Chapter 15
Chromatin Replication and Histone Dynamics

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Abstract Inheritance of the DNA sequence and its proper organization into chromatin is fundamental for genome stability and function. Therefore, how specific chromatin structures are restored on newly synthesized DNA and transmitted through cell division remains a central question to understand cell fate choices and self-renewal. Propagation of genetic information and chromatin-based information in cycling cells entails genome-wide disruption and restoration of chromatin, coupled with faithful replication of DNA. In this chapter, we describe how cells duplicate the genome while maintaining its proper organization into chromatin. We reveal how specialized replication-coupled mechanisms rapidly assemble newly synthesized DNA into nucleosomes, while the complete restoration of chromatin organization including histone marks is a continuous process taking place throughout the cell cycle. Because failure to reassemble nucleosomes at replication forks blocks DNA replication progression in higher eukaryotes and leads to genomic instability, we further underline the importance of the mechanistic link between DNA replication and chromatin duplication.

Keywords DNA replication • Nucleosome assembly • Histone chaperone • Histone recycling • Chromatin • Epigenetics

15.1 Introduction

Genome function in eukaryotes is regulated by chromatin, a complex structure consisting of DNA, histones, RNA and a large number of structural and regulatory proteins. Chromatin compacts the genome, restricting access to the DNA template

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in a manner that is dependent on chromatin composition and chemical modifications on histones and DNA. Distinct chromatin states can be inherited through mitotic cell division, and this contributes to the correct execution of developmental programmes by establishment and maintenance of gene expression patterns. Duplication of chromatin structures in dividing cells thus impinges on the maintenance of epigenetic states and cell identity. However, while our knowledge about DNA replication is comprehensive, the process of chromatin duplication remains poorly understood. In this chapter we will discuss how chromatin is replicated with the emphasis on histone dynamics during DNA replication and maintenance of histone-based information.

The nucleosome consists of 147 base pairs of DNA wrapped around a histone octamer. Each octamer is composed of one centrally located H3-H4 tetramer, flanked by two H2A-H2B dimers. Histones come in different flavours, so-called variants, which along with the large array of modifications in the histone tails holds information important for the chromatin state. The current view is that the preservation of such histone-based information contributes to inheritance of chromatin states. Pioneering electron microscopy studies of replicating chromatin in *Drosophila* embryos revealed that nucleosomes are formed very rapidly after on the daughter DNA strands (McKnight and Miller 1977). Since then, we have learned that chromatin replication can be divided into five steps (Fig. 15.1): (1) disassembly

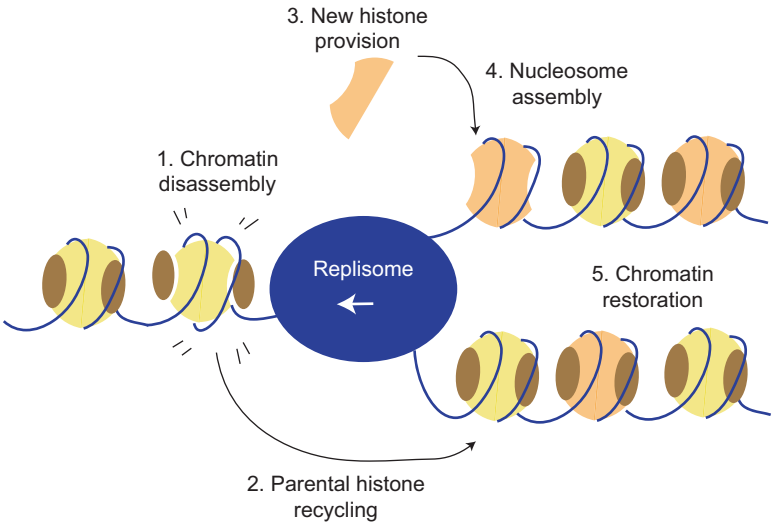

Fig. 15.1 DNA replication in the context of chromatin. The process can be divided into five steps: (*1*) disassembly of chromatin ahead of the replisome, (*2*) recycling of parental histones in a presumably random fashion onto the two daughter strands, (*3*) supply of newly synthesized histones to sites of ongoing DNA replication, (*4*) nucleosome assembly on leading and lagging strands from 50% newly synthesized histones to 50% old recycled histones, (*5*) restoration of marks on DNA and histones and reassociation of other chromatin components including structural and regulatory proteins

of chromatin immediately ahead of the replication machinery, (2) recycling of evicted parental histones onto newly replicated DNA, (3) supply of newly synthesized histones to the sites of ongoing DNA replication, (4) nucleosome assembly from recycled parental and new histones to maintain nucleosome density on the two new daughter DNA strands and (5) restoration of marks on DNA and histones and association of additional chromatin components to restore the chromatin structure. Here we cover mainly steps 1–4 as step 5 is separated from the replication process. Further, we discuss current challenges to understand the dynamic and complex processes of chromatin replication and epigenome maintenance.

15.2 Replication Through Nucleosomes

The DNA molecule is wrapped in a left-handed manner around histone octamers to form nucleosomes (Fig. 15.2). Nucleosomes represent a barrier for DNA-based processes such as transcription, DNA replication and DNA repair. This section focuses

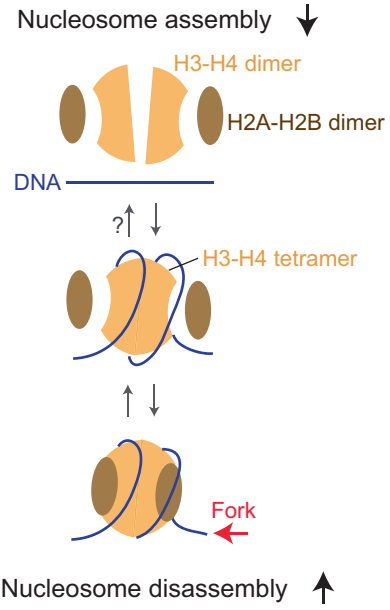

Fig. 15.2 Nucleosome assembly and disassembly processes. In the nucleosome, a histone octamer is wrapped by 147 bp of DNA in a left-handed manner. Nucleosome disassembly (*bottom* to *top*) probably relies on unwinding of DNA by the helicase arriving at the proximal H2A-H2B dimer. This will first release a H2A-H2B dimer and then lead to full disruption of the octamer upon release of the H3-H4 tetramer. Parental histone H3-H4 do not mix with new histone H3-H4, suggesting that separate deposition pathways may exist (see main text). Newly synthesized histones H3-H4 are transported as dimer, which assemble into tetramers in the process of nucleosome assembly

on recent advances in understanding of how nucleosomes are transiently disassembled ahead of replication fork and how evicted histones are recycled on the newly replicated daughter strands.

15.2.1 Chromatin Disassembly Ahead of the Replisome

To sustain an elongation rate of 1.5–2 kb per minute, one nucleosome has to be evicted every 7 second ahead of the replisome. The eviction mechanism involves local destabilization of one (maximally 2) nucleosomes ahead of each replication fork (Gasser et al. 1996; Sogo et al. 1986). Unzipping of the DNA duplex using optical tweezers is sufficient to provoke octamer eviction in vitro (Shundrovsky et al. 2006). However, in vivo studies and recent reconstituted replication systems suggest that a coordinated effort of histone chaperones and chromatin remodellers along with other events allow fork progression through chromatin.

15.2.1.1 Histone Chaperones

Histone chaperones are defined proteins that handle non-nucleosomal histones in vivo and mediate the assembly of nucleosomes from histones in vitro. FACT, consisting of a heterodimer of Spt16 and SSRP1 in humans, is currently viewed as the key chaperone involved in chromatin disruption. FACT binds H2A-H2B dimers (Belotserkovskaya et al. 2003; Orphanides et al. 1999) and H3-H4 tetramer (Tsunaka et al. 2016) and serves a key role in transcription where it aids nucleosome disruption ahead of the RNA polymerase and restores the chromatin template behind (Hammond et al. 2017). Evidence from several organisms have linked FACT to DNA replication, and multiple interactions between FACT and replisome components have been reported (Hammond et al. 2017). However, the exact function of FACT in replication has been difficult to dissect as genetic analysis is hampered by its role in transcription. However, a recent study reconstituting DNA replication on a nucleosomal template from recombinant proteins revealed that FACT is required and sufficient to allow replisome progression in this system (Kurat et al. 2017). How FACT permits the progression of replisomes through nucleosome arrays remains to be determined. As FACT interacts with the replisome (Foltman et al. 2013), one possibility is that FACT promotes nucleosome disassembly as it collides with nucleosomes. As FACT is an extremely abundant protein (one per five nucleosomes in yeast McCullough et al. 2015), another possibility is that FACT destabilizes nucleosomes ahead of the replisome. Since FACT has also been proposed to deposit parental (Foltman et al. 2013) and new histones (Yang et al. 2016) behind the fork, it will be important to dissect these functions from disassembly. Indeed, both nucleosome disassembly ahead and reassembly behind replication fork may control elongation speed, as discussed in Sect. 15.3.

15.2.1.2 ATP-Dependent Chromatin Remodelling Complexes

Chromatin remodellers are large multi-protein complexes that come in different flavours and allow the access to DNA by altering the structure, composition and/or position of nucleosomes. They were first found to play a key role in transcriptional regulation and are now recognized to be part of most chromatin-based processes including DNA replication (Narlikar et al. 2013). Several chromatin remodelling complexes have been suggested to destabilize or remove nucleosomes ahead of replisomes. Recent studies of reconstituted replication on a chromatin template did not find a requirement for ATP-dependent remodelling for fork progression, but the activity of INO80 and ISWIA enhanced the elongation rate (Kurat et al. 2017). Consistent with this, members of the INO80 and ISW2 complex promote efficient fork progression in *S. cerevisiae* (Iida and Araki 2004; Vincent et al. 2008). In mammals, members of the ISWI family (WICH and ACF) and of the INO80 complex have been shown to promote fork progression (Collins et al. 2002; Lee et al. 2014; Poot et al. 2004). However, chromatin remodellers also play an important role behind the fork in establishing nucleosome spacing and position, as discussed in Sect. 15.4. Thus, whether slow fork speed in vivo reflects a function of these remodellers ahead or behind the fork remains unclear.

15.2.1.3 Other Mechanisms Promoting Nucleosome Disassembly

Progression of the replisome creates positive torsional stress of the DNA molecule ahead of the fork. In vitro studies show that nucleosome assembly stalls when DNA is under positive torsional stress (Gupta et al. 2009) and that H2A/H2B dimers could dissociate from the H3-H4 tetramer (Bancaud et al. 2006, 2007). Positive torsional stress could thus induce a structural change in nucleosome that aids disassembly and fork progression (reviewed in Teves and Henikoff 2014). Another mechanism that may aid chromatin disruption is phosphorylation of the linked histone H1. Phosphorylation of histone H1 by cyclin A-CDK2 can decompact chromatin fibres (Contreras et al. 2003) by increasing the dynamic exchange of histone H1. Given that Cdk2 may travel with the fork, it could trigger decompaction of replication domains to facilitate replisome progression (reviewed in Alabert and Groth 2012).

15.2.2 Histone Recycling at Replication Forks

Nucleosomal histones carry modifications that are important for regulation of genome function; thus how they are handled during DNA replication impinges not only on fork progression but also on maintenance of epigenetic states. Histone octamers dissociate upon nucleosome disruption, releasing two H2A-H2B dimers and two H3-H4 dimers under physiological salt conditions (Hammond et al. 2017).

Most evidence suggest that the H3-H4 tetramer remains intact in the process of being recycled onto newly replicated DNA (Fig. 15.2, see below), in which case additional factors like histone chaperones must be involved to maintain the tetrameric state (Hammond et al. 2017). H2A-H2B dimers are also recycled (Alabert et al. 2015), but this process remains poorly understood.

15.2.2.1 H3-H4 Transfer

Upon release from nucleosome, H3-H4 could be transferred as a tetramer or further split into dimers. This has been a major question in the field because of the implicates it has on inheritance of histone-based information; if new and old histone dimers mix after DNA replication, histone modification could be evenly distributed onto the two daughter stands and copied from old histones to new ones within one nucleosome. However, despite the appealing nature of this model, most evidence argue that there is no or only little mixing of new and old histones H3-H4 dimers in the process of DNA replication (Annunziato 2005; Xu et al. 2010). Two complementary technologies have been used to analyse the fate of H3-H4 tetramers upon recycling during DNA replication, differential metabolic labelling of pre-existing and newly synthesized histones (Jackson 1990; Yamasu and Senshu 1990) and differential tagging on newly synthesized and pre-existing histones (Katan-Khaykovich and Struhl 2011; Prior et al. 1980; Xu et al. 2010). Results obtained by both approaches suggest that parental histone H3-H4 do not mix with new histone H3-H4 dimer, which support the idea that the H3-H4 tetramer is transferred as an entity. However, it remains possible that the H3-H4 tetramers split into dimers transiently and reassociate as nucleosomes are assembled on newly replicated DNA. In either case, the absence of new and old H3-H4 dimer mixing in nucleosomes suggests that there are distinct pathways for replication-coupled deposition of new and old histones H3-H4. In relation to epigenetic cell memory, this argues that a potential copy-paste mechanism to propagate histones must operate between nucleosomes rather than internally between tails in the same nucleosome.

15.2.2.2 Mechanisms of Histone Recycling

Pioneer studies of SV40 in vitro replication systems have suggested that histones remain in close proximity to the replisome during recycling (reviewed in Annunziato 2013); however it remains unclear how accurate the recycling process is. Will old histones reassociate with the same DNA sequence or will there be some displacement relative to their original position? A study in yeast used mathematical modelling to address this question, and they estimated that old histones would be recycled roughly within 400 bp of their original position (Radman-Livaja et al. 2011). Recent insights into histone-binding activities within the replisome provide some mechanistic insight into this process of histone segregation (Fig. 15.3).

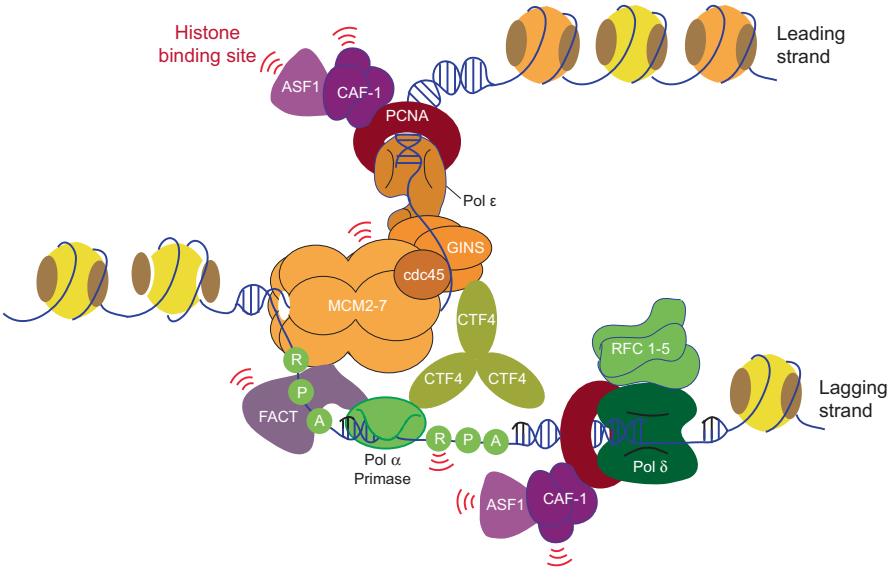

Fig. 15.3 Contact between histones and the replisome. Contact points between histones and replication proteins are indicated in red

MCM2, part of the replicative helicase (MCM2-7), has histone chaperone activity (Ishimi et al. 2001) and binds histone H3-H4 in human (Groth et al. 2007) and yeast (Foltman et al. 2013). Recent crystal structures revealed that MCM2 can chaperone either a H3-H4 tetramer (Richet et al. 2015; Huang et al. 2015) or a H3-H4 dimer in complex with the ASF1 chaperone (Huang et al. 2015; Wang et al. 2015). The histone-binding domain of MCM2 binds H3-H4 tetramers by mimicking DNA in the nucleosome (Huang et al. 2015), which provide a very attractive binding mode for a chaperone involved in recycling. Indeed, MCM2 can handle all H3 variants including H3.3 and CENPA (Huang et al. 2015), and modification of the histone tails should not affect binding. Thus, MCM2 could act as an acceptor of evicted histone H3-H4 genome wide. Consistent with this, mutations of MCM2 histone-binding domain reduce silencing in yeast (Foltman et al. 2013) and slow down cell proliferation in cancer cell lines (Huang et al. 2015). Whether MCM2 operates alone or in conjunction with additional chaperones remains unclear. MCM2 form a co-chaperone complex together with ASF1 in which both chaperones make contact with a H3-H4 dimer (Groth et al. 2007; Huang et al. 2015; Wang et al. 2015). This co-chaperone interaction occurs at replication sites in mammalian cells (Huang et al. 2015), but it remains unclear whether it contributes to normal recycling of H3-H4, through a transient dimeric state, or mainly operates to catch evicted histone upon replication stress as this leads to accumulation of the complex with parental histones (Huang et al. 2015; Jasencakova et al. 2010).

MCM2 also forms a co-chaperone complex with FACT (Foltman et al. 2013). This chaperone travels with the replisome (Alabert et al. 2014; Foltman et al. 2013; Gambus et al. 2006) and can make contact to several replisome components including MCM4 (Gambus et al. 2006; Tan et al. 2006), Polα and RPA1 (VanDemark et al. 2006; Wittmeyer et al. 1999; Zhou and Wang 2004). FACT can bind H3-H4 tetramers (Stuwe et al. 2008; Tsunaka et al. 2016) as well as H2A-H2B dimers (Belotserkovskaya et al. 2003; Orphanides et al. 1999). Recently structural work showed that the binding of H2A-H2B and H3-H4 is not mutually exclusive, and therefore, in theory, FACT could transfer a H3-H4 tetramer together with at least one H2A-H2B dimer. This may happen through a co-chaperone interaction with MCM2, as the structural and biochemical data supports that they can bind H3-H4 simultaneously. FACT is required for replisome progression (Abe et al. 2011; Kurat et al. 2017; Tan et al. 2006); however, as mentioned in Sect. 15.1, genetic studies on FACT are complicated by its multiple functions in transcription and chromatin maintenance.

15.3 New Histone Provision

During replication, pre-existing parental histones are recycled as described above, and in addition newly synthesized histones are delivered and deposited onto the newly synthesized daughter DNA strands to maintain nucleosome density. In human cells about 33 million new nucleosomes have to be assembled in each S phase. To match this high demand for nucleosome assembly, the biosynthesis of new histones and their transfer to replicating DNA are regulated in multiple ways. The replication-dependent canonical histones (H3.1, H3.2, H4, H2A, H2B and H1) are multicopy genes, which are induced at the onset of DNA replication and tightly regulated at both the transcription and post-transcription levels (reviewed in Marzluff et al. 2008). The shortage or excess of histones can block DNA replication and trigger genomic instability in yeast and mammals (Groth et al. 2007; Gunjan and Verreault 2003; Han et al. 1987; Kim et al. 1988; Meeks-Wagner and Hartwell 1986; Mejlvang et al. 2014; Nelson et al. 2002). The massive production of canonical histones also represents a logistic challenge: newly synthesized histones need to be efficiently guided to sites of ongoing replication, a process that is carried out by histone chaperones (reviewed in Hammond et al. 2017). Histone chaperones are a broad class of proteins that handle non-nucleosomal histones in vivo and in vitro can mediate the assembly of nucleosomes when mixed with histones and DNA.

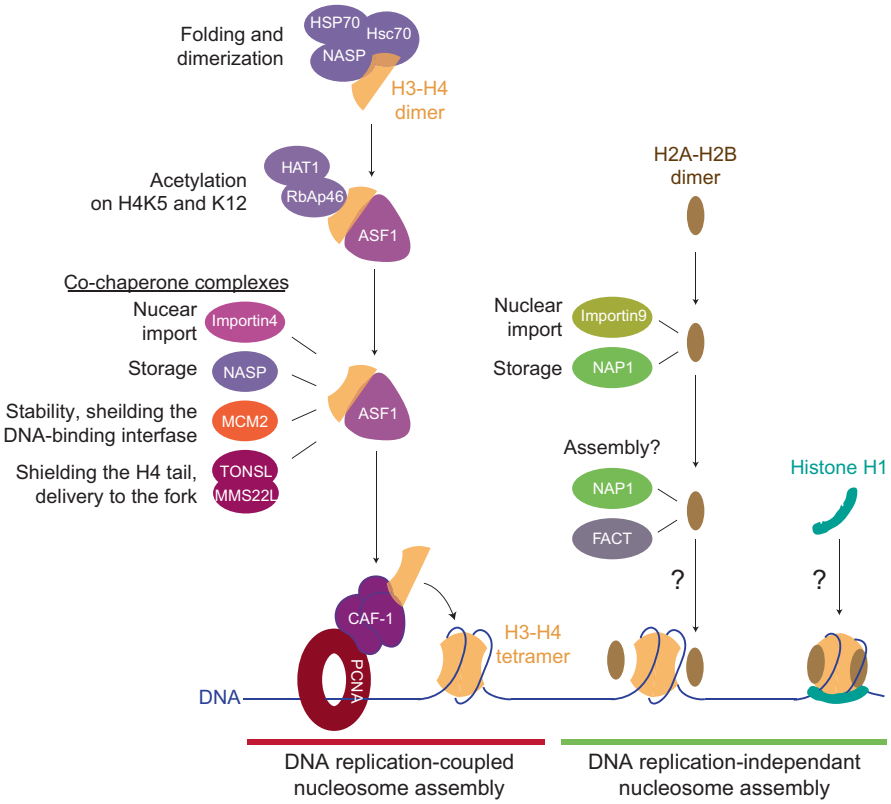

Fig. 15.4 Network of histone chaperones, handling histones from their site of synthesis to their deposition onto newly replicated DNA (See text for details)

15.3.1 Delivery of Newly Synthesized H3.1/H3.2-H4

Soon after their synthesis, histones H3.1/H3.2 and H4 engage with histone chaperones and are transported to sites of DNA replication as dimers (Benson et al. 2006; Tagami et al. 2004). The histone supply chain involves a network of specialized chaperones that are connected via histone-dependent and histone-independent interactions that allow 'on-the-go' modification of the histone, handover of histones between different chaperone complexes and culminates with CAF-1 that assembles H3.1-H4 dimers and newly replicated DNA into tetrasomes, which are completed into nucleosomes upon the addition of H2A-H2B (reviewed in Hammond et al. 2017) (Fig. 15.4).

15.3.1.1 Histone H3.1-H4 Chaperone Network

Biochemical and proteomic characterization of protein complexes interacting with soluble histones has uncovered a large number of histone chaperones, and the list is still growing. While it remains unclear exactly how all these chaperones are organized with respect to each other in the supply chain, the position and role of several key players have been identified based on both biochemical and functional studies. Initially, rapidly after histone synthesis in the cytoplasm, histone H3.1/H3.2/H3.3-H4 can interact with HSC70, HSP90 and NASP that promote the folding and formation of histone dimers (Alvarez et al. 2011; Bowman et al. 2017; Campos and Reinberg 2010). NASP is found in co-chaperone complexes with ASF1 and RBAP46-HAT1 (catalyses H4 acetylation, discussed below) and thus presumably works upstream of these in the network (Bowman et al. 2017; Haigney et al. 2015; Jasencakova et al. 2010).

ASF1 is a central histone H3-H4 chaperone that then engages with several additional histone chaperones, histone modifiers and other accessory partners to form dynamic multi-protein complexes that carry out specialized tasks in histone supply. These tasks include nuclear import (IMPORTIN-4), acetylation of histone tails (RBAP46-HAT1) and regulating the storage pool of soluble histones (NASP or MCM2) (reviewed in Hammond et al. 2017) (Fig. 15.4). These interactions represent co-chaperone relationships where two or more chaperones bind histones concomitantly. ASF1 interacts with the H3 alpha2-alpha3 helices that constitute the H3-H4 tetramerization interface (English et al. 2006; Natsume et al. 2007). Thus, a major consequence of ASF1 binding is to maintain H3-H4 dimeric, while leaving other histone interfaces available for other chaperones like MCM2 (Groth et al. 2007; Huang et al. 2015), RBAP46 (Haigney et al., 2015; Jasencakova et al. 2010), VPS75 (Hammond et al. 2016), NASP (Bowman et al. 2017; Jasencakova et al. 2010) and TONSL (Saredi et al. 2016). It remains to be understood how this dynamic network of multi-chaperone interactions is regulated. However, ASF1 is subject to phosphorylation by the S phase active kinases TLK1 and TLK2 (Sillje and Nigg 2001; Sillje et al. 1999), which are targets of the checkpoint kinase Chk1 (Groth et al. 2003; Krause et al. 2003). TLKs specifically target histone-free ASF1, and phosphorylation in turn promotes histone binding and interaction with downstream chaperones like CAF-1 (Klimovskaia et al. 2014). In contrast, Codanin-1 can act as a negative regulator of histone supply by sequestering ASF1 in the cytoplasm (Ask et al. 2012). Once ASF1 translocate with H3-H4 dimers into the nucleus, it makes direct contact with the two downstream chaperones CAF-1 and HIRA (De Koning et al. 2007; Mello et al. 2002; Tang et al. 2006; Tyler et al. 2001) and, somehow, hand over histone H3-H4 dimers to these chromatin-bound complexes. Whereas CAF-1 deposits canonical histones H3.1/H3.2-H4 in a replication-coupled manner (Sect. 15.3.3) (Smith and Stillman 1989; Tagami et al. 2004), HIRA deposit H3.3-H4 in a replication-independent manner (Ray-Gallet et al. 2002; Tagami et al. 2004) and may serve a gap-filling role under conditions where replication-coupled nucleosome assembly is impaired (Ray-Gallet et al. 2011).

15.3.1.2 Post-translational Modifications of Newly Synthesized H3.1-H4

Newly synthesized histones H4 are acetylated on lysine 5 and 12 by the histone acetyl transferase HAT1 in complex with RBAP46 (Kleff et al. 1995; Parthun et al. 1996; Sobel et al. 1995). This histone H4 di-acetylation is a highly conserved hallmark of new histones, yet its exact function remains unclear. It may contribute to a wide range of events including import of histones into the nucleus (Blackwell et al. 2007; Glowczewski et al. 2004), repair of replication forks (Barman et al. 2006) and CAF-1-dependent chromatin assembly in vivo (Ejlassi-Lassallette et al. 2011; Zhang et al. 2013). Acetylation of new histones generates an open and accessible chromatin organization behind the replication fork (Annunziato and Seale 1983), in part by delaying binding of the linker histone H1 until the acetylations are removed by histone deacetylases 10–20 min after fork passage (Alabert et al. 2014; Perry and Annunziato 1989; Sirbu et al. 2011). Histone H3 is also acetylated, but the sites and abundance differ between species. In yeast, ASF1-bound histone H3 is subject to acetylation on lysine 56 by Rtt109 (Driscoll et al. 2007; Han et al. 2007; Tsubota et al. 2007), and this is a very abundant modification which has major implications for genome stability (Masumoto et al. 2005). H3 K56ac promotes binding to the downstream chaperones CAF-1 and RTT106 and thus facilitates nucleosome assembly (Burgess et al. 2010; Li et al. 2008). The function of H3K56ac does not appear to be conserved in mammals, as H3K56ac is not very abundant (Jasencakova et al. 2010; Ray-Gallet et al. 2011; Xu et al. 2011). In human, soluble H3 is acetylated on lysine 14 and 18 (Jasencakova et al. 2010; Loyola et al. 2006), but their functions remain unclear. Soluble histone H3.1/H3.2 can also be monomethylated on lysine 9 by SETDB1 (Loyola et al. 2006) both early on in the process of translation (Rivera et al. 2015) and late in the assembly pathway as part of a heterochromatin-associated CAF-1 complex containing HP1a and SetDB1 (Loyola et al. 2009). Pre-deposition mono-methylation of H3K9 can prime for the establishment of H3K9me3, which is associated with repressive chromatin (Loyola et al. 2009; Pinheiro et al. 2012); however H3K9me1 may also serve additional roles in the assembly process itself.

15.3.2 Delivery of Newly Synthesized H2A-H2B

The supply chain that delivers histone H2A-H2B to newly replicated DNA appears to be less complex (reviewed in Hammond et al. 2017), although this may reflect that fewer studies have focused on H2A-H2B chaperones. NAP1, the main chaperone for soluble H2A-H2B, stabilizes the H2A-H2B dimer (D'Arcy et al. 2013) and together with Importin-9 facilitates nuclear import (Straube et al. 2010). The binding of NAP1 prevents unscheduled accumulation of H2A-H2B on DNA (Andrews et al. 2010; D'Arcy et al. 2013), and NAP1 is a prime candidate to deliver H2A-H2B to newly formed tetrasomes to complete nucleosome assembly. However, given that FACT is also a histone H2A-H2B (Belotserkovskaya and Reinberg 2004; Hondele

et al. 2013) and it is present at replication forks, it might also contribute to H2A-H2B deposition (reviewed in Hammond et al. 2017).

15.3.3 De Novo Deposition

Based on the average replication rate of 1.5–2 kb/min, about one nucleosome assembles on leading and lagging strands every 7 second. Electron microscopy data of replication intermediates suggest that the first nucleosome is assembled about 225 and 285 bp behind the fork for leading and lagging strands, respectively (Gasser et al. 1996). The nucleosome assembly is a stepwise process: the H3-H4 tetramer assembles first followed by two H2A-H2B dimers (Hammond et al. 2017; Smith and Stillman 1991) (Fig. 15.4). While new and old H3-H4 dimers are segregated into separate nucleosomes, new and old H2A-H2B dimers can associate with both new and old H3.1-H4 tetramers (Jackson 1987; Xu et al. 2010). Deposition of histone H1 occurs later as part of the chromatin maturation process, probably depending on deacetylation of the new histones and nucleosome remodelling to establish order nucleosome arrays (Alabert et al. 2014; Perry and Annunziato 1989).

15.3.3.1 Mechanisms of Nucleosome Assembly

Under physiological salt concentrations, histones bind nonspecifically to naked DNA and do not form nucleosomes, and efficient nucleosome assembly thus relies on the concerted action of histone chaperones (reviewed in Hammond et al. 2017). The key chaperone responsible for histone H3.1-H4 deposition onto newly replicated DNA is CAF-1 (Smith and Stillman 1989), which receives histone dimers from ASF1 (Tyler et al. 1999). CAF-1 is composed of three subunits: p150, p60 and RBAP48 (reviewed in Hammond et al. 2017). CAF-1 p150 binds directly to PCNA via a dual PIP-box motif (Moggs et al. 2000; Shibahara and Stillman 1999), elegantly coupling CAF-1 activity to DNA synthesis. ASF1 binds directly to the B domain of the CAF-1 p60 subunit (Mello et al. 2002; Tang et al. 2006; Tyler et al. 2001) through a surface that is an opposite site to the H3-H4-binding site on ASF1 (English et al. 2006; Natsume et al. 2007) (Fig. 15.4). While CAF-1 potentially could bind H3-H4 through RBAP48 (Verreault et al. 1996), recent data argue that it is mainly the p150 subunit that interacts with histones (Kim et al. 2016; Liu et al. 2016). CAF-1 has been found to mainly bind H3.1-H4 dimers (Tagami et al. 2004), but biophysical studies of CAF-1 function show that two CAF-1 complexes may work together to assembly a tetramer during assembly (Mattiroli et al. 2017); Sauer et al. 2017).

In addition to CAF-1, additional pathways for replication-coupled nucleosome assembly are emerging. However, their relative importance and integration with each other remain unclear. RTT106, a yeast chaperone with no clear mammalian homolog, works together with CAF-1 in replication-coupled nucleosome assembly

(Clemente-Ruiz et al. 2011; Li et al. 2008), but it is not clear how it is recruited to sites of DNA replication. Yeast RPA, a critical constituent of DNA replication machinery that binds ssDNA, was recently found to bind histone H3-H4 and facilitate replication-coupled deposition of new histones H3-H4 (Liu et al. 2017). In yeast, FACT has also been implicated in new histone H3-H4 deposition (Yang et al. 2016), and there is evidence that yeast ASF1 interacts with RFC3, part of the clamp loader (Franco et al. 2005). In addition, MCM2 is able to bind both new and old histones at the replication fork and could thus play a role in new histone deposition together with ASF1 (Huang et al. 2015; Jasencakova et al. 2010), although interaction with MCM2 is not a prerequisite for H3.1-H4 incorporation in mammalian cells (Huang et al. 2015). A critical question for future research is whether any of these deposition pathways are specific to either new or old histone H3-H4 and how their activities are coordinated to ensure balanced deposition of new and old H3-H4 on both leading and lagging strands as predicted in the current models.

15.4 Coordination of Nucleosome Assembly and Fork Progression

In yeast, cells can complete one round of DNA replication in the absence of newly synthesized histones (Kim et al. 1988; Prado and Aguilera 2005). In mammals, inhibition of histone production rapidly blocks replisome progression (Mejlvang et al. 2014; Seale and Simpson 1975; Weintraub 1972), reflecting a requirement to coordinate DNA replication and nucleosome assembly. The pool of newly synthesized histones represents only about 1% of the total number of histones in the cells, which explain the need for high rates of histone biosynthesis throughout S phase. The prediction is that replication rates will be sensitive to small changes in new histone availability, as it is the case for deoxyribonucleotides (Earp et al. 2015). However, whereas lack of dNTPs rapidly induces a checkpoint response in part due to excessive DNA unwinding at replication forks (Zeman and Cimprich 2014), lack of histones and impaired nucleosome assembly slows down replication forks without exposing ssDNA and triggering a strong checkpoint response (Groth et al. 2007; Mejlvang et al. 2014). Nevertheless, forks arrested due to lack of nucleosome assembly do become unstable over time giving rise to DNA damage and genomic instability (Hoek and Stillman 2003; Mejlvang et al. 2014; Ye et al. 2003). While replication-coupled nucleosome assembly is not required for fork progression in yeast, it is necessary to maintain integrity of advancing forks. Indeed, mutants defective in replication-coupled chromatin assembly show large chromosomal rearrangements, elevated sister chromatid exchange and loss of replication fork integrity (Clemente-Ruiz et al. 2011; Clemente-Ruiz and Prado 2009; Myung et al. 2003; Prado et al. 2004).

One mechanism proposed to directly link nucleosome assembly and replisome progression involves nucleosome assembly on the lagging strand. Here, histones

can be in principle be deposited onto the growing Okazaki fragment once sufficient DNA has been synthesized. Consistent with this idea, the two enzymes important for Okazaki fragment processing, FEN1 and DNA ligase I, can operate efficiently on a nucleosomal substrate (Chafin et al. 2000; Huggins et al. 2002). Intriguingly, Okazaki fragment size is close to the size of a nucleosome repeat (about 200 bp in human cells), suggesting a link between the two processes. This link has now been supported by in vitro and in vivo work in yeast (Devbhandari et al. 2017; Kurat et al. 2017; Smith and Whitehouse 2012). In vivo mapping of Okazaki fragments in yeast revealed that the ligation sites between Okazaki fragments are found in close proximity to the nucleosome dyads and that Okazaki fragment length is increased in CAF-1 mutants deficient in nucleosome assembly (Smith and Whitehouse 2012). More recently, elegant systems for reconstituting DNA replication in vitro demonstrated that nucleosome assembly determines Okazaki fragment length by restricting Pol delta (Polδ) progression (Devbhandari et al. 2017). The evidence thus support a model in which newly assembled nucleosomes will block Polδ progression as the polymerases reaches the nucleosome, which in turn is the signal to release the polymerase and ligate the fragment to the lagging strand. Consistent with this, PCNA unloading, which requires ligation of Okazaki fragments (Kubota et al. 2015), is also impaired when nucleosome assembly is impaired (Mejlvang et al. 2014). Nucleosome assembly on the lagging strand would therefore prime for Polδ release, Okazaki fragment maturation and PCNA unloading, potentially tethering replisome progression to chromatin assembly. How the interplay and potential interdependency between DNA synthesis by Pol epsilon and PCNA-dependent nucleosome assembly operate on the leading strand remains unclear.

15.5 Epigenome Maintenance

DNA replication leads to a genome-wide disruption of chromatin along with two-fold dilution of histone modification as new histones are deposited to double the nucleosome content (Alabert et al. 2015; Alabert and Groth 2012). How chromatin-based information is restored on the two new daughter DNA strands remains a major question. The mechanisms governing restoration of DNA methylation have been extensively studied, and here the maintenance DNA methyltransferase DNMT1 is recruited to hemi-methylated sites in new DNA shortly after fork passage through a mechanism that involves the UHFR1/2 cofactors directly recognizing hemi-methylated CpG sites (Almouzni and Cedar 2016). This section focuses on the restoration of nucleosome positioning and histone modifications. Reincorporation of histone variants and restoration of high-order structures are reviewed elsewhere (Dileep et al. 2015; Talbert and Henikoff 2017).

15.5.1 Nucleosome Positioning

The position of nucleosomes contributes to gene regulation by controlling access to DNA. Accordingly, active promoters and enhancers consist of well-defined nucleosome-free regions (NFR). At these loci, newly assembled nucleosomes thus have to be repositioned to match the pre-replicative state. Nucleosome positioning behind replication forks has recently been analysed genome-wide in yeast and *Drosophila*. In yeast, nucleosomes realign according to their initial profile within minutes (Fennessy and Owen-Hughes 2016; Vasseur et al. 2016). The positioning occurs more rapidly in gene bodies of highly transcribed genes, suggesting a role for transcription elongation (Vasseur et al. 2016). However, transcription elongation-independent mechanisms are also involved in nucleosome positioning, and transcription factors have been suggested to work as so-called rulers that define upstream and downstream nucleosome occupancy patterns (Fennessy and Owen-Hughes 2016; Vasseur et al. 2016). In *Drosophila*, nucleosome positioning is blurred after DNA replication with nucleosome gain at NFR and weakening of strong promoter nucleosome positioning (Ramachandran and Henikoff 2016). One hour later, nucleosomes largely regain patterns, an occupancy pattern resembling the pre-replicative state. These observations suggest that in *Drosophila*, but not in yeast, nucleosomes may outcompete transcription factor during chromatin assembly, potentially creating a window for switching expression states by forming a post-replicative chromatin state characterized by a more uniform nucleosome occupancy pattern.

15.5.2 Histone Post-translational Modifications

A 1:1 mix of newly synthesized and recycled histones is assembled into nucleosomes on the daughter DNA strands (Alabert et al. 2015) (Fig. 15.1), leading to a twofold dilution of histone modifications as the new histones carry mainly pre-deposition-specific acetylation marks. How the pre-replication level of histone modifications is restored after DNA replication is a central question in epigenetics, given that histone modifications sustain transcriptional programmes and must be stably maintained across cellular division (Pengelly et al. 2013). Mass spectrometry data in human cells and genetic analysis in *C. elegans* and fission yeast support that histones retain their modifications during recycling (Alabert et al. 2015; Audergon et al. 2015; Gaydos et al. 2014; Ragunathan et al. 2015; Scharf et al. 2009; Xu et al. 2011). Once incorporated, newly synthesized histones must acquire modification similar to the old ones in the locus where they are incorporated. Several studies have followed this process (Alabert et al. 2015; Pesavento et al. 2008; Scharf et al. 2009; Xu et al. 2011; Zee et al. 2012) and conclude that restoration of the pre-replicative

histone modification level is a slow process continuing even in G1 after cell division. This argues that the level of histone modification at a given site oscillates during the cell cycle. It remains unclear how these fluctuations affect chromatin-based processes such as transcription. However, new histones were recently shown to provide a signature of post-replicative chromatin that is recognized by a protein complex involved in homologous recombination (Saredi et al. 2016). This signature relies on the unmethylated state of histone H4 K20, which remains unmodified on new histones until late G2 (Alabert et al. 2015; Pesavento et al. 2008), at which time almost all new histones acquire mono- and di-methylation at this site in a stepwise fashion. In this way, unmethylated H4K20 marks regions of chromatin that have been replicated as good substrates for homologous recombination (Saredi et al. 2016).

How newly assembled histones acquire modifications to restore the pre-replicative state remains unclear. Several histone modifiers bind to PCNA (reviewed in Alabert and Groth 2012) which could serve as a landing platform and provide a direct link between DNA replication and histone modification. However, the slow rates with which marks are established on the new histones question the idea of a tight coupling to the replication process. One possibility is that the replication machinery and parental histones recruit activities that prime histones towards a certain modification state (e.g. H3K9me1 and H3K27me1 are imposed very rapidly, Alabert et al. 2015) and that general chromatin maintenance mechanisms known from studies of transcriptional regulation ensure that the final modification state is restored prior to the next round of cell division. These processes are under intense studies since they are instrumental for maintenance of epigenetic states in cycling cells and likely to play an important role in both organismal development and disease.

15.6 Concluding Remarks

Our understanding of how histones are handled at replication forks is continuing to expand. In the future it will also be important to understand how non-nucleosomal proteins, such as regulatory factors, structural proteins and transcription factors, reassociate on daughter strands and whether their occupancy pattern is affected by DNA replication. Another exciting area of research is to understand how replication stress impinges on the ability of cells to maintain chromatin states (reviewed in Dabin et al. 2016; Svikovic and Sale 2016). As shown in this chapter, nucleosome dynamics are tightly linked to replisome progression, and it remains unclear how fork arrest, processing and collapse will affect the process of chromatin duplication.

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