003; Reinke and Horz, 2003] as well as exchange, histones [Krogan et al., 2003; Kobor et al., 2004; Mizuguchi et al., 2004]. Importantly, specific histone-DNA interactions need to be disrupted and reestablished during cell cycle in order to allow faithful and rapid duplication of DNA, as well as associated chromatin structures. To achieve this, it is plausible that ATP-dependent chromatin remodeling may play important regulatory roles to facilitate the many steps of the replication process. DNA replication itself is a highly complex process involving the coordinated activity of many factors that function in all phases of the cell cycle. We will summarize recent findings linking chromatin remodeling to DNA replication and address the potential roles of ATP-dependent chromatin remodeling in the key stages of DNA replication.

BEFORE S PHASE

DNA replication starts before the S phase transition with the ordered assembly of a multiprotein complex, the pre-replicative complex (pre-RC). Formation of the pre-RC begins with ORC (origin recognition complex) binding to replication origins. Although the mechanism of ORC recruitment differs among eukaryotes, the assembly of pre-RC is conserved among all eukaryotes. ORC recruits the initiation factors Cdc6 and Cdt1 to origins, which are required for loading of the Mcm2-7 proteins that function as the replicative helicase during S phase [Takeda and Dutta, 2005]. As mentioned above, ORC recruitment differs between organisms. In the budding yeast *Saccharomyces cerevisiae*, ORC recruitment depends on the recognition of specific DNA sequences, such as an 11 base pair element in the autonomously replicating sequences (ARS) [Bell and Stillman, 1992]. In the fission yeast *S. pombe*, AT rich elements appear to be sufficient for specifying a functional origin [Okuno et al., 1999; Segurado et al., 2003]. However, in higher eukaryotes, the organization of origins is more complex and difficult to define. Furthermore, it seems that epigenetic factors and chromatin structure might be important in defining origins in higher eukaryotes.

Given that ORC binding may occur in the context of chromatin, the question arises whether chromatin remodeling has a role during DNA replication initiation. Insights into this issue came from early work on the role of nucleosome configuration adjacent to replication origins. Early experiments pointed to the notion that nucleosome positioning interferes with the ability of an origin to initiate replication when nuclesomes form at the origin.

Simpson et al. showed deletions that move the ARS core from an accessible region of the yeast minichromosome into a stable nucleosome provoked a reduction in the copy number of the plasmid [Simpson, 1990]. These results suggest a negative role of chromatin structure in pre-replication. In contrast, results obtained by Lipford and Bell [2001] suggest correct positioning of nucleosomes adjacent to ARS1 by ORC is important for efficient replication initiation. Based on nucleosome mapping, plasmid stability measurement and 2D gel analyses, they found that a plasmid with an altered nucleosome structure next to the ORC binding site showed a reduction in DNA replication initiation efficiency, while the ORC binding pattern remained unaltered. Furthermore, alteration of the ORC-dependent nucleosome configuration of a yeast origin compromised origin function by disrupting pre-RC formation supporting a positive role for nucleosomes at the origin [Lipford and Bell, 2001]. Finally, it was shown that the SWI/SNF remodeling complex was required for replication initiation in a yeast minichromosome assay [Flanagan and Peterson, 1999]. These authors assessed the stability of minichromosomes as a measurement of origin replication function, and found that minichromosomes containing ARS1, ARS307, or ARS309 were not significantly altered by inactivation of the SWI/SNF complex. In contrast, the stability of a minichromosome that contains ARS121 was dramatically reduced in the *swi/snf* mutant compared to the wild-type.

Together, these studies provide indirect evidence that chromatin remodeling may be required to move nucleosomes around the replication origin either to unmask the ORC-binding site, or to configure the nucleosomes around the ORC-binding site to precise positions, allowing ORC to bind and function efficiently. ATP-dependent chromatin remodeling complexes are candidates for achieving such nucleosomal movements. If indeed chromatin remodeling complexes are needed to enhance ORC-binding or function, these complexes themselves need to be recruited to the replication origin. One mechanism could be through binding to ORC directly, or by interacting with other replication initiating factors, such as Cdc6 and Cdt1. Another potential mechanism could be the direct binding of chromatin remodeling complexes to replication origins, which could be mediated either through DNA-binding or by recognition of a specific DNA replication histone code. The INO80 class of ATP-dependent chromatin remodeling complexes is of particular interest, since these complexes contain hexameric helicases, Rvb1 and Rvb2, which can potentially be used to unwind DNA during replication initiation. The involvement of the INO80 class and other classes of ATP-dependent chromatin remodeling complexes in replication initiation remains to be investigated.

THE G1/S TRANSITION

During S phase, pre-RCs initiate replication by promoting origin unwinding and facilitating the recruitment of replicative DNA polymerases. This process is regulated by a set of replication factors, the activities of cyclin-dependent kinases (CDKs), and the Dbf-dependent kinase (DDK). This cell cycle regulation of DNA replication ensures that DNA replicates just once during S phase of each division cycle. Then, several replication factors must be loaded in order to pass through the G1/S transition. The MCM complex is thought to be the replicative helicase, and its loading correlates with the licensing and activation of a replication origin [Zhou et al., 2005]. As mentioned above, ATP-dependent chromatin remodeling is thought to be important for the repositioning of nucleosomes preceding the binding of DNA replication factors. Therefore, cell cycle changes in chromatin remodeling and histone modifications at eukaryotic origins might also be important regulatory features controlling replication and licensing factor access to DNA. Interestingly, in a recent study, Zhou et al. found that the dyad symmetry (DS) region of origin of plasmid replication (OriP) was flanked by nucleosomes that undergo chromatin remodeling and histone deacetylation at the G1/S border of the cell cycle [Zhou et al., 2005]. These changes correlated with MCM3 binding in the G1/S phase of the cell cycle, suggesting that cell cycle changes in chromatin are coordinated with replication licensing at OriP. The authors also found that SNF2h (a member of the Swi/Snf family) was enriched at DS in G1/S arrested cells. Moreover, depletion of SNF2h inhibited OriP replication and decreased G1/S associated binding of MCM3. These results are consistent with a role of SNF2h in the remodeling of nucleosomes, which facilitates the loading of MCM3.

ATP-dependent chromatin remodeling could potentially play several roles during the G1/S transition. As suggested by the SNF2h study, chromatin remodeling may be needed to reconfigure nucleosomes once the pre-RC complex is formed and to facilitate the loading of MCM proteins. Subsequently, the reconfigured chromatin structure may not be conducive to initiation events, such as ORC binding. The reconfiguration of chromatin at the G1/S transition would therefore be an important way to ensure that initiation only happens once at a given origin. Similarly, the recruitment of ATP-dependent chromatin remodeling complexes at G1/S transition can be achieved by either interactions with G1/S specific replication factors or by recognition of a particular pattern of histone modifications at the G1/S transition. Another potential mechanism to achieve tight regulations of chromatin remodeling during the cell cycle could be through cell-cycle dependent expression or post-translational modifications of chromatin remodeling complexes. These possible mechanisms remain to be addressed experimentally.

MOVING ALONG WITH THE REPLICATION FORK

The final step in replication initiation is the loading of the replicative polymerases. DNA polα is recruited to origins and synthesizes short RNA primers for leading and lagging strand synthesis. DNA polα is the only polymerase that can initiate synthesis de novo on single-stranded DNA. After primer synthesis, polymerase switching occurs, which replaces DNA polα with DNA polδ and/or polε. Processive DNA synthesis requires DNA polδ and DNA polε to associate with the ring-shaped processivity factor, proliferating cell nuclear antigen (PCNA), which encircles DNA and topologically links the polymerase to DNA. PCNA is loaded onto the DNA template by the clamp loader, replication factor C (RFC) [Takeda and Dutta, 2005]. After loading of polymerase the replication fork is established and it starts moving along the euchromatin and heterochromatin.

There are several ways in which ATP-dependent chromatin remodeling can potentially contribute at this stage. The loading of DNA polymerases and PCNA may be facilitated by local reconfiguration of nucleosomes. In this case, interactions between chromatin remodeling complexes and subunits of DNA polymerases, PCNA, or RFC may provide the necessary recruitment mechanism. Perhaps more importantly, in order for replication to proceed through chromatin, it might be necessary to pave the way for the replication fork to move without obstacles. In this regard, chromatin remodeling complexes might have an important role during fork movement. Interestingly, two remodeling complexes have been implicated in heterochromatin replication. First, RNAi-mediated depletion of ACF1-ISWI (ATP-utilizing chromatin assembly and remodeling factor 1) has been showed to impair the replication of heterochromatin in HeLa cells [Collins et al., 2002]. In this study, the authors demonstrated that ACF1 in complex with SNF2h was required for efficient DNA replication through highly condensed chromatin and proposed that this complex might facilitate this process by remodeling the chromatin structure to allow the movement of the replication fork. A second study from the same lab showed that the WSTF (Williams syndrome transcription factor) interacted with PCNA directly to target chromatin remodeling by SNF2h to replication foci [Poot et al., 2004]. RNAi depletion of WSTF or SNF2h caused a compaction of newly replicated chromatin and increased the amount of heterochromatin markers.

Moreover, the authors proposed that the WSTF-SNF2h complex would have a role in chromatin maturation and the maintenance of epigenetic patterns through DNA replication [Poot et al., 2005]. Chromatin remodeling by WSTF-SNF2h might keep an open chromatin state after the replication fork passes, thus creating a window of opportunity for the epigenetic machinery to copy all the epigenetic marks, passing them on to the next generation with high fidelity. Although it seems that the WSTF-SNF2h complex has a direct role in replication, its precise function during elongation remains to be investigated. Nonetheless, these studies suggest that ATP-dependent chromatin remodeling plays important roles during the progression of DNA replication, either by clearing the path for the replication fork, or by allowing efficient transmission of epigenetic memory. Since chromatin is quickly reassembled after DNA replication, mostly through replication-coupled chromatin assembly pathways, such as the CAF1 and ASF1 pathways, it is also possible that ATP-dependent

chromatin remodeling complexes facilitate replication coupled chromatin assembly by enhancing the movements of histones in and out of the nucleosomes. The involvement of ATP-dependent chromatin remodeling in fork progression and its relationship with replication-coupled chromatin assembly should be further investigated.

STALLED REPLICATION FORK

Elongating replication forks stall when they encounter DNA lesions or when nucleotide pools are depleted. Replication forks appear to be able to sense these conditions since checkpoints are activated during S phase. In this regard, it is of interest to highlight the involvement of chromatin remodeling activities in response to DNA damage. Several recent studies have directly implicated chromatin remodeling activities in DNA repair [Morrison et al., 2004; van Attikum et al., 2004]. These studies showed that the ATP-dependent INO80 chromatin remodeling complex was directly recruited to sites of DSB (double strand break), and such recruitment required interaction with γ-H2AX, the phosphorylated form of histone H2AX, in response to DSBs. These results raise questions about how chromatin remodeling activities might be involved in DNA repair. It is thought that chromatin remodeling might affect DNA repair by providing the repair machinery with an exposed or open chromatin environment that might facilitate the recruitment of DNA repair proteins. However, it can also be argued that chromatin remodeling is needed to form a compact chromatin structure, which would hold broken DNA ends close to each other. Furthermore, chromatin remodeling might also assist in the restoration of the chromatin structure after the DNA damage has been repaired. Interestingly, it was shown that the histone chaperone CAF1, important for replication-coupled chromatin assembly, deposits histones onto DNA after repair. CAF1 is recruited to the sites of NER (nucleotide excision repair) and single strand break repair, probably through interaction with PCNA, an essential molecule in the replication fork [Ehrenhofer-Murray, 2004]. Therefore, proteins such as PCNA provide a good example of a dual role in both DNA replication and repair, since many PCNA-interacting proteins for both functions have being discovered [Maga and Hubscher, 2003]. Because of the intimate links between DNA replication and repair, chromatin remodeling complexes, which assists DNA repair, might also play a role in DNA replication, particularly at stalled replication forks.

Regulation of DNA replication forks is tightly linked to the DNA damage and DNA replication checkpoint controls. When replicative polymerases encounter a lesion during DNA replication, the replication fork stalls. Then, DNA polymerases capable of bypass synthesis have to be loaded. Recent observations have led to the conclusion that PCNA, due to its interaction with polδ could be located at the point of polymerase stalling and would play a role as a recruiting platform, allowing the switch from replicative to translesion polymerases required to resume the replication fork. This observation suggests a window in which chromatin remodeling complexes may play a role during replication fork stalling. PCNA or other components, which already move with the replication fork might be able to recruit chromatin remodeling complexes in order to assist with replication fork reestablishment after the stall. The roles of chromatin remodeling complexes at the stalled replication forks might be similar to those proposed above for sites of DNA repair as previously discussed.

Another mechanism by which ATP-dependent chromatin remodeling complexes may affect stalled replication forks is through the checkpoint responses. Recent studies have implicated several DNA replication factors in mediating the checkpoint response, such as PCNA, RFC, and RPA. It is possible that chromatin remodeling complexes may interact with these replication proteins at the stalled replication fork in order to efficiently activate checkpoints, either by facilitating the access of checkpoint proteins to stalled replication fork, or though direct activation of checkpoints. It is also possible that chromatin remodeling complexes exert their function after checkpoint activation by assisting the downstream DNA repair process or the loading of alternative polymerases. Finally, stability of the replication fork after the stall is important to avoid a collapse of the fork, and chromatin remodeling complexes might play an important role at stabilizing chromatin structure during the stall and the reestablishment of the fork. Given the multiple potential roles of chromatin remodeling at stalled replication forks and in other steps

of replication discussed above, it would be important to begin investigating the involvement of a specific chromatin remodeling complex in a systematic way to reveal the contribution of ATP-dependent chromatin remodeling to DNA replication.

SUMMARY

The view of chromatin remodeling complexes solely as transcriptional regulators has been challenged in the past few years with discoveries that implicate ATP-dependent chromatin remodeling in a variety of other nuclear processes. Notably, a link between chromatin remodeling complexes and DNA repair has recently been established, and emerging studies have also suggested that DNA replication might be regulated by chromatin remodeling as well. In this context, we proposed several potential roles and mechanisms by which ATP-dependent chromatin remodeling might contribute to DNA replication. Further investigations along these lines will likely reveal even broader impacts of chromatin on nuclear processes.

REFERENCES

Annunziato AT. 2005. Split decision: What happens to nucleosomes during DNA replication? J Biol Chem 280(13):12065–12068.

Becker PB, Horz W. 2002. ATP-dependent nucleosome remodeling. Annu Rev Biochem 71:247–273.

Bell SP, Stillman B. 1992. ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. Nature 357(6374):128–134.

Boeger H, Griesenbeck J, Strattan JS, Kornberg RD. 2003. Nucleosomes unfold completely at a transcriptionally active promoter. Mol Cell 11(6):1587–1598.

Collins N, Poot RA, Kukimoto I, Garcia-Jimenez C, Dellaire G, et al. 2002. An ACF1-ISWI chromatin-remodeling complex is required for DNA replication through heterochromatin. Nat Genet 32(4):627–632.

Ehrenhofer-Murray AE. 2004. Chromatin dynamics at DNA replication, transcription and repair. Eur J Biochem 271(12):2335–2349.

Flanagan JF, Peterson CL. 1999. A role for the yeast SWI/SNF complex in DNA replication. Nucleic Acids Res 27(9):2022–2028.

Kobor MS, Venkatasubrahmanyam S, Meneghini MD, Gin JW, Jennings JL, et al. 2004. A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. PLoS Biol 2(5):587–599.

Krogan NJ, Keogh MC, Datta N, Sawa C, Ryan OW, et al. 2003. A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Mol Cell 12(6):1565–1576.

Lipford JR, Bell SP. 2001. Nucleosomes positioned by ORC facilitate the initiation of DNA replication. Mol Cell 7(1): 21–30.

Maga G, Hubscher U. 2003. Proliferating cell nuclear antigen (PCNA): A dancer with many partners. J Cell Sci 116(Pt 15):3051–3060.

McNairn AJ, Gilbert DM. 2003. Epigenomic replication: Linking epigenetics to DNA replication. Bioessays 25(7): 647–656.

Mizuguchi G, Shen X, Landry J, Wu WH, Sen S, et al. 2004. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 303 (5656):343–348.

Morrison AJ, Highland J, Krogan NJ, Arbel-Eden A, Greenblatt JF, et al. 2004. INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. Cell 119(6):767–775.

Okuno Y, Satoh H, Sekiguchi M, Masukata H. 1999. Clustered adenine/thymine stretches are essential for function of a fission yeast replication origin. Mol Cell Biol 19(10):6699–6709.

Poot RA, Bozhenok L, van den Berg DL, Steffensen S, Ferreira F, et al. 2004. The Williams syndrome transcription factor interacts with PCNA to target chromatin remodelling by ISWI to replication foci. Nat Cell Biol 6(12):1236–1244.

Poot RA, Bozhenok L, van den Berg DL, Hawkes N, Varga-Weisz PD. 2005. Chromatin remodeling by WSTF-ISWI at the replication site: Opening a window of opportunity for epigenetic inheritance? Cell Cycle 4(4):543–546.

Reinke H, Horz W. 2003. Histones are first hyperacetylated and then lose contact with the activated PHO5 promoter. Mol Cell 11(6):1599–1607.

Segurado M, de Luis A, Antequera F. 2003. Genome-wide distribution of DNA replication origins at A+T-rich islands in Schizosaccharomyces pombe. EMBO Rep 4(11):1048–1053.

Simpson RT. 1990. Nucleosome positioning can affect the function of a cis-acting DNA element in vivo. Nature 343(6256):387–389.

Takeda DY, Dutta A. 2005. DNA replication and progression through S phase. Oncogene 24(17):2827–2843.

van Attikum H, Fritsch O, Hohn B, Gasser SM. 2004. Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. Cell 119(6):777–788.

Zhou J, Chau CM, Deng Z, Shiekhattar R, Spindler MP, et al. 2005. Cell cycle regulation of chromatin at an origin of DNA replication. Embo J 24(7):1406–1417.