Biological Chemistry

Chromatin replication: TRANSmitting the histone code

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Efficient overcoming of the nucleosomal barrier and accurate maintenance of associated histone marks during chromatin replication are essential for normal functioning of the cell. Recent studies revealed new protein factors and histone modifications contributing to overcoming the nucleosomal barrier, and suggested an important role for DNA looping in survival of the original histones during replication. These studies suggest new possible mechanisms for transmitting the histone code to next generations of cells.

Chromatin | nucleosome | replication | histones | segregation

During cell division in eukaryotes, the DNA replication complex efficiently moves through chromatin and the chromatin structure is recovered at a high rate during/after replication. Several recent studies have considerably contributed to our understanding of the mechanism of chromatin replication and the fate of core histones during this process.

Overcoming the nucleosomal barrier to replication

Eukaryotic genome is composed of nucleosomes, each containing a 145–148 bp DNA segment tightly wrapped around the histone octamer in 1.65–1.7 superhelical coils (*1, 2*). Nucleosomal organization limits DNA accessibility to various DNA-binding proteins and enzymes progressing along DNA, like DNA and RNA polymerases. Arrays of nucleosomes are further folded, forming higher order chromatin structure. In eukaryotes, replication of DNA organized in chromatin is highly efficient and the replication fork proceeds at the average rate of 1-2 kb per minute *in vivo* (*3, 4*). However, recent *in vitro* studies have shown that nucleosomes form a high barrier for a replisome (*5-9*), raising a question about the mechanisms allowing the high rate of chromatin replication *in vivo*.

In order to study the mechanisms of chromatin replication, highly purified *in vitro* experimental systems that contained mononucleosomes assembled on short DNA fragments containing nucleosome positioning sequences and the minimal bacteriophage T7 replisome were utilized in recent studies (*5, 6*). During replication through the nucleosome, T7 replisome pauses in several regions inside of the nucleosome, especially at the +(41–65) region (41-65 bp from the nucleosome boundary) (*6*). The pausing pattern and efficiency of the replication are dramatically affected by the exonuclease activity on the T7 DNA polymerase. The exonuclease activity of DNA polymerase increases the fidelity of DNA replication through removal of misincorporated dNTPs. Kinetic analysis of the nucleosomal pausing pattern revealed that the exonuclease activity is important for resolving the non-productive complexes containing DNA polymerase paused during replication through chromatin (*6*). This observation is consistent with the results of previous studies suggesting that the exonuclease activity is required for high processivity of replication *in vivo* (*10, 11*). The authors have proposed that the exonuclease activity is required to facilitate recovery from non-productive intermediates formed after backtracking of DNA polymerase (*6*). Similar mechanisms involving backtracking of RNA polymerase and recovery of from the resulting non-productive intermediates after RNA cleavage by protein factor TFIIS have been proposed to operate during transcription through chromatin (*12, 13*).

In eukaryotes, replication through chromatin requires multiple proteins and assembly steps, making its analysis *in vitro* a difficult task. In a remarkable recent work the authors assembled a purified yeast replication system *in vitro* (*14*) and analyzed the rate of replication through chromatin (*7-9*). Similarly to T7 replisome, *in vitro* assembled yeast replisome progresses through chromatin template at much lower rate as compared with the rate observed *in vivo*. Several protein factors were required to recapitulate the rate of leading and lagging strand replication through chromatin *in vivo* (*8, 9*). These factors include histone chaperone FACT and FACT-associated protein Nhp6 (*8*) that induce nucleosome unfolding (*15*) and thus likely destabilize the nucleosome structure. Since nucleosomes are partially unfolded during replication (*6*), additional unfolding introduced by FACT could facilitate replication through chromatin. In the presence of FACT, the nucleosome remodelers INO80 and ISW1A, and the lysine acetyltransferases Gcn5 and Esa1 further increase the rate of chromatin replication (*8*). Histone chaperone Nap1 also facilitates progression of the replisome through chromatin (*7*). Protein factors associated with active replisome Mrc1 (yeast claspin homolog) and Csm3/Tof1 (proteins associated with Mrc1) together with PCNA are required for the maximum rate of leading strand synthesis by Pol ε on chromatin templates (*9*). In addition, MCM2-7 helicase having histone H3-H4 chaperone activity promotes replication through chromatin (*16*). Thus the nucleosome is a strong obstacle for replication and multiple factors including histone chaperones, nucleosome remodelers, histone acetylation and other protein factors are required to achieve the high rate of replication through chromatin observed *in vivo*.

Segregation of parental histones during replication

Accurate maintenance of specific histone modifications (histone code) targeted to specific chromatin regions is essential for maintaining normal cellular functions (*17*). Nucleosomes can survive DNA replication *in vitro* in the absence of newly synthesized core histones (*8, 18, 19*), suggesting that nucleosome survival is an intrinsic property of chromatin replication. Early studies using labeled histones suggested that the original histones H3 and H4 remain in the cell during several rounds of cell division (reviewed in (*19, 20*)). Several studies have addressed the fate of histones and nucleosomes during replication. In pioneering studies from Alberts laboratory, it was shown that bacteriophage T4 replisome progression through chromatin is not accompanied by nucleosome disruption and that nucleosomes can be distributed to both new DNA molecules *in vitro* (*21*). Similarly, during replication of minichromosomes by SV40 replisome the old nucleosomes are immediately and nearly quantitatively transferred to newly synthesized DNA (*22*) and are distributed

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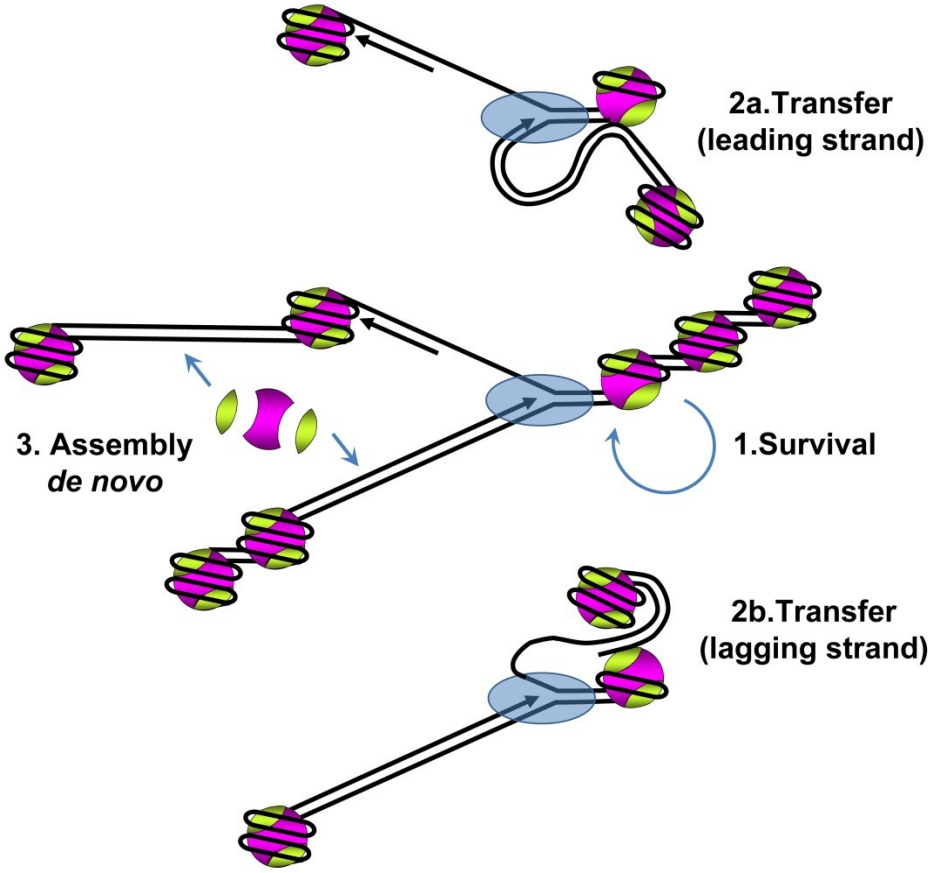

Figure 1. The proposed mechanisms of chromatin recovery during/after replication. Recent studies suggest several pathways: (1) parental histone octamers can survive at the nearly original positions on DNA, possibly through formation of a very small intranucleosomal DNA loop during replication, or (2) are transferred within the (400-1000)-bp nucleosome-free DNA region immediately upstream of the replication fork to either leading (2a) or lagging strand (2b), likely through formation of a larger DNA loop. The looping likely occurs through the DNA-binding surfaces of the histone octamer that are transiently exposed during replication. Alternatively, (3) nucleosomes are assembled *de novo* from newly synthesized histones at the nucleosome-free DNA regions.

between both new DNA molecules (*23*). In yeast cells, old histone H3 remains associated with new DNA after replication and H3-H4 tetramer splitting does not occur (*24*). In agreement with these studies, parental canonical histone H3.1 is preserved after several rounds of cell division without H3-H4 tetramer splitting in human cells (*25, 26*). These findings suggest that at least entire parental H3-H4 tetramers, and possibly the parental histone octamers are segregated to the newly replicated DNA, although loss of some H2A-H2B dimers that occurs during Pol II transcription through chromatin *(13)* cannot be excluded. Furthermore, the majority of parental histones after replication are localized within 400 bps from their original locations in the yeast after replication (*27*).Taken together, previous studies have suggested that nucleosomes and the associated histones can survive replication, segregate to both new DNA molecules and remain at nearly original positions on DNA after this process.

More recently, using the minimal T7 replisome and short mononucleosomal templates it has been shown that ~50% of intact nucleosomes survive replication and remain within ~30 bp from their original positions on DNA after replication of the leading DNA strand *in vitro*, suggesting that nucleosomes are nearly equally segregate to both newly synthesized DNA molecules (*6*). Histone survival on DNA during replication *in vitro* can also involve nucleosome translocation, provided that an extended region (~1000 bp) of nucleosome-free DNA is available (*5*). Since ~600-1000-bp nucleosome-depleted DNA regions were observed upstream of the replication fork *in vivo* (*28*), it is likely that some nucleosomes would be translocated there. Indeed, the average distance of nucleosome translocation during replication in yeast is ~400 bp (*27*). Importantly, the presence of the nucleosome-free regions upstream of the replication fork resulted in facilitated replication through nucleosomes, suggesting that the upstream DNA can interact with the replicating nucleosomes (*6*), likely through formation of a DNA loop (*5, 6*). The mechanism of DNA looping required for nucleosome translocation is unknown; however, replication likely involves partial and transient uncoiling of nucleosomal DNA from the octamer surface during replisome progression through a nucleosome. The exposed DNA-binding surfaces of the octamer could support DNA looping, as was proposed for transcription through chromatin by RNA polymerase II (*29-31*).

Thus recent data obtained in the model systems involving T7 replisome and yeast reconstituted system are consistent and suggest multiple pathways of nucleosome segregation during chromatin replication (Fig. 1). Nucleosomes can survive during replication at the nearly original positions on DNA, likely through

transient formation of a very small intranucleosomal DNA loop (*6*). Alternatively, nucleosomes can be translocated to the (400-1000)-bp nucleosome-free DNA regions localized immediately upstream of the replication fork. This translocation likely involves transient formation of larger DNA loops. The choice between the pathways is likely determined by the rate of replication and by the size of the nucleosome-free DNA regions. Finally, nucleosomes are assembled *de novo* from newly synthesized histones at the nucleosome-free DNA regions available after nucleosome translocation and survival of the original histones (Fig. 1).

Both nucleosome translocation and assembly de novo after replication are tightly coupled with replication and occur at a high rate *in vivo* (*19*). Although the model systems faithfully recapitulate the basic features of chromatin replication, multiple other factors are required to achieve efficient nucleosome translocation *in vitro*. Thus it has been proposed that the histone chaperones, such as MCM2-7 and FACT, remove the histones in front of the replisome and transfer them to the newly synthesized DNA behind replication fork, thus facilitating replication through chromatin (*8, 16, 32*). However, histone chaperones could facilitate chromatin replication by alternative mechanisms. FACT facilitates transcription through chromatin by transiently interacting with histone H2A-H2B surface of the nucleosome, transiently exposed during progression of RNA polymerase II (*33*). Several protein factors are involved in nucleosome assembly *de novo* after replication. The major histone chaperone for nucleosome assembly is CAF1 that interacts with newly synthesized H3-H4 tetramer ((*34, 35*), reviewed in (*20, 21*)).

In summary, recent *in vitro* studies have established the basic mechanisms of chromatin replication and nucleosome survival during this process, and identified multiple protein factors dictating the rate of replication. The current model of chromatin replication involving nucleosome and histone survival during this process and the short-range nucleosome translocation (Fig. 1) also suggests a mechanism for the inheritance of the histone code that is reestablished after replication through spreading of the histone modifications from parental nucleosomes to the adjacent *de novo* assembled nucleosomes (*17*). One example of this mechanism is the H3K27 trimethylation by PRC2 (Polycomb Repressive Complex 2) that could bind H3K27-trimethylated nucleosomes and catalyze H3K27me3 at adjacent nucleosomes (*36, 37*). The other predictions of the proposed model of chromatin replication remain to be evaluated.

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