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Chapter 16

Replicating – DNA in the Refractory Chromatin Environment

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1. Introduction

The replication of DNA is a process found throughout the prokaryotic and the eukaryotic kingdoms. Although the basic aim of this process is the duplication of the genetic information, the mechanisms leading to replication are different in prokaryotes and in eukaryotes. A major divergence between the two kingdoms corresponds to the nature of the substrate of the replication process [1]. Indeed, while the genetic information in prokaryotic cell is recovered in the nucleoid, the eukaryotic genome is found in the nucleus and the genetic material is associated with proteins. The tight interaction of the DNA molecule with proteins forms the chromatin, and for replication as well as for the other cellular processes that require the access to the genetic material, the chromatin is the actual substrate [2]. This organization of the eukaryotic genome in chromatin generates additional constraints to enzymatic activities. Therefore, it is required for the replication machinery to over-rule the refractory environment of chromatin.

Although the arrangement of the genetic material with proteins is an inhibitory environment, it is also required for packaging the molecule of DNA within the confined nuclear volume and for organizing the genome. Therefore, defects in the genetic material packaging affect genome stability and cell viability. Importantly, as replication results in the doubling of DNA, it is required for the cell to synthesize DNA-associated proteins and to form chromatin. This process known as replication-coupled chromatin assembly implies the copy of the epigenetic information carried by the histone proteins [3].

In the present chapter, we define the general features of chromatin, primarily on the basis of the fundamental sub-unit, the nucleosome, and the constraints that this structure generates for creating a refractory environment to replication. In addition to the view of the single nucleosome, as chromatin can be viewed as a polymer of nucleosomes which

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are highly ordered, the impediment of the replication machinery induced by higher chromatin order is discussed. Although replication activity should be inhibited by the chromatin, we review the mechanisms developed by eukaryotic cells to over-rule this non-permissive environment. Genetic experiments have shown that chromatin structure is essential for cell viability. We review the data providing evidence that the genome stability is, at least partly, inherent to chromatin assembly during replication, and the histone requirement in this process.

2. Chromatin: From the nucleosome sub-unit to the higher order structure

The basic chromatin sub-unit is the nucleosome, which is composed of the association of histone proteins with DNA [4]. The histone proteins are the most abundant nuclear proteins and are divided in four classes, H2A, H2B, H3 and H4, respectively. We distinguish in the histone protein two regions, the histone fold domain which is involved in the histone-histone and histone-DNA interactions, and the histone tail domain located at the N-terminal part of the protein, which is unstructured and extends out of the nucleosome [2, 5](Figure 1A). The association of the histones via their fold domain is highly conserved throughout the eukaryotic kingdom. Indeed, H3 is always associated with H4 and H2A with H2B forming therefore heterocomplexes H3/H4 and H2A/H2B (Figure 1B, upper panel). The histone pairing is done by three helixes of the fold domain of two histone counterparts which adopt a specific 'handshake' structure. The first high resolution crystal structure of the histone octamer in absence of DNA revealed that the histone octamer was organized in a tripartite structure wherein the H3/H4 complex formed a central tetramer which is flanked by two H2A/H2B dimers [6, 7](figure 1B, lower panel). Interestingly, while the histone fold domains were clearly resolved in the crystal, the unstructured tail domains were unseen. Although the histone octamer arrangement in presence of DNA confirmed the tripartite structure of the histone octamer, details of the edge of histone tails revealed the exit of these unstructured domains from the nucleosome [8].

It has been believed that the basic nature of the histones allowed the neutralization of the DNA phosphodiester backbone. However, the structure of the nucleosome at 1.9 Å resolution substantially improved the clarity of the electron density and revealed the presence of over 3000 water molecules and 18 ions [9]. The water molecules within the nucleosome promote the formation of hydrogen-bond bridges between the histone and the DNA molecule, like balls in a ball-bearing. Therefore, the water molecules enable the accommodation of intrinsic DNA conformational variation and promote the nucleosome mobility by limiting the rigidity of the nucleoprotein complex. The nucleosome crystal structures provided important information on the interactions between the histones and showed that the histone-DNA association is not only due to electrostatic interactions between the positively charge histones and the negatively charge DNA as it was primarily believed.

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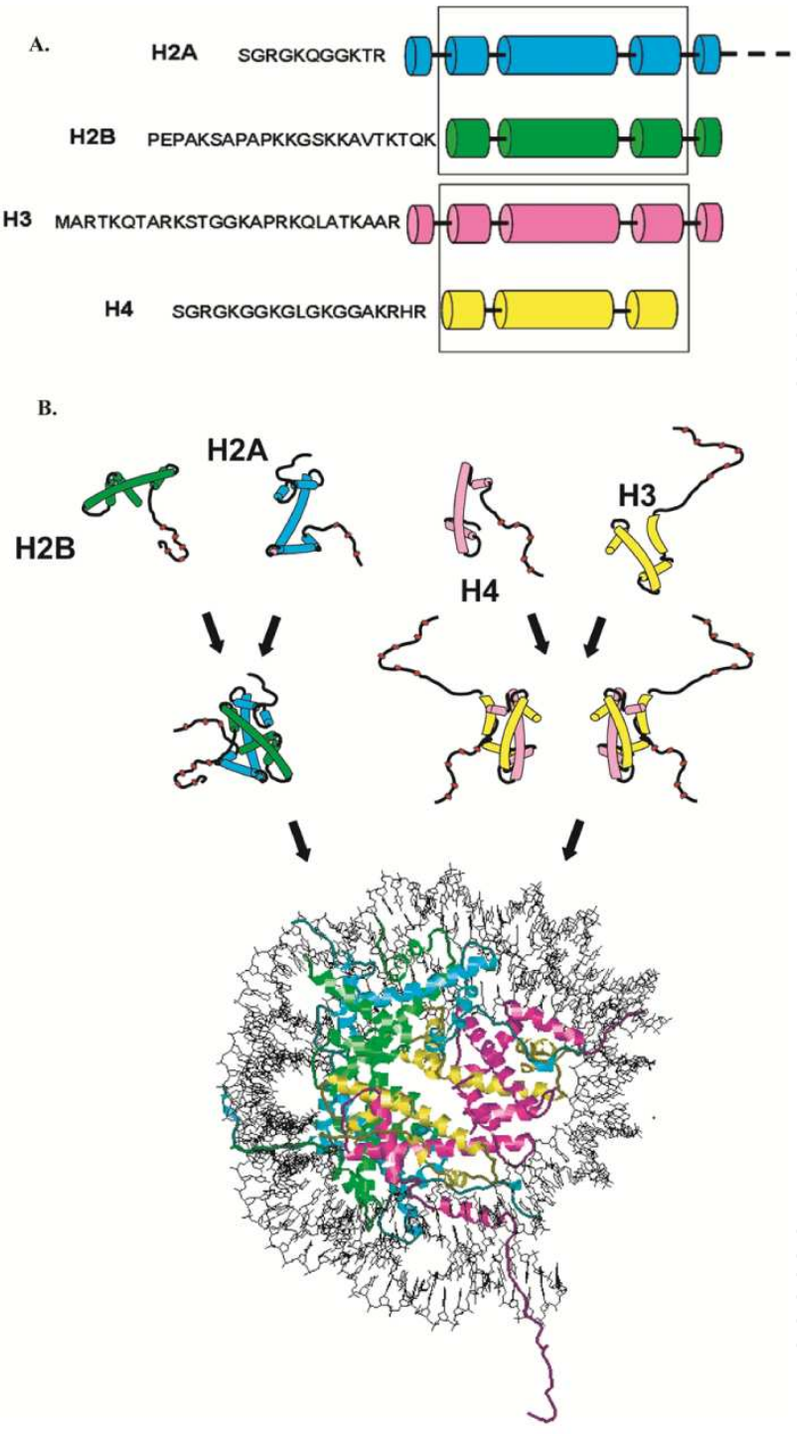

Figure 1. Histones and nucleosome formation: (A) schematic representation of the core histones. The boxes indicate the helixes of the histone fold domain, which is involved in the histone-histone interactions between H2A/H2B, and H3/H4. The amino-acid sequences correspond to the conserved sequence of the unstructured histone tail domain. (B) Individual core histones H2A (green), H2B (blue), H3 (yellow) and H4 (magenta) first heterodimerize to form the H2A/H2B and the H3/H4 complexes. The different complexes can either under different stringencies or with the help of histone chaperones associate together to form the nucleosome composed of a central tetramer of H3/H4 flanked by two heterodimers of H2A/H2B, and wrapped by 146 base pairs of DNA.

The demonstration of the labile interactions between the DNA molecule and the histone octamer was performed by the development of an elegant biochemical approach examining the accessibility of specific DNA sites within the nucleosomal DNA [10, 11]. In these experiments, the authors used a known nucleosome-positioning DNA sequence from the 5S gene, and by directed mutagenesis, restriction sites were generated at precise position within the DNA sequence. Nucleosome core particles were reconstituted with the different DNA sequences and purified by sucrose gradient centrifugation. The accessibility of the specific DNA sequences was examined as a function of time by adding to the nucleosome core particles the restriction enzymes. The quantitative analyses of the digested nucleosomal DNA reflect the accessibility of precise positions within the nucleosome core particles corresponding to the loss of histone-DNA contacts. Interestingly, the results revealed that DNA sequences engaged in the histone-DNA interactions are accessible to the restriction enzymes, and the accessibility gradually decreased when the restriction site is placed at proximity of the diad axis [12]. It was thus proposed that within the nucleosome core particle, dissociation of the histone-DNA contacts enables the transient exposure of DNA stretches to the solvent. Using a similar strategy, Widom and colleagues have also examined the contribution of the histone tail domains in the accessibility of nucleosomal DNA [13]. The results revealed that the removal of the histone tail domains leads to up to 14-fold increase in the site exposure within the nucleosomal DNA. Therefore, the tail domains within the nucleosome are also involved in the stabilization of DNA-histone fold domain interactions possibly by repressing the intrinsic dynamic nature of DNA.

The packaging of DNA in the nucleosome is a dynamic structure in conformational equilibrium, transiently exposing stretches of DNA off the histone surface, as demonstrated in model systems. Importantly, the binding of linker histone nearby the dyad axis to DNA restricts the flapping of the arms of DNA at the entry and at the exit of the nucleosome [14]. Although the analyses of the nucleosome behavior are very informative on the potential mobility of the nucleosome, it is obvious that the nucleosome is not recovered as a single subunit in living cell but rather found as a nucleosome polymer. Thus, the mobility of a considered monomer is possibly modulated by the surrounding nucleosomes. The analyses of a dinucleosome template generated from the 5S gene revealed a spontaneous mobility of the core histones which is restricted by the presence of the linker histone [15]. To better understand the function of the histones in the chromatin folding, it was required to examine templates that contained more than one or two nucleosomes. Using defined oligonucleosome models systems, the molecular mechanisms through which the histones modulated the chromatin folding were investigated [16]. These experiments revealed that the core histone tails play a critical function in the chromatin folding, as demonstrated by the removal of the tail domains *in vitro* [17, 18]. Interestingly, analyses of histone acetylation mimics on the chromatin fiber folding exhibited effects on the self-association properties of model nucleosome arrays, which depended upon the histone carrying the acetylation mimics and the number of mimics within the nucleosomes [19]. Such *in vitro* approaches using reconstituted nucleosomes systems are performed under particular pH and salt conditions. Additionally, acetylated histones increase chromatin solubility. Even if this can potentially biased the results, these investigations provide important features for understanding the physico-chemi-

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cal parameters that facilitates or relieves the folding chromatin. But to date, the actual arrangement of the nucleosomes in the fiber is not yet well-determined. Nonetheless, experimental data have enabled to propose different models, the solenoid model and the zig-zag model, and it is possible that both models are juxtaposed in the nucleus [20, 21].

3. Relieving the chromatin inhibition

The ordered structure of chromatin represents the primary barrier to access the genetic information. On the basis of *in vitro* studies, the linker histones are proposed to be involved in the high-ordered chromatin structures [22]. Although the linker histone is not essential in protozoans [23, 24], the knock-out experiments in mouse revealed critical functions [25]. Indeed, in higher eukaryotes, the linker histones are composed of about eight subtypes which can compensate each other in some extend. However, upon the deletion of three subtypes, the synthesis compensation fails and embryonic lethality is observed. To attempt to gain insight in the function of the linker histone, analyses of the histone modifications have been carried out and reported a correlation between the cell cycle and the phosphorylation of the C-terminal tail domain [26, 27]. Surprisingly, while the genetic analyses revealed that preventing the phosphorylation of linker histone affects the chromatin organization leading to an increase of the nuclear volume, a raise in the linker histone phosphorylation was also detected in mitosis [28, 29]. Nonetheless, at the G1/S phase transition, linker histone is also found as substrate of cyclin-dependant kinase Cdk2, wherein the phosphorylation of the C-terminal tail leads to a relaxation of chromatin structure which might facilitate DNA replication [30, 31]. More recently, knock-down experiments of the linker histone in the slime mold *Physarumpolycephalum* showed a significantly faster rate of genome duplication, which was caused by a lost in the regulation of replication origin firing rather than the increase in the replication fork propagation [32]. Clearly, it has been evidenced that the linker histone affect the compaction of chromatin into the nucleus, and its release is required for the initial transition from non-permissive to permissive chromatin, but the actual mechanisms remain unclear.

Undoubtedly, if the primary inhibition for DNA replication is the higher levels of chromatin structure, relieving the high order of chromatin leaves the core histones associated with DNA, which is still an impediment for DNA accessibility. Thus, the next step is the release of the parental core histones to allow replication machinery to process all along the DNA molecule. To reach this goal, several concerns have to be taken into account. A bevy of studies have attempted to address the segregation of parental histones during replication, but the results are often controversial and many questions still need to be addressed. The fate of parental nucleosomes deals mainly with two overlapping key questions : do they dissociate from DNA during replication ? and, how are they transferred behind the replication fork ?

In vitro studies claimed chromatin replication without histone displacement. Initially showed in prokaryotic *in vitro* system [33], same conclusions were drawn from eukaryotic system studies [34]. In contrast, other studies evidenced that parental nucleosomes dissoci-

ate from DNA [35, 36]. The main argument for a non-displacement was that radioactively-labeled histone octamers are not reassembled onto a large excess of competitor DNA templates, suggesting that they do not dissociate from initial DNA matrix [34]. The idea that nucleosomes could partially relax to allow the passage of DNA processing machineries without complete dissociation is a matter of intense debate in the chromatin field, where the problematic of DNA accessibility is essential for most chromatin activities including replication, transcription and repair. Regarding replication, although no definite model can be drawn, it is commonly believed that disrupted parental nucleosomes are bound to specific protein chaperones which would transfer the core histone building blocks behind the replication fork.

The tripartite structure of the histone octamer implies that the removal of the H3/H4 from the nucleosome is associated with a displacement of the histone dimers H2A/H2B. However, two hypotheses could be postulated for lost of the nucleosomal structure, either the entire octamer is evicted or this is performed by the successive displacement of the different building blocks composing the histone octamer. Experimental approach for studying parental histone segregation implies the possibility of discriminating the old pool of histones and the new one [37]. By preventing the synthesis of new histones using translation inhibitors, like cycloheximide and puromycine, would enable the analysis of parental nucleosome transfer, though such treatments impair replication progression. Still, one can argue that as the replication process requires a tight regulation of the histone supply, impairing this regulation profoundly impact the replication leading to the replication fork blocks. Thus, most conclusions from these experiments have to be taken with caution. Original studies using this approach coupled with micrococcale digestion (enzyme allowing specific digestion of internucleosomal DNA) revealed that the size of the fragments obtained were consistent with DNA size protected by the histone octamer. So it was originally proposed that the parental nucleosomes are dissociated ahead of the replication fork and transferred behind with no detectable intermediate. Whether the experimental design led to artifacts remains likely.

Importantly, several studies using different approaches have demonstrated a distinct mobility for the H2A/H2B and the H3/H4 in living cells [36, 38]. On the basis of the different motions of the H2A/H2B and the H3/H4, one can reasonably believe that the octamer building blocks dissociate during cellular processes. Moreover, *in vitro* experiments for reconstituting or destabilizing nucleosome revealed the presence of basic heterocomplexes of H3/H4 tetramer and H2A/H2B dimer [37]. At physiological conditions, the heterotetramer H3/H4 prepared from chromatin and in absence of histone chaperones is the most stable form of the complex in solution [39]. Even if it has been claimed that a very transient dimeric state can exist, the absence of demonstration of the H3/H4 dimers led to the anchored view that parental nucleosomes split into two H2A/H2B dimers and a H3/H4 tetramer, and are then reassembled behind the fork, with the central tetramer H3/H4 deposited first [40, 41].

The simplest view regarding the dissociation of the parental core histone from DNA could be that the driving force of the replication fork progression is sufficient for overriding the histone-DNA interactions by the only action of replication specific proteins as helicases [42]. This model involves that core histones in presence of DNA spontaneously form nucleosomal

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structures with a tripartite organization. Unfortunately, *in vitro* experiments demonstrated that such arrangement of the histone octamer required either high salt concentrations or chaperone proteins to assist the proper loading of the histones in a tripartite structure [43]. A more comprehensive view was provided by a study by Groth *et al* [44] showing that the major H3/H4 histone chaperone ASF1 (Anti-Silencing Factor 1) forms a complex with the putative replicative helicase MCM2-7 (Minichromosome Maintenance Complex), via a H3/H4 bridge. On the basis of the *in vitro* capability of ASF1 to assemble chromatin, it has been proposed that this chaperone might be involved in the recycling and the transfer of parental H3/H4 histones directly coordinated by the DNA replication process.

Concerning H2A/H2B, picture is even less clear. Chaperones, like NAP1 (Nck-associated protein 1) and FACT (Facilitates Chromatin Transcription) might be involved. The heterodimeric complex FACT, a chromatin-modifying factor initially described to promote nucleosome rearrangement during RNA polymerase II-driven transcription through H2A/H2B dimer destabilization [45], was shown to be involved in DNA replication. FACT interacts with DNA polymerase α, and in human with the MCM helicase to act on DNA unwinding [46]. Recently, a conditional knock-out of one of the FACT subunit in DT40 chicken cells (Structure-Specific Recognition Protein 1, SSRP1) showed apparent impairment in replication fork progression [47]. Even if the precise mechanisms are still to be elucidated because this complex interacts with H2A/H2B and H3/H4 in multiple ways, the synergized action of histone chaperones and replication actors is actually an attractive model of coordinated nucleosome eviction/reassembly and DNA replication during S-phase.

It is known for a long time that chromatin assembly is an ATP-dependent process [48], so it is not surprising that ATP-dependent chromatin remodeling factors have been implicated in the release of the chromatin structure. Most studies focused on nucleosome movement during transcription, but strong arguments of their involvement during replication exist. The ISWI-class of ATP-dependent remodeling family interacts with several proteins in complexes, among them ACF1 (ATP-utilizing Chromatin assembly and remodeling Factor) and WSTF (Williams syndrome transcription factor). Depletion experiments demonstrated that ACF1 is critical for efficient DNA replication of highly condensed regions of mouse cells [49], and that WSTF, targeted to replication foci via its interaction with the processivity factor PCNA (Proliferating Cell Nuclear Antigen), promotes DNA replication by preventing premature maturation of chromatin [50].

4. Reforming chromatin behind the replication fork

Chromatin reassembly behind the replication fork is a rapid process. Electron microscopic studies and psoralen cross-linked nucleosome used, have clearly shown random distribution of the nucleosomal structures on both strand of the nascent DNA, with no apparent free-DNA [35]. By blocking protein synthesis with different inhibitors, it was demonstrated that half of the nucleosome pool came from random segregation of recycled parental ones, whereas the other half came from newly synthesized histones. In proliferating cells, the histone biosynthe-

sis is coupled with the cell cycle progression. The vast majority of histones (the canonical histones) are massively produced at the beginning of the S phase, mainly by transcriptional activation of histone genes and improvement of pre-mRNA processing and stability, that begins during G1 phase (reviewed in [51, 52]). Through a feedback regulation reducing drastically the half-life of histone mRNAs, the amount of proteins then decreased at the end of S-phase until the baseline level is reached. However, experiments using replication blocking agents showed distinct synthesis profiles between H3/H4 and H2A/H2B, illustrating that specific level of regulation may exist [53]. Some specific histones (histone variants), used for deposition and exchange of nucleosomes outside of the S-phase (replication-independent chromatin assembly), are produced throughout the cell cycle. Although this aspect presents a great interest, the present chapter focuses on the regulation of the canonical histone proteins at the onset of DNA replication (for reviews about histone variants see [54, 55]).

Once the histones are synthesized, they are rapidly delivered to the site of replication and assembled into chromatin. Because these proteins are highly basic proteins, histones tend to promptly bind non-specifically to nucleic acids with a higher affinity to RNA than DNA, and they do not spontaneously form nucleosomes. To allow correct transfer into the nucleus and efficient deposition onto DNA, histone chaperones play a dual function, they neutralize the histone charge to prevent the formation of aggregates and they address the histones at precise locations within the nucleus [56].

The supply of histone is a tightly regulated process. Any events leading to replicational stress (as DNA damage for example) disturb the fine balance between histone supply and demand and have deleterious effects on the cell. Histone chaperone have critical roles in regulating this process. Consistently, deletion of the major histone H3/H4 chaperones CAF-1 (Chromatin Assembly Factor 1) or ASF1 in various organisms impair S-phase progression [57, 58]. In human, it was shown that ASF1 exists in a highly mobile soluble pool that buffered the histone excess [59]. In the budding yeast *S. cerevisiae*, ASF1 depletion impairs cell cycle progression and generates chromosome instability [60]. In this organism, it was shown that the up-regulation of the amount of histone in the cells leads to the degradation of the excess histones by a Rad53 kinase-dependent mechanism [61].

4.1. Transport into the nucleus

The nuclear import of the histone complexes is among the first levels of regulation. Several groups have attempted to define the mechanisms by which the histone supply might be regulated. The role of specific domains within newly synthesized histones essential for transport (and also formation of nascent chromatin) was first addressed using powerful genetic approaches in the yeast *S. cerevisiae.* Pioneer studies performed in budding yeast emphasized the essential role of both N-terminal H2A/H2B tails for cell viability (reviewed in [62]). Fusion protein experiments using fluorescent tracers led to the assumption that nuclear localization signals (NLS) are present in the N-terminal non-structured domain of histone proteins, and their interaction with karyopherin or importins would promote nuclear import of newly synthesized histones [63, 64]. Nevertheless, incorporation experiments of exogenous histones in the slime mold *Physarum polycephalum* showed that H2A/H2B dimers

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lacking both tail regions still localized to the nucleus. It was thus concluded that the tails of H2A/H2B are dispensable for nuclear import. However, the chromatin assembly analyses revealed that at least one tail is necessary for the deposition of the dimer complex into chromatin [65]. Conversely, studies using a similar strategy of incorporation of exogenous histones in *Physarum* to examine the fate of the H3/H4 complexes exhibited a function of the amino-terminal domains in nuclear import. Indeed, the histone H3/H4 dimers lacking H4 tail are inefficiently imported, while H3 tail was found dispensable in this process, but impaired nucleosome assembly coupled to replication [66].

By extending out of the nucleosomal structure, the exposed N-terminal regions of histones are subjected to active post-translational modifications. These marks, when imposed on assembled histones, have been shown to impact on the overall nature of the chromatin [67]. Newly synthesized histones are also characterized by a specific pattern of post-translational modifications, imposed in the cytoplasm shortly after synthesis. For example, newly synthesized H4 are diacetylated at lysine 5 and 12 by the holoenzyme HAT1 (Histone Acetyl Transferase 1), and these acetyl groups are rapidly removed after the assembly of histones into chromatin [68]. Despite the conservation of the H4 diacetylation throughout the evolution, the actual function in histone nuclear import and/or chromatin assembly remains undetermined. In Drosophila embryos, the RCAF complex comprises ASF1, acetylated H3K14, and diactetylated H4K5K12 [60] and in human, the CAC complex is composed of diacetylated H4K5K12 and CAF-1 [69]. This highlights an essential role of this dual signature for the formation of a complex between H3/H4 and the major chaperones associated to replication. However, as revealed by the co-crystal structure, ASF1 interacts with the C-terminal region of H3 [70], so the precise role of the post-translational modifications is not obvious. Strikingly, all described chaperones so far do not interact with the unstructured tails of histones. To conclude, even if the requirement of the amino-terminal regions of the histones has been evidenced for the assembly of chromatin and/or regulation of histones, their precise involvements in the overall process still necessitate investigations.

4.2. Mechanism of chromatin reassembly

Albeit the two DNA strands run in opposite directions, the progression of the replication fork is unidirectional. To reconcile that, during the replication process one daughter strand is synthesized continuously (the leading strand) whereas the other (the lagging strand) is build by short stretches of DNA named Okazaki fragments, ligated afterwards. Does this particular mode of duplication have an impact on parental nucleosomes segregation ? Even if adjacent "old" histones tend to segregate together, no clear preference for the leading or lagging strand have been demonstrated, mainly because the studies did not clearly discriminate the two strands. A recent study suggests that nucleosome positioning onto the lagging-strand could determined the length of Okazaki fragment in *S. cerevisiae*, via interaction with the enzyme polymerase pol δ, responsible for the extension of the nascent DNA chain through the 5′ end of an Okazaki fragment [71]. By purifying Okazaki fragments, and performing the alignment onto the yeast genome, they demonstrated that they strikingly mapped with nucleosome positions. Once again, these experiments nicely illustrated the coupling between the DNA replication and the chromatin assembly.

The apparent higher sensitivity to nuclease digestion of newly synthesized chromatin compared to bulk chromatin suggests that new chromatin is not completely mature. Even though it was shown that specific post-translational modifications carried by newly synthesized histones and the absence of linker H1 histone could at least partially outline a more relaxed chromatin state, the reasons for the detection of the greater DNA accessibility in replicated chromatin remain actually elusive.

Newly synthesized H3/H4 are sequestered into the cytoplasm by ASF1, probably through interaction with several other chaperones, like the histone acetyltransferase HAT1, heat-shock proteins as HSC70 (Heat Shock Cognate 70 kDa protein), HSP90 (Heat Shock Protein 90), and NASP (Nuclear Autoantigenic Sperm Protein). The recent involvement of NASP as part of a cytosolic H3/H4 histone buffering complex is surprising, as this protein was initially described as an H1 chaperone [72, 73]. Indeed, in the nucleus ASF1 synergize with CAF1 via direct interaction with the p60 subunit. CAF1 was described to promote chromatin assembly *in vitro* [74]. This evolutionary conserved trimeric protein complex is recruited to site of DNA synthesis through interaction of the p150 subunit with the replication processivity factor PCNA, linking again chromatin assembly to replication fork progression [58]. As for parental histones, pioneer experiments using pulse-labeled histones suggested a sequential deposition of newly synthesized histones, with a H3/H4 tetramer assembled first, follow by the deposition of two H2A/H2B dimers.

The deposition model of nucleosomes, based on the stable tetrameric nature of histone H3/H4, was recently revisited [75]. Tagami and colleagues purified predeposition chromatin assembly complexes from HeLa cells stably expressing epitope-tagged histone H3.1 isoform (the replicative histone). The analyses of the immunoprecipitated tagged histones from purified nucleosomes and from the predeposition complexes showed that whereas about 50% of H3 in the nucleosomal fraction contained the epitope tag, all the histone complex in the predeposition complexes were tagged. It was thus concluded that H3/H4 complex is deposited onto DNA as dimer rather than tetramer. Biochemical, crystallographic and NMR analyses of ASF1 in complex with H3 (and sometimes H4) confirmed the dimeric nature of H3/H4 bound to the chaperone [70, 76, 77]. Furthermore, the structural data pointed out that the H3/H4 heterodimer binds ASF-1 at critical residues for H3/H3 interaction in the nucleosome, thus physically blocking the formation of a H3/H4 heterotetramer. This model has been reinforced by mutations of amino acids at critical regions. The dimeric nature of H3/H4 is also supported by a paper analyzing the composition of centromeric nucleosomes in the fruit fly *Drosophila*. At this particular genomic location, the nucleosome would exist in interphase as a stable tetramer, as a complex of single copy of CenH3-H2A-H2B and H4 has been identified [78].

5. Concluding remarks

The semi-conservative mode of replication of DNA ensures that the genetic information is faithfully transmitted to the daughter cells after mitosis. In higher eukaryotes, as the DNA is replicated, the chromatin environment has to be removed and subsequently restored. Here,

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we have reviewed an overview of the actual mechanisms that can sustain this operation. The studies described and cited in this chapter are based upon different experimental approaches, which might potentially present caveats inherent to the experimentations. Even though profound advancements have been reported during the past few years to clarify the factors involved in the transport and delivery of histones, basic concerns still have to be unraveled.

It is generally believed that the histone post-translational modifications impact chromatin structure and the chromatin activities through the recruitment of different effectors and modulators. Beside the mechanistic comprehension of the process of DNA replication in the chromatin context, underlying question addressed is how the chromatin organization and the information carried by histones are maintained or altered during replication. Indeed, the demonstration of the link between chromatin replication and cell differentiation suggests that the S-phase is a window of great opportunity for modulating the epigenetic regulations in a genetic program. However, in this context, the alterations of the chromatin structure and the histone modifications have not yet been fully elucidated.Three models can emphasize the nucleosome reorganization behind replication fork (Figure 2): (A) the entire parental octamer is transferred to form nucleosome and newly synthesized histones fill up the gaps. (B) The parental nucleosome splits into building blocks composed of a tetramer of H3/H4 and dimers of H2A/H2B, and the blocks are redistributed onto the two strands of DNA. The new histones are utilized for achieving the formation of the octamer. (C) The recently advanced dimeric nature of H3/H4 paved a new avenue for future investigations. The splitting of the tetramer could lead to mixed nucleosome, composed of parental and new histones.

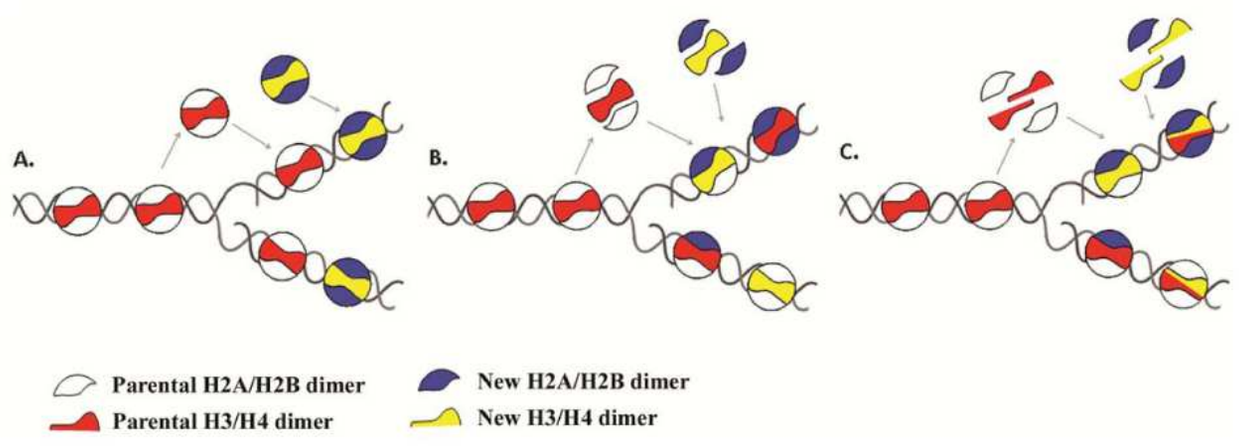

Figure 2. Working models of nucleosome reorganization during DNA replication. (A) Parental nucleosome is transferred as intact unit, without disruption of the octamer, leading to nucleosome fully constituted either of old or of new histones. (B) Parental nucleosome splits into H3/H4 tetramer and H2A/H2B dimers. In this model, new and old H3/H4 cannot coexist in the same nucleosome behind the replication fork. (C) Parental nucleosome splits into H3/H4 and H2A/H2B dimers, leading to mixed nucleosomes composed of old and new histones in each nucleosome building block.

In any considered model, the epigenetic information associated with the histone marks need to be copied from parental histones to newly synthesized ones. Concerning DNA methyla-

tion, the inheritance is a better-characterized process. In mammals, this modification mainly occurs on CpG dinucleotide (a cytosine followed by a guanine). The anti-parallelism of the DNA molecule, and the semi-conservative mode of DNA replication, ensure that the PCNA-interacting DNA methyltransferase DNMT1 easily copy the parental pattern onto the virgin daughter strand. To date, the mechanisms of the histone modification inheritance remains unclear. Most likely, future works in the field will attempt to address this issue.

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