Genome-Wide Dynamics of Chromatin Maturation Following DNA Replication

by

Mónica Paola Gutiérrez

University Program in Genetics and Genomics
Duke University

Date: _____
Approved:

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David MacAlpine, Supervisor

_____
Beth Sullivan

_____
Thomas Petes

_____
Alexander Hartemink

Dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in the University Program in Genetics and Genomics
in the Graduate School of Duke University
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Abstract

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Abstract

All DNA-templated events, including replication and gene transcription, occur in the context of the local chromatin environment. The passage of the replication machinery results in disassembly of chromatin, which must be re-assembled behind the replication fork to re-establish the epigenetic state of the cell. Many of the factors and mechanisms regulating DNA replication and chromatin assembly have been identified from elegant *in vitro* biochemical experiments, work in model systems like *Saccharomyces cerevisiae*, or novel proteomic approaches. In spite of current advances in the field, it is still not clear how the chromatin landscape is organized and re-assembled during this process.

Current methods, while informative, lack the genome-wide base-pair resolution required to assess the dynamics of chromatin assembly and maturation in a spatial-temporal manner. To overcome the limitations of these studies, I have taken advantage of an epigenome mapping technique based on micrococcal nuclease (MNase) digestion followed by paired-end sequencing. This approach facilitates the analysis of chromatin structure by capturing not only nucleosomes, but also smaller DNA binding protein footprints in a factor-agnostic manner. I have developed a technique based on this approach that generates Nascent Chromatin Occupancy Profiles (NCOPs) to study the dynamics of chromatin assembly following passage of the DNA replication fork at a genome-wide level and at single base-pair resolution in *S. cerevisiae*. It employs a nucleoside analog to specifically enrich for nascent chromatin,

which can be captured following a chase over different periods of time. Thus, NCOPs resolve the structure of nascent and mature chromatin, facilitating the analysis of chromatin maturation across the entire genome.

Using NCOPs, I provide a comprehensive description of the maturation process across different genomic regions and the dynamics of small DNA binding factor association with nascent and mature chromatin states. Our results support previous work characterizing the structure of nascent chromatin as being more disorganized and having poorly positioned nucleosomes. Importantly, using positioning and occupancy scores, I provide new details on the structure of nascent and mature chromatin at intergenic regions, including replication origins, and at highly transcribed and poorly transcribed genes. I uncovered that local epigenetic footprints have the potential to shape the dynamics of chromatin assembly, generating a chromatin maturation landscape that is dependent on the parental chromatin. Finally, I resolved patterns of transcription factor occupancy with nascent and mature chromatin, and observed transient factor association in the nascent state.

In all, this work provides insight into the dynamics of chromatin assembly, and allows for genome-wide and base-pair resolution investigation of chromatin maturation. The genomic and bioinformatic approaches developed here open the door for further investigation of the dynamics of epigenetic inheritance and the role of known and unknown players in re-establishing the eukaryotic epigenome following passage of the DNA replication fork.

A mi mamá, por ser ejemplo de vida y de mujer. Su sacrificio, amor y apoyo incondicional hicieron este sueño posible.

Este logro es para ti.

Contents

Abstract **iv**

List of Tables **x**

List of Figures **xi**

List of Abbreviations **xiii**

Acknowledgements **xv**

1 Introduction **1**

1.1 DNA replication 2

1.2 Chromatin overview 3

1.2.1 Histones 3

1.2.2 Histone post-translational modifications 5

1.3 DNA replication dependent chromatin assembly 7

1.3.1 Nucleosome disassembly ahead of the fork and parental histone recycling 8

1.3.2 Nucleosome assembly behind the DNA replication fork 9

1.3.3 From newly replicated nascent chromatin to a mature state . 11

1.3.4 Chromatin assembly on the leading and lagging strands 12

1.4 Chromatin assembly during transcription and DNA repair 14

1.5 Epigenetic inheritance 16

1.6 Genomic tools for analysis of chromatin 19

1.7 Thesis roadmap 23

2 Nascent Chromatin Occupancy Profiling Reveals Locus and Factor Specific Chromatin Maturation Dynamics Behind the DNA Replication Fork **25**

2.1 Introduction 25

2.2 Results 29

2.2.1 Profiling nascent chromatin occupancy 29

2.2.2 Locus specific differences in the re-establishment of chromatin architecture 33

2.2.3 Nucleosomes exhibit distinct patterns of positioning and occupancy genome wide 39

2.2.4 Actively and passively replicated origins have distinct maturation dynamics 42

2.2.5 Transient association of DNA binding factors with nascent chromatin 44

2.3 Discussion 48

2.4 Acknowledgements 52

2.5 Methods 53

2.5.1 Yeast strains 53

2.5.2 Chromatin occupancy profiling 53

2.5.3 Chromatin preparation 53

2.5.4 Click reaction and streptavidin affinity capture 54

2.5.5 Flow cytometry 54

2.5.6 Sequencing library preparation 55

2.5.7 Data Analysis 55

2.5.8 Sequence Alignment 55

2.5.9 Analysis of chromatin organization and structure 55

2.5.10 Signal normalization of histone post-translational modifications, H2A.Z variant occupancy, and NET-seq 56

2.5.11 Individual nucleosome positioning and occupancy scores . . . 56

2.5.12 Data Availability 58

3 Chromatin Assembly Activity of Chd1 and CAF-1 Change the Maturation Kinetics Genome-Wide 59

3.1 Introduction 59

3.2 Results 62

3.2.1 Gene body chromatin maturation is impaired in *chd1*Δ yeast . 62

3.2.2 Chromatin assembly and chromatin remodeling activities of Chd1 at gene structures 66

3.2.3 chromatin assembly at origins of replication is severely impaired in *cac1*Δ cells 67

3.2.4 Proper chromatin structure is necessary for transcription factor re-association with chromatin 70

3.3 Discussion 74

3.4 Methods 77

3.4.1 Yeast stains 77

4 Conclusions 78

4.1 Future directions 84

Bibliography 88

Biography 106

List of Tables

1.1 Histone variants 4

1.2 Histone chaperones of the chromatin assembly pathway in yeast . . . 15

List of Figures

1.1 Main histone post-translational modifications in humans and yeast . . 6

1.2 Chromatin assembly following DNA replication 10

1.3 MNase epigenome mapping 20

2.1 Enrichment of EdU labeled chromatin at sequences proximal to early origins 31

2.2 EdU-labeling and recovery of chromatin surrounding early origins of DNA replication 32

2.3 Nascent chromatin occupancy profiles 34

2.4 Experimental conditions to capture nascent and mature chromatin . . 35

2.5 Nucleosome organization for nascent and mature chromatin from a 5 or 10 min EdU pulses 37

2.6 Genome-wide nucleosome positioning and occupancy in nascent and mature chromatin 40

2.7 Chromatin maturation at actively and passively replicated origins . . 43

2.8 Transcription factor association with nascent and mature chromatin . 46

2.9 Chromatin profiles at general regulatory factor binding sites 47

2.10 Nucleosome positioning and occupancy schematic 57

3.1 Nascent chromatin occupancy profiles in *chd1*Δ and *cac1*Δ 63

3.2 Chromatin structure of *cac1*Δ and *chd1*Δ cells at fast and slow maturing genes 65

3.3 Chromatin structure of *cac1*Δ and *chd1*Δ cells at highly and poorly expressed genes 67

3.4 NCOPs at the efficient origins *ARS805* and *ARS1* 69

3.5 Nucleosome positioning of *cac1*Δ and *chd1*Δ cells at actively and passively replicated origins 70

3.6 Transcription factor footprint in *chd1*Δ and *cac1*Δ cells 71

3.7 Chromatin maturation of *cac1*Δ and *chd1*Δ cells at Abf1p and Reb1p binding sites 73

List of Abbreviations

ACF	Autocorrelation Function
ARS	Autonomously Replicating Sequence
Asf1	Anti-Silencing Function 1
ATP	Adenosine Triphosphate
CAF-1	Chromatin Assembly Factor 1
Chd1	Chromatin organization modifier, Helicase, and DNA-binding domains
ChIP	Chromatin Immunoprecipitation
EdU	5-Ethynyl-2'- Deoxyuridine
FACT	Facilitates Chromatin Transcription
Hir	Histone Regulator
HTZ1	Histone Two A Z1
HU	Hydroxyurea
Mcm2	Minichromosomal Maintenance 2
MNase	Micrococcal Nuclease
NCOPs	Nascent Chromatin Occupancy Profiles
NET-seq	Native Elongating Transcript Sequencing
ORC	Origin Recognition Complex
PCNA	Proliferating Cell Nuclear Antigen
PTM	Post Translational Modification
Rtt106	Regulator of Ty Transposition

TF	Transcription Factor
TSS	Transcription Start Site
WT	Wild Type

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1

Introduction

DNA replication is the basis for genetic inheritance in all living organisms. During DNA replication, the entirety of the genetic material is duplicated with high fidelity once every cell cycle. In 1958, soon after the discovery of the DNA double helix, Arthur Kornberg purified the enzyme required to synthesize it (Lehman et al., 1958; Bessman et al., 1958). In his own words 'I never thought that I would discover a phenomenon utterly unprecedented in biochemistry: an absolute dependence of an enzyme for instruction by its substrate serving as a template' (Kornberg et al., 1989). This phenomenon and the 'polymerase' enzyme Kornberg discovered are so essential to life that both are conserved across all the different domains of life.

The DNA of eukaryotic cells is contained within the cell nucleus in association with proteins, forming a complex structure called chromatin. The higher order structure of chromatin is the chromosome, which helps compartmentalize the genetic material. Together, the complex of DNA and proteins form the epigenome, referring to the idea that all DNA-bound proteins control access to the sequence of DNA and in doing so regulate all DNA-dependent processes. As DNA is replicated, the local chromatin structure and epigenetic state of the parent chromosome is disrupted by

the passage of the replication machinery and must be subsequently re-assembled on each of the new daughter strands. Thus, DNA replication plays an integral role in propagating the parental epigenetic state to newly copied sequences (MacAlpine and Almouzni, 2013). This dynamic chromatin environment allows for locus-specific epigenetic states associated with distinct cell types and developmental stages (Méchali, 2010).

My thesis work focuses on two of these principles, that the chromatin architecture regulates all DNA-templated processes and DNA replication is necessary to propagate the chromatin state of the mother cell to newly synthesized DNA.

1.1 DNA replication

Replication is a conserved and essential process required for the maintenance and integrity of the genome, and it is precisely regulated to ensure that the genetic material is copied only once per cell cycle (Bell and Dutta, 2002). Start sites of DNA replication, termed origins, are selected by the binding of the origin recognition complex (ORC) to DNA. ORC is a heterohexameric protein complex that promotes DNA replication by serving as a scaffold for the association of other replication factors (Bell and Dutta, 2002). In G1-phase of the cell cycle, ORC recruits additional factors to the origin resulting in the loading of the Mcm2-7 replicative helicase and the formation of the pre-replication complex (pre-RC) (Alabert and Groth, 2012). Assembly of the pre-RC 'licenses' the origin for activation in the subsequent S-phase (Méchali, 2010).

ORC binding and recognition of replication origins is a complex process. In *Saccharomyces cerevisiae*, ORC binds to an 11 base pair consensus sequence necessary, but not sufficient, for replication origin activity (Bell, 2002). Of the thousands of potential motif sites in the yeast genome, only a few hundred are occupied by ORC, indicating that other epigenetic features are likely needed to define origin lo-

cations (Xu et al., 2006). Despite the high homology of the ORC proteins across all eukaryotes, ORC exhibits little sequence specificity in higher eukaryotes including *Drosophila melanogaster* (Remus et al., 2004) and humans (Vashee et al., 2003), and a conserved sequence motif has not emerged. This suggests that the chromatin environment is the primary determinant for ORC localization and origin selection in higher eukaryotes. In fact, nucleosome positioning, chromatin remodeling, and the presence of histone variants known to be found at replication origins are important for ORC binding (Deal et al., 2010; Eaton et al., 2010, 2011; MacAlpine et al., 2010).

1.2 Chromatin overview

In the late 1800s, German biologist and anatomist Walther Flemming first coined the term *chromatin* when he observed an intricate scaffold within the nucleus that could be easily stained using techniques he had developed (Flemming, 1879). The smallest subunit of chromatin, the *nucleosome*, was first described in 1975 as the complex of ∼200 bps of DNA (146 base-pairs plus linker DNA) and 8 histone proteins (Oudet et al., 1975). The idea that nucleosome formation could modulate the accessibility to the DNA sequence and regulate processes that use DNA as a template has been transformative in itself, and has offered a new perspective to many areas of study, including the DNA replication field. Thus, to understand the importance of the nucleosome unit, we need to evaluate its individual components.

1.2.1 Histones

Histones were first discovered in the late nineteenth century as nuclear proteins (Kossel, 1884). However, it was not until almost a century later that their role in forming chromatin and their properties in modulating DNA templated processes were characterized. There are four main canonical histone variants, H2A, H2B, H3 and H4. These are highly conserved across all eukaryotic species (Mariño-Ramírez

Table 1.1: *Major histone variants.* Nomenclature varies across species. (Kamakaka and Biggins, 2005; Sarma and Reinberg, 2005)

Histone	Function	Organism
H3	Canonical Core	Eukaryotes
H3.3	Transcription activation	Metazoans
CenH3	Kinetochore assembly	Eukaryotes
H4	Canonical Core	Eukaryotes
H2A	Canonical Core	Eukaryotes
H2A.X	DNA repair and recombination	Metazoans
H2A.Z	Transcription activation and repression	Eukaryotes
H2B	Canonical Core	Eukaryotes

et al., 2005), and together form the octamer at the core of nucleosomes by assembling a tetramer of (H3 - H4)$_2$ and two dimers of H2A-H2B. Other variants exists that can take the place of the canonical histone proteins and confer a distinct biological function to the genomic location where they are located. Unlike the canonical histones, their variants are deposited in a replication independent manner. In this way, the replacement of canonical histones with specific variants makes the chromatin architecture highly dynamic.

While many histone variants exist in higher eukaryotes, H2A.Z and CenH3 are the only variants in *S. cerevisiae*, replacing H2A and H3 respectively (Eriksson et al., 2012). CenH3 is found at the chromosome centromeres, locations responsible for kinetochore attachment and subsequent chromosome segregation during mitosis. H2A.Z is a versatile variant that has been associated with a variety of roles, including transcription activation and repression, RNA Pol II elongation, heterochromatin maintenance, DNA replication, DNA repair, chromosome segregation, and genome stability (Henikoff and Smith, 2015). In yeast, the presence of H2A.Z is correlated with

poorly transcribed genes (Guillemette et al., 2005; Mavrich et al., 2008); however, it is not known whether its function in positioning nucleosomes at the promoters of these genes facilitates their transcription. Nonetheless, the current evidence suggests a role for H2A.Z in chromatin remodeling by which, whether its deposition or by recruitment of other chromatin remodelers, aids in organizing the chromatin environment at yeast promoters (Guillemette et al., 2005; Mavrich et al., 2008).

1.2.2 Histone post-translational modifications

One of the key structural features of the core histone proteins is their N-terminal tails, which extend out from the nucleosome. Their N-terminal tails are rich in lysine residues that can be modified by covalent post-translational addition of, primarily, acetyl and methyl groups (Figure 1.1). Other less frequent lysine modifications include SUMOylation, ubiquitination, and ribosylation (MacAlpine and Almouzni, 2013). Earlier studies found that removal of these histone tails causes a decrease in chromatin condensation, indicating that they are critical for modulating chromatin dynamics (Allan et al., 1982). The high lysine content increases the positive charge of the tails; thus, making lysine residues a critical source of regulation. For instance, adding a negatively charged acetyl group leads to reduced affinity with DNA, prompting a more open chromatin state (Bowman and Poirier, 2015). Tails in H3 and H4 are preferentially used as sites of epigenetic regulation. H2A and H2B are also post-translationally modified, but their modifications do not seem to play a widespread role in chromatin architecture and their function is much less understood (Wyrick and Parra, 2009). Consequently, many post-translational modifications (PTMs) exist with unidentified roles.

The histone PTMs not only change the chromatin architecture, but in doing so they also modulate DNA-templated processes. Initial *in vitro* observations on the dynamics of histones with DNA showed that highly acetylated histones that remained

bound to DNA did not interfere with RNA polymerase activity (Allfrey et al., 1964), sparking interest in understanding how acetylation and methylation affect gene expression (Allfrey et al., 1964; Pogo et al., 1966). Years of work led to the *histone code* hypothesis, proposing that classes of histone PTMs, and combinations thereof, provide a readout for an epigenetic signature across different genomic locations (e.g. open vs condensed chromatin) and biological phenomena (e.g. active vs. repressed gene states) (Turner, 2000; Jenuwein and Allis, 2001).

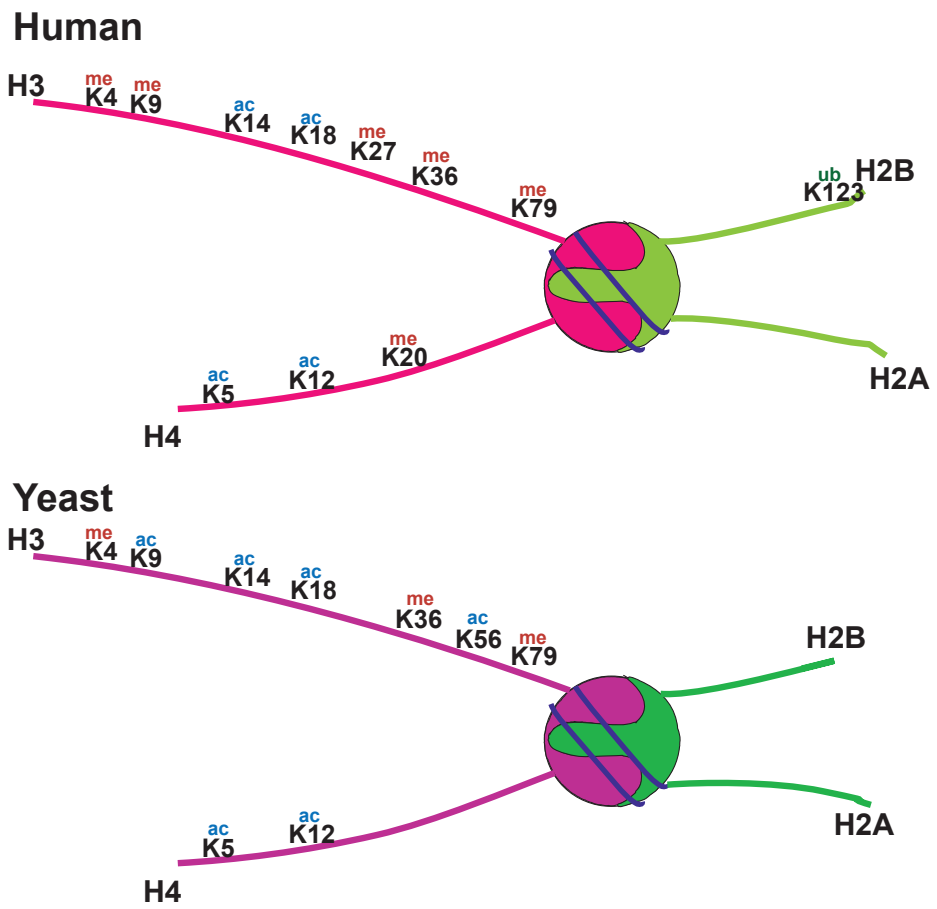

FIGURE 1.1: *Main histone post-translational modifications in humans and yeast.* Most lysine residues can be either methylated or acetylated. Represented here are the most common modified forms of these residues at each position. K= lysine, me=methylation; ac= acetylation; ub=ubiquitination. Adapted from (Bannister and Kouzarides, 2011; Millar and Grunstein, 2006)

PTMs largely influence the epigenetic landscape and have the potential to control cellular processes like DNA replication. Studies in *S. cerevisiae* found that origin sequences located in the highly condensed telomeres were able to start replication efficiently when translocated to another genomic location, and the opposite was true when an efficient origin was placed in a telomeric region (Ferguson and Fangman, 1992), demonstrating that the location of replication origins along the genome regulates their activity. In line with this, recruitment of a histone acetylase to a late origin led to its earlier activation, and deletion of a deacetylase caused global early origin firing in *S. cerevisiae* (Vogelauer et al., 2002), indicating that histone acetylation influences the firing of replication origins. Similarly, early activating origins of replication are found in euchromatic locations enriched in highly acetylated nucleosomes in the fruit fly *D. melanogaster* (Eaton et al., 2011). In another example of histone PTM regulation of the replication program, monomethyltransferases of H3K27 were shown to be necessary to prevent re-replication of heterochromatic regions rich in transposon sequences in *Arabidopsis thaliana*, (Jacob et al., 2010). Thus, histone PTMs are important not only to regulate the time of activation, but they also prevent uncontrolled replication.

1.3 DNA replication dependent chromatin assembly

The development of sequencing technologies has provided a detailed look into the organization of nucleosomes and the characterization of chromatin features across the genomes of organisms from yeast to humans. Despite our understanding of the chromatin organization genome-wide, however, we do not know how the architecture is re-established following DNA replication, transcription, and repair. Nucleosome assembly during these DNA-templated processes becomes even more important in the context of epigenetic inheritance, since the blueprints of epigenetic information are stored in the histones and their PTMs.

1.3.1 Nucleosome disassembly ahead of the fork and parental histone recycling

The nature of the DNA replication program dictates that all proteins, including histones and other DNA binding proteins, need to be partially displaced ahead of the fork to facilitate passage of the replication machinery. However, the process by which nucleosomes are disassembled is not well understood. DNA unwinding could serve as the first step of this process as it has been shown that unwinding leads to nucleosome eviction (Shundrovsky et al., 2006). Thus, it is plausible that helicase activity ahead of the fork primes nucleosomes for disassembly. Furthermore, it is likely that this process of nucleosome disassembly is aided by chromatin remodelers and histone chaperones. Although there is not direct evidence of their activity, the remodeling factors Ino80 and Isw2 may have a role in fork progression as they have been found at sites of active replication (Vincent et al., 2008; Lee et al., 2014). In this context, fork progression could be tied to the remodeling of nucleosomes ahead of the fork to facilitate passage of the replication machinery through disassembly of parental nucleosomes.

Unlike chromatin remodelers, the role of histone chaperones has been linked directly to the recycling of histones. The histone chaperone activity of MCM2, a subunit of the replicative helicase, is central to histone recycling and it is responsible for symmetrical segregation of parental histones into the leading and lagging strands (Gan et al., 2018; Petryk et al., 2018). This process occurs through formation of a complex between MCM2, an H3-H4 dimer, and the H3-H4 chaperone ASF1 (anti-silencing function 1), which subsequently transfers the dimers to CAF-1 (chromatin assembly factor 1) complex and Rtt106 (regulator of Ty transposition) for deposition behind the fork (Clemente-Ruiz et al., 2011; Huang et al., 2015; Sauer et al., 2017). CAF-1 is associated with PCNA (proliferating cell nuclear antigen), the DNA sliding clamp, facilitating proper histone deposition behind the fork. While much is known

about the dynamics of H3-H4 dimer and tetramer recycling, our understanding of how H2A and H2B are recycled is less clear. In contrast to the complete recycling of parental H3-H4 behind the fork, the pool of parental H2A-H2B do not seem to be recycled it is entirety (Alabert and Groth, 2012). However, FACT (facilitates chromatin transcription) is a versatile histone chaperone capable of associating with H3-H4 dimers and preferentially with H2A and H2B, and it has been shown to be both necessary and sufficient to assemble nucleosomes *in vitro* (Kurat et al., 2017). FACT has also been found in complex with MCM2 and the histone proteins (Foltman et al., 2013; Yang et al., 2016), indicating that this association may facilitate FACT recycling of H2A and H2B at the fork. This deposition of parental histones behind the replication fork is important for re-establishment of the epigenetic landscape and serves as the basis of epigenetic memory. Indeed, recent work has shown that the proper recycling of parental histones reproduces the histone post-translational modification landscape of parental chromatin (Reverón-Gómez et al., 2018).

1.3.2 Nucleosome assembly behind the DNA replication fork

The process of nucleosome assembly following replication requires the synthesis and deposition of new histones. Newly synthesized H3-H4 dimers are carried by ASF1 from the cytosol to CAF-1. Newly synthesized H3-H4 histones are differentiated by their actylation marks, particularly at H3K56 in *S. cerevisiae* (Yu et al., 2018) and H4K5 in mammalian cell lines (Petryk et al., 2018). Recent work revealed that $(H3 - H4)_2$ tetramers are formed by association of two CAF-1-H3-H4 complexes in a reaction that requires DNA (Sauer et al., 2017), indicating that tetramers are formed on the DNA template and not prior to deposition. An important observation is that new H3-H4 does not form tetramers with old H3-H4. Early studies aiming at understanding the stability of the histone proteins used fluorescence and radiolabeled amino acids to mark and trace newly synthesized histones. The results revealed that

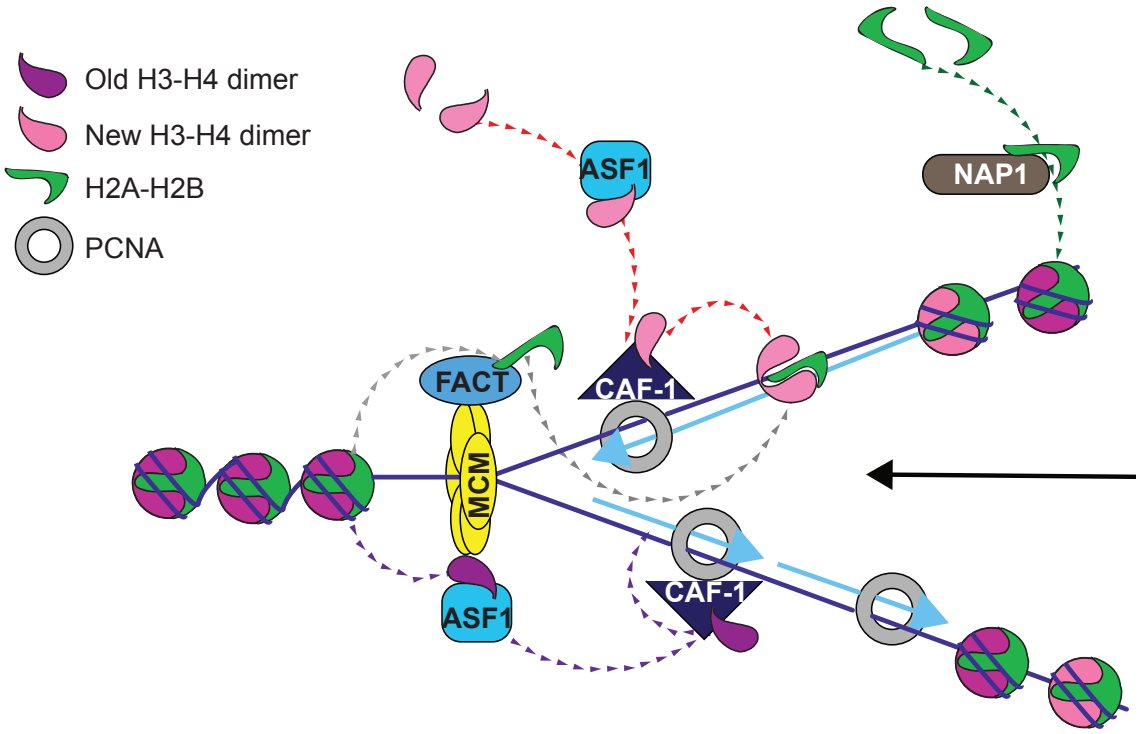

FIGURE 1.2: *Chromatin assembly following DNA replication.* Nucleosomes ahead of the fork are destabilized with the help of ATP-dependent chromatin remodelers and DNA unwinding by the MCM2-7 helicase. Interactions between the helicase and the histone chaperones ASF1 and FACT contribute to nucleosome disassembly and parental histone recycling. ASF1 shuttles both parental histones (through its association with MCM2) and new H3-H4 dimers for deposition to CAF-1. CAF-1 association with PCNA facilitates proper histone delivery to both leading and lagging strands. H2A-H2B parental dimers are recycled with the help of FACT, and newly synthesized H2A-H2B are shuttled by NAP1.

new H3-H4 were found almost exclusively with new H3-H4 tetramers, with little evidence of tetramer formation of new and old H3-H4 (Prior et al., 1980; Jackson, 1990). This indicates that (H3 - H4)$_2$ tetramers are conserved during chromatin assembly in a manner that persists through subsequent cell cycles. This work in conjunction with current studies allows for a model in which the process of new H3-H4 deposition is tightly regulated and independent from the recycling of parental H3-H4, despite the fact that the ASF1 and CAF-1 intermediaries facilitate both

mechanisms. Formation of the (H3 - H4)$_2$ tetramer on DNA primes the deposition of two H2A-H2B dimers by NAP1 to complete the octamer (Ito et al., 1996; Park and Luger, 2006).

1.3.3 From newly replicated nascent chromatin to a mature state

Electron microscopy showed that the distance between the most recently deposited nucleosome and the branch point of the replication fork is on average 225 bp on the leading strand and 285 bp on the lagging strand (Sogo et al., 1986), indicating that nucleosome formation is rapid and happens as soon as enough double stranded DNA is available (Cusick et al., 1989). While nucleosome deposition occurs very rapidly, it takes between 15 and 20 minutes for nucleosomes to reach the organization and structure of pre-replicative chromatin (Annunziato and Seale, 1982). Chromatin can therefore be classified into into two states; 1) nascent and 2) mature chromatin. Nascent chromatin is the state of the most recently replicated chromatin and it is characterized by hypersensitivity to nucleases (Cusick et al., 1989), enzymes that cleave the DNA into smaller fragments. This hypersensitivity is influenced by the highly acetylated nature of nascent chromatin caused by the deposition of new histones (Annunziato and Seale, 1983).

A multitude of enzymes are necessary for nascent chromatin to reach the state of the parental chromatin state, in a process referred to as chromatin maturation. One group of enzymes involved in this process are the histone deacetylases (HDACs), which are enriched at replication forks (Sirbu et al., 2011; Alabert et al., 2014) and remove the acetylation marks of newly synthesized histones. The importance of this process in underscored by the fact that inhibition of deacetylation induces DNA damage in a replication dependent manner (Conti et al., 2010). Furthermore, failure to remove the acetylation marks on histones through inhibition of deacetylases has been shown to cause defects in heterochromatin formation, leading to faulty

chromosome segregation during mitosis (Taddei et al., 2001).

Another group of proteins involved in chromatin maturation are ATP-dependent chromatin remodelers. In human cell lines, the remodelers SMARCAD1 and BAZ1B (Williams syndrome transcription factor) are found at active replication forks (Sirbu et al., 2013; Alabert et al., 2014), perhaps to aid in the positioning of nascent nucleosomes. Moreover, the remodeler SNF2H interacts with PCNA at active elongating forks (Poot et al., 2004), and both SNF2H and SNF2L were identified through isolation of proteins on nascent DNA (iPOND) as factors associated behind the fork on nascent chromatin of human cells (Sirbu et al., 2013). These observations indicate that remodeling on nascent chromatin is necessary to re-establish the parental chromatin architecture. Reconstitution of chromatin assembly in an *in vitro* system showed that INO80 and ISW1A increased the incorporation and organization of nucleosomes in the presence of FACT, and together stimulated the rate of replication (Kurat et al., 2017). Not only were chromatin remodelers important to assemble chromatin in this system, their role was enhanced in the presence of acetyltransferases (Kurat et al., 2017), indicating that histone acetylation promotes chromatin remodeling. Altogether, these results highlight that the native state of chromatin following passage of the replication fork stimulates chromatin remodeling and maturation toward a parental chromatin state.

1.3.4 Chromatin assembly on the leading and lagging strands

The differences in the distance between the first assembled nucleosome and the branch point of the replication fork at the leading and lagging strands (Sogo et al., 1986; Cusick et al., 1989) suggests that there are distinct chromatin assembly dynamics at the fork. In spite of these differences, the maturation process needs to be completed equally on both strands of DNA in order to re-establish the epigenetic state of parental chromatin. Recent work has started to uncover the mechanisms of

chromatin assembly at the lagging strand by deep sequencing of Okazaki fragments in *S. cerevisiae*. First, it was found that Okazaki fragment termini coincided with nucleosome dyads, indicating that the length of Okazaki fragments is determined by deposition of nucleosomes behind the fork (Smith and Whitehouse, 2012). To further support this hypothesis, deletion of each of the subunits of CAF-1 led to longer Okazaki fragments (Smith and Whitehouse, 2012). Thus, in the absence of a nucleosome, the polymerase is able to extend the fragments past their average known length.

Analyses of the dynamics of parental histone deposition have started to elucidate how they are recycled in the leading and lagging strands. Current studies have reported a small strand bias of parental histone recycling that is exacerbated when the dynamics of chromatin assembly and DNA replication are perturbed. Histone segregation is almost identical in the two daughter strands. In yeast cells, however, the bias of parental histone deposition is slightly skewed toward the lagging strand (Yu et al., 2018). Interestingly, depletion of two subunits of the leading strand polymerase Polϵ, Dpb3 and Dpb4, decreased parental histone deposition on the leading strand (Yu et al., 2018). Given the small preference of parental histone deposition toward the lagging strand, Dpb3 and Dpb4 might be important to ensure proper histone segregation behind the DNA replication fork by shuttling old histones for deposition into the leading strand. In parallel, mutations in the histone binding domain of yeast Mcm2 prevented parental $(H3\text{-}H4)_2$ deposition onto the lagging strand (Gan et al., 2018). This process appears to involve the histone transfer from Mcm2 to Ctf4 and Polα to ensure symmetrical parental histone inheritance on newly synthesized DNA. Similarly, mouse embryonic stem cells (mESC) exhibit a biased parental histone deposition toward the leading strand while newly synthesized histones demonstrate a slight bias toward the lagging strand (Petryk et al., 2018). This bias was increased when the histone binding activity of MCM2 was mutated (Petryk et al., 2018), fur-

ther affirming that MCM2 is important for histone recycling to the lagging strand. These results emphasize that MCM2 is not only important for histone recycling but is relevant to ensure symmetrical histone segregation behind the DNA replication fork. Together, these observations suggest that active mechanisms involving multiple protein complexes mediated primarily by MCM2 are necessary in both the leading and the lagging strands to ensure proper histone inheritance behind the replication fork.

1.4 Chromatin assembly during transcription and DNA repair

The chromatin architecture is not only altered during DNA replication, but it is also disrupted during transcription. Following passage of Pol II, nucleosome assembly occurs simultaneously with elongation to prevent cryptic transcription from starting within the coding sequence (Joshi and Struhl, 2005; Fleming et al., 2008). Thus, this process occurs in a manner that prevents histone exchange and preserves the chromatin footprint. Co-transcriptional methylation of H3K36 serves as a safeguard mechanism against this possible histone exchange by stabilizing pre-existing nucleosomes (Gossett and Lieb, 2012). Furthermore, H3K36me3 is critical for recruitment of chromatin remodelers that help retain this nucleosomes and promote their positioning in *S. cerevisiae* (Smolle et al., 2012). Interestingly, Chd1 is also important to re-establish the chromatin architecture at this actively transcribed genes, but its recruitment is not dependent on methylated H3K36 (Hennig et al., 2012). Loss of Chd1 causes decreased monoubiquitylation at H2BK123, another mark that is necessary to aid in nucleosome re-assembly during transcription elongation (Lee et al., 2012). FACT recognizes ubiquitylated H2A-H2B dimers and facilitates histone dissociation during transcription (Venkatesh and Workman, 2015). It is plausible that FACT-mediated nucleosome re-assembly is regulated by Chd1 (Venkatesh and Workman, 2015). Thus, it is likely that the same histone chaperones responsible for removing histones ahead of the transcription machinery are responsible for their re-deposition.

Table 1.2: *Histone Chaperones of the chromatin assembly pathway in yeast* (Bannister and Kouzarides, 2011; Millar and Grunstein, 2006)

Chaperone	**Histone**	**Function**
Asf1	H3-H4	Transport from cytosol Histone transfer to CAF-1 Regulation of Rtt109 acetylation of new H3 at K56
CAF-1	H3-H4	Dimer deposition and tetramer formation
Rtt106	H3-H4	Dimer deposition and tetramer formation
Hir	H3-H4	Replication-independent chromatin assembly
Nap1	H2A-H2B	Transport from cytosol and deposition
FACT	H2A-H2B	Removal and deposition

Unlike transcription, new histone deposition during DNA repair is necessary to replenish nucleosomes at the repair site. In this regards, there are many parallels in the reconstitution of chromatin in DNA repair with DNA replication-dependent chromatin assembly. CAF-1 and Asf1 work together to assemble nucleosomes during nucleotide excision repair in human cell lines (Mello et al., 2002). Similarly, the histone chaperones FACT and HIRA (histone regulator A) are recruited at sites of UV damage (Dinant et al., 2013; Adam et al., 2013), while CAF-1 and Hir (yeast homologue of human HIRA) are found at double strand breaks (DSBs) in yeast (Brachet et al., 2015). HIRA is reponsible for replacing the variant H3.3 at gene promoters in human cells, and it is likely that its role in DNA repair is to restore the epigenetic footprint and facilitate transcription following DNA repair (Polo and Almouzni, 2015; Dabin et al., 2016). However, the only other H3 histone variant in yeast is the centrometic variant CenH3. It has been shown that Hir and Asf1 form a complex that promotes histone assembly in a replication-independent manner (Green et al., 2005); thus, Hir recruitment to DSBs may be a result of a specific DNA-repair chromatin restoration pathway.

1.5 Epigenetic inheritance

The concept of epigenetic inheritance is based on the notion that the position and occupancy of proteins along the DNA, the information stored on the histone post-translational modifications, and, in higher eukaryotes, the methylation landscape across the DNA, are transmitted from one generation to the next. The process of DNA replication challenges this idea of epigenetic inheritance. As the proteins ahead of the fork are partially disassembled, newly synthesized histones are required, and the synthesis of DNA leads to a hemimethylated state. This presents an opportunity to alter the epigenetic state of the cell, which can lead to changes in cell identity (Ishiuchi et al., 2015; Cheloufi et al., 2015), alteration on the regulation of protein-DNA interactions and transcriptional programs (Xu and Corces, 2018), or cause genomic instability (Ye et al., 2003; Prado et al., 2004).

Parental histones account for half the supply available for deposition behind the fork; thus, newly synthesized histones are required to re-establish the chromatin landscape. A consequence of this phenomenon is that new histones carry different PTMs from those in parental chromatin, requiring the removal of these marks in order to re-write the PTMs of the parental chromatin state. The primary mark of new yeast H3 is K56ac, while new H3 in humans are marked by K14ac, K18ac (Probst et al., 2009; Alabert et al., 2015). In contrast, new H4 is acetylated at K5 and K12 in both yeast and higher eukaryotes (Probst et al., 2009; Alabert et al., 2015). Failure to remove these marks can cause changes to the epigenetic landscape, as acetylation promotes open chromatin, including changes in gene expression and accessibility to regulatory sites (Bannister and Kouzarides, 2011). This underscores the importance of re-establishing the epigenome to the parental state.

Parental histones are key factors for preservation of the epigenetic state since their PTMs serve as *cis* templates to establish the local chromatin marks onto the newly

deposited histones. Given that nascent chromatin is composed of equal amounts of new and old histones (Alabert et al., 2015), it is likely that neighboring old histones facilitate the transmission of PTM states by recruiting enzymes such as methyltransferases to spread parental histone marks (Ragunathan et al., 2015). Although post-translationally modified parental histones are quickly deposited behind the replication fork in close proximity to their native locations (Reverón-Gómez et al., 2018), the time required to re-establish the epigenetic marks on nascent chromatin varies. For instance, while the re-establishment of the repressive marks H3K27me3 and H3K9me3 is delayed until the next G1 phase, the parental profile of H3K4me3 is restored following S-phase in G2 (Alabert et al., 2015; Reverón-Gómez et al., 2018). The fact that these marks are established with different kinetics could also present an opportunity to change the epigenetic landscape in a manner that leads to developmental and cell-type specific programs.

Histone variants of H3 and H2A substitute their canonical counterparts to regulate genome function throughout the cell cycle in a replication independent manner (Henikoff and Smith, 2015). Given that they are deposited at specific genomic features (e.g. centromeres, gene promoters, etc), it is possible to hypothesize that their prevalence during and following replication is important to preserve their epigenetic footprint. A clear example of this phenomenon is found at centromeres, the chromosomal locations where kinetochore assembly takes place. Centromeres are characterized by the specific H3 variant cenH3 (CENP-A in humans) and, in higher eukaryotes, lack a precise DNA sequence or motif (Henikoff and Smith, 2015). Thus, centromeric maintenance is driven by the local epigenetic state. Indeed, it was shown that CENP-A is faithfully inherited following S-phase (Jansen et al., 2007), and the newly deposited H3 variant is replaced with new CENP-A specifically at the vicinity old CENP-A in G1 prior to the next round of replication (Jansen et al., 2007; Ross et al., 2016). This underscores the idea that parental histones are critical in

re-establishing the local epigenetic state across cell cycles.

Overall, epigenome inheritance regulates aspects of cell identity and differentiation, as well as genome stability. The importance of proper chromatin assembly and its role in differentiation and development was highlighted in studies in which the reduction of CAF-1 induces cell reprogramming (Cheloufi et al., 2015). This reduction in chromatin assembly led to a more open chromatin environment at regulatory enhancer regions that allowed for pluripotency in mouse embryonic fibroblasts (Cheloufi et al., 2015) and increased the emergence of totipotent 2-cell-stage like cells in embryonic stem cells (Ishiuchi et al., 2015). These studies demonstrate that programmed changes in the chromatin assembly dynamics could have a strong influence in the cellular plasticity required during early stages of development. Most importantly, it emphasizes that changes to the process of chromatin assembly lead to differences in cell transcriptional profiles.

Changes in the nucleosome assembly dynamics are important during development, but what happens when unprogrammed changes occur? Defects in the nucleosome assembly pathway caused by decreased CAF-1 activity can activate cell cycle checkpoints as a result of stalled replication forks in somatic cells (Ye et al., 2003). Similarly, deletion of Asf1 causes accumulation of double strand breaks that lead to high rates of recombination in yeast (Prado et al., 2004; Clemente-Ruiz et al., 2011). Regulation of DNA replication through the histone chaperones is also important for genome maintenance. In human cells, ASF1 regulation is carried out, in part, by Codanin-1, a protein that is mutated in congenital dyserythropoietic anaemia type I (CDAI) (Ask et al., 2012). Molecularly, this disease is characterized by unusual condensation of heterochromatin identified through electron micrographs (Heimpel et al., 1971) and chromatin bridges between nuclei (Heimpel et al., 2010). Codanin-1 negatively regulates ASF1 activity through direct binding and, conversely, deletion of the protein caused higher association of ASF1 to chromatin and increased DNA

replication compared to controls (Ask et al., 2012). The potential chromatin maturation defects caused by the lack of ASF1 regulation may be responsible for the abnormal heterochromatin condensation seen in these bone marrow cells.

Active and increased proliferation is one of the hallmarks of cancer (Hanahan and Weinberg, 2011). In this regard, CAF-1 and ASF1 have been described as reliable prognostic markers in a variety of cancers. High levels of CAF-1 have been linked to patient prognosis in a variety of malignancies including breast, prostate, and colon cancers (Polo et al., 2004; Staibano et al., 2009), and it has been proposed as a proliferation marker with the potential to predict a patients response to therapy (Polo et al., 2004). Similarly, the human ASF1b isoform has emerged as a strong marker in breast cancer since its increased levels predicted metastasis and the severity of tumors (Corpet et al., 2011). While it is likely that higher chaperone activity is a result of the increased proliferative behavior, these higher than normal levels can lead to similar chromatin defects observed in CDAI.

1.6 Genomic tools for analysis of chromatin

In the advent of next generation sequencing, many techniques have been developed to study the structure of chromatin. These techniques require enzyme digestion or chemical reactions that probe chromatin structure based on DNA accessibility and protein occupancy. One such technique uses the endo-exo nuclease micrococcal nuclease (MNase) to digest the linker DNA between nucleosomes and other DNA-binding proteins. Early studies used MNase to examine nucleosome organization in bulk and low throughput experiments (Noll, 1974; Reeves and Jones, 1976; Annunziato and Seale, 1982; Stillman, 1986; Cusick et al., 1989). The use of MNase has been extended to recover DNA fragments of different sizes that, after paired-end high throughput sequencing, can be used to infer the genome-wide occupancy of nucleosomes, transcription factors, and protein complexes at a base pair resolution

(Henikoff et al., 2011; Belsky et al., 2015). This approach, termed MNase epigenome mapping, generates a factor agnostic view of the chromatin architecture.

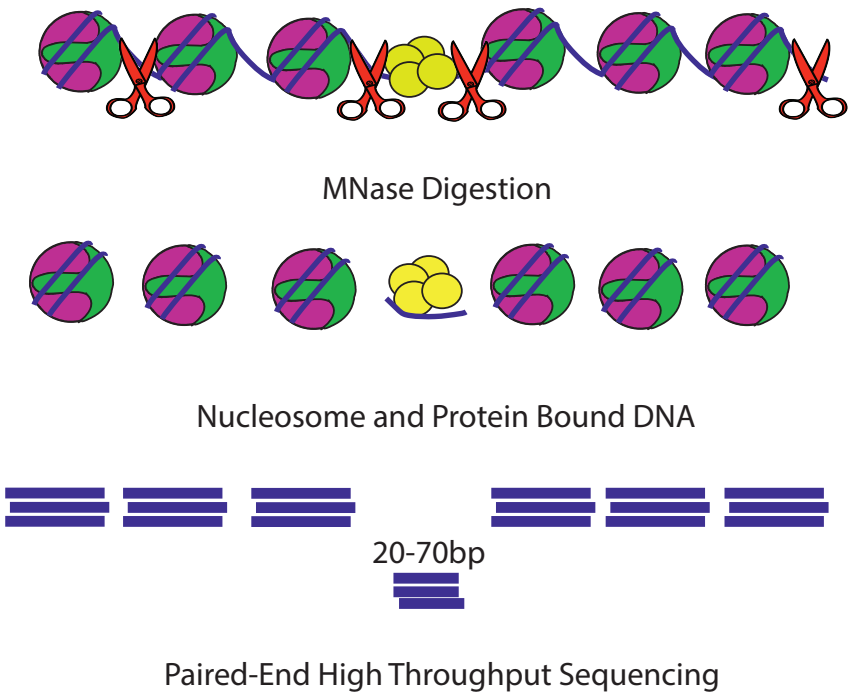

FIGURE 1.3: *MNase epigenome mapping.* MNase digests any unprotected DNA. Protein-bound DNA is recovered and these fragments of different sizes representing the footprints of nucleosomes and small DNA-binding proteins are sent for paired-end high throughput sequencing.

There are many advantages to MNase epigenome mapping. First, the factor agnostic footprinting of chromatin allows for a detailed map of all protein-DNA interactions at the time of digestion in one single experiment. It does so without the need of antibodies or epitope tags, which makes this technique amenable to a variety of model systems from yeast to human cells lines (Zentner and Henikoff, 2012). All of this increases the efficiency of MNase at a relatively low cost. In this context, MNase-seq offers many advantages compared to other methods aiming at determining the occupancy of a single protein (ChIP-seq) or only capable of resolving regulatory sites (DNase-seq) (Tsompana and Buck, 2014). Furthemore, MNase-seq provides the

great advantage of determining the wide-spread chromatin changes, or lack thereof, resulting from perturbations in proteins (e.g. knock out and over-expression) or induced by environmental cues in a locus specific and temporal manner.

Despite all the benefits of MNase-seq epigenome mapping, some drawbacks have been reported. The most relevant is the digestion bias of the nuclease toward A/T rich sites (Dingwall et al., 1981; Chung et al., 2010), which can alter the precise mapping of nucleosome positions. However, it has been shown that MNase does not affect the outcome of nucleosome positioning maps (Allan et al., 2012) and protocols have been developed that decrease experimental variability across MNase digestion (Rizzo et al., 2012). Furthermore, while one of the advantages of MNase is the factor agnostic nature of the technique, this also means that the exact identity of transcription factors and other small DNA binding proteins is unknown. This problem can be partially resolved by motif analysis (Bailey and Elkan, 1994; McLeay and Bailey, 2010; Grant et al., 2011) using the sequence fragments of the protein footprints of interest. In addition, experiments that combine MNase with chromatin immunoprecipitation followed by sequencing generate a high resolution map of protein occupancy (Teves and Henikoff, 2011; Skene and Henikoff, 2015) and provide a solution to some of the common drawbacks in standard chromatin immunoprecipitation protocols (Zentner and Henikoff, 2012; Skene and Henikoff, 2015).

Chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) enriches for a protein of interest that is associated with chromatin, and the protein-bound DNA is then sequenced to determine the sites of occupancy by the factor. Although this approach requires *a priori* knowledge of the protein of interest, ChIP-seq can be applied to a variety of organisms to determine the occupancy of transcription factors, protein complexes, histone post-translational modifications, and any other protein associated with DNA. The Encyclopedia of DNA Elements (ENCODE) and model organism ENCODE (modENCODE) have pioneered the standardization of experi-

mental and computational protocols for ChIP-seq and provided a library of chromatin profiles across different cell lines and model systems (ENCODE Project Consortium, 2012; modENCODE Consortium et al., 2010; Landt et al., 2012). However, this massive effort exemplifies the disadvantages of this approach. The experiments carried out by the ENCODE and modENCODE consortia were an undertaking that required protocol optimization for each organism and cell line, given the many factors and conditions that were being studied. This underscores the difficulty of standardizing ChIP-seq protocols (Furey, 2012). The fact that ChIP-seq profiles one factor at a time means that it is necessary to carry out multiple experiments in order to footprint multiple factors, which increases the time and cost of the experimental design. This is further affected by the fact that antibody deficiencies and epitope recognition are common issues arising from this approach (Landt et al., 2012).

The most common method to fragment the DNA for ChIP-seq is through sonication, which generates fragment sizes averaging 200 bp that are then sequenced in single-end mode. This significantly limits the resolution to hundreds of base pairs, offering only an approximation of the factor's binding site (Zentner and Henikoff, 2012). To contrast some of the limitations of this technique, ChIP-exo was developed. In this approach, standard ChIP is performed followed by lambda λ exonuclease digestion (Rhee and Pugh, 2011). The nuclease digests DNA in the 5' to 3' direction until it encounters a barrier (e.g. a DNA-bound protein), at which point the enzyme can not continue. This approach significantly increases the base pair resolution of the standard ChIP-seq protocol and can be easily applied to systems were standard ChIP has been optimized (Rhee and Pugh, 2011).

Other techniques are concerned with the study of regulatory regions of the genome such as promoters and enhancers. These genomic locations are often depleted of nucleosomes and, therefore, are the binding sites of transcription factors, regulatory complexes, protein machineries (e.g. the transcriptional machinery), and enhancer

elements. One such technique relies on deoxyribonuclease I (DNase I) digestion of DNA. DNase I is a large endonuclease that can only access regions of open chromatin that are free of nucleosomes, and these sites are termed DNase hypersensitive sites (DHS) (Tsompana and Buck, 2014). The ENCODE consortium used DNase-seq to determine the variability in the regulatory landscape across 125 cell lines (ENCODE Project Consortium, 2012). A more recent alternative to this technique is ATAC-seq (Assay for TransposaseAccessible Chromatin with highthroughput sequencing). It takes advantage of hyperactive Tn5 transposase which fragments the genome at regulatory regions and tags it at these sites in a protocol that requires only two steps (Buenrostro et al., 2013). There are many advantages to ATAC-seq as it reduces the experimental time significantly, decreases loss of DNA material, does not require formaldehyde fixation, and uses less input material compared to DNase-seq (Buenrostro et al., 2013, 2015). However, these techniques together share the same drawbacks of MNase-seq, in which the identity of the factors associated with the regulatory sites needs to be validated by other methods such as ChIP-seq.

1.7 Thesis roadmap

In order to systematically and comprehensively evaluate the dynamics of chromatin architecture genome-wide, innovative approaches such as MNase epigenome mapping are required to provide the precise location of nucleosomes and DNA binding proteins (e.g. transcription factors). During my thesis work, I used this assay to develop a new approach to monitor the re-establishment of chromatin structure following passage of the DNA replication fork in *S. cerevisiae*.

In chapter two, I describe a novel approach to study the dynamics of chromatin maturation at a genome-wide level and at a base-pair resolution with the aim to understand the locus and temporal differences in chromatin maturation behind the replication fork. I used bioinformatic approaches to assess the dynamics of matura-

tion within gene bodies, and elucidate differences in the kinetics within individual genes. I also determined the positions of bulk chromatin nucleosomes and use this information to determine genome-wide nucleosome positioning and occupancy in both nascent and mature chromatin. This approach allowed me to study chromatin maturation within intergenic regions and at origins of replication. I also explored the kinetics of small DNA-binding protein re-association with chromatin following passage of the replication fork.

Chapter three expands on work from chapter two, as I analyze the role of the chromatin remodeler Chd1 and the histone chaperone CAF-1 during the chromatin maturation process. I uncovered new evidence for the role of Chd1 in establishing the nascent chromatin architecture in a manner that appears to be independent of its known chromatin remodeling activity. Most notably, I find proof that proper chromatin architecture is critical to allow regulatory factor re-association with chromatin after replication.

In chapter four, I discuss the implications of this work and the contributions to the fields of DNA replication and chromatin biology. I also reflect on the future direction of my studies and ways in which this work can help elucidate how specific epigenetic states are inherited in a DNA replication-dependent manner.

2

Nascent Chromatin Occupancy Profiling Reveals Locus and Factor Specific Chromatin Maturation Dynamics Behind the DNA Replication Fork

2.1 Introduction

Chromatin organization is essential to maintain and regulate almost all aspects of genome function. The distribution and phasing of histone octamers on the DNA as well as the location of DNA binding proteins such as transcription factors define the regulatory landscape of the genome and govern transcription (Jiang and Pugh, 2009; ENCODE Project Consortium, 2012). The chromatin landscape is dynamic and responds to both developmental and environmental cues to modulate cell type specific gene expression programs. In addition to regulating gene expression, the local chromatin environment is also critical for other DNA templated processes such as DNA replication and repair (MacAlpine and Almouzni, 2013; Dabin et al., 2016; Gutiérrez and MacAlpine, 2016). Despite the central role of chromatin in genome function, every cell cycle the chromatin landscape must be disassembled ahead of the replication fork and then re-assembled behind the fork to preserve epigenetic

memory.

Elegant genetic and biochemical experiments have elucidated many of the factors and mechanisms involved in the assembly of chromatin behind the DNA replication fork (Smith and Stillman, 1989; Chang et al., 1997; Li et al., 2008; Tyler et al., 1999; Schlesinger and Formosa, 2000; Luk et al., 2007). The conserved chromatin assembly factor 1 (CAF-1), an H3-H4 histone chaperone, was first identified promoting histone deposition onto replicating SV40 DNA in vitro (Smith and Stillman, 1989). CAF-1 is coupled to the replisome via an interaction with PCNA to ensure rapid histone deposition at the replication fork (Shibahara and Stillman, 1999). Another H3-H4 histone chaperone, ASF1, cooperates with MCM2 at the fork to capture parental H3-H4 dimers which are then assembled into H3-H4 tetramers by CAF-1 for deposition on the nascent DNA (Huang et al., 2015; Richet et al., 2015; Sauer et al., 2017). The H2A/H2B histone chaperone, NAP-1, completes the assembly of the histone octamer on the DNA to form the nucleosome (Chang et al., 1997; Mosammaparast et al., 2002). The ordered deposition of histone octamers behind the replication fork is critical for viability, genome stability and the maintenance of epigenetic state (Exner et al., 2006; Jasencakova et al., 2010; Cheloufi et al., 2015; Ishiuchi et al., 2015).

The assembly of nascent chromatin is tightly coupled to the replication fork. Early electron microscopy studies found a similar density of nucleosomes ("beads on a string") on both the parental and nascent DNA strands (McKnight and Miller, 1977), indicating that histone deposition and nucleosome formation must occur rapidly behind the replication fork. Consistent with these observations, reconstitution of replication-coupled assembly revealed that nucleosome assembly occurred within ∼250 bp of the replication fork (Sogo et al., 1986; Cusick et al., 1989; Gasser et al., 1996). Despite the rapid deposition of the histone octamer behind the fork, nascent chromatin is deferentially sensitive to nuclease digestion as compared to ma-

ture chromatin (>20 minutes post replication) (DePamphilis and Wassarman, 1980; Klempnauer et al., 1980; Annunziato and Seale, 1982; Stillman, 1986), suggesting that nucleosomes are non-uniformly spaced in nascent chromatin. Together, these results underscore the complex and dynamic process by which chromatin matures following the rapid deposition of the histone octamer.

The organization of mature chromatin is dictated by many factors, including primary DNA sequence (Segal et al., 2006; Mavrich et al., 2008), the presence of pioneer factors (Bai et al., 2010; Li et al., 2015; Yan et al., 2018), and active transcription (Weiner et al., 2010). Conserved and stereotypical patterns of chromatin organization have emerged from micrococcal nuclease (MNase)-based studies of nucleosome positioning in a wide variety of eukaryotic organisms (Cui and Zhao, 2012). Genes typically have well phased nucleosomes starting with the +1 nucleosome at the transcription start site (TSS) and proceeding into the gene body. Promoter regions are commonly marked by a nucleosome-free region (NFR) which are thought to accommodate regulatory factors. Similarly, well-positioned nucleosomes are observed flanking origins of DNA replication and their positioning is a determinant of origin function (Berbenetz et al., 2010; Eaton et al., 2010; Belsky et al., 2015).

The study of nascent chromatin has been facilitated by the use of nucleoside analogs that allow for the affinity capture and purification of newly synthesized DNA. The enrichment of labeled nascent chromatin has been used in proteomic studies to identify proteins and protein networks associated with normal, stalled and collapsed replication forks (Sirbu et al., 2013). Similarly, others have described the maturation of post-translational histone modifications and the identification of new replisome factors important for maintaining genome stability (Alabert et al., 2014). While these proteomic studies provided a wealth of data on the network of proteins that ensure the stability and progression of the DNA replication fork and the temporal order in which chromatin modifications occur, they fail to reveal information about

locus-specific differences in chromatin maturation.

Recent work by multiple groups have combined the power of 5-ethynyl-2-deoxyuridine (EdU) labeling of nascent DNA with MNase nucleosome mapping to ascertain the positioning of nucleosomes genome-wide in nascent and mature chromatin (Fennessy and Owen-Hughes, 2016; Vasseur et al., 2016). Specifically, nascent DNA is labeled by a short pulse of EdU followed by a longer chase period to allow chromatin maturation. The EdU-labeled DNA is purified and subjected to next-generation sequencing following digestion with MNase. These studies focused on the chromatin maturation dynamics of nucleosomes within gene bodies and their rapid re-acquirement of nucleosomal organization and phasing, highlighting the role of transcription and histone chaperones in shaping the chromatin landscape. Importantly, studies in Drosophila found that the re-establishment of chromatin architecture at gene regulatory elements (e.g. promoters and enhancers) was dependent on the re-association of transcription factors (Ramachandran and Henikoff, 2016).

We have combined MNase epigenome mapping (Henikoff et al., 2011; Belsky et al., 2015) with EdU labeling of recently replicated DNA to generate nascent chromatin occupancy profiles (NCOPs) allowing us to holistically explore chromatin maturation dynamics in *S. cerevisiae*. We are able to resolve, at near nucleotide resolution, the maturation of nucleosomes and smaller DNA binding factors providing a factor agnostic view of chromatin assembly dynamics throughout the genome. We found that nascent chromatin was less organized than mature chromatin; however, there were locus specific differences in the maturation kinetics that were predicted by the epigenetic landscape. For example, poorly transcribed genes marked with the histone variant H2A.Z exhibited rapid chromatin maturation. Our results also confirm the role that site-specific DNA binding factors have in establishing chromatin organization and nucleosome positioning following passage of replication fork (Yadav and Whitehouse, 2016; Yan et al., 2018). Strikingly, we also identified origin specific

differences in chromatin maturation that were dependent on whether an origin initiated DNA replication or whether it was passively replicated from a neighboring fork, which may suggest an active mechanism to re-establish origin chromatin architecture at efficient origins. Finally, our factor agnostic approach to studying chromatin maturation revealed sites of transient occupancy by DNA-binding factors behind the replication fork, underscoring the plasticity of the chromatin landscape.

2.2 Results

2.2.1 Profiling nascent chromatin occupancy

We developed nascent chromatin occupancy profiles (NCOPs) to provide a factor-agnostic view of protein-DNA occupancy on newly synthesized DNA at nucleotide resolution. Specifically, we combined the power of labeling nascent DNA with the nucleoside analog EdU (Sirbu et al., 2011) with MNase-based epigenome mapping (Henikoff et al., 2011; Belsky et al., 2015; Ramachandran and Henikoff, 2016). To demonstrate the sensitivity and specificity of NCOPs, we took advantage of the intra-S-phase checkpoint to specifically label newly synthesized DNA proximal to early origins of DNA replication. Briefly, yeast cells engineered to incorporate EdU (Viggiani and Aparicio, 2006) were arrested in G1 by addition of the yeast mating hormone, α-factor, and subsequently released into media containing 200 mM hydroxyurea (HU) and 130 μM EdU. HU treatment depletes nucleotide pools which results in replication fork stalling and activation of the intra-S-phase checkpoint to prevent further origin activation (Santocanale and Diffley, 1998; Shirahige et al., 1998). Only those sequences proximal ($\sim$10 kb) to early activating efficient origins will incorporate EdU into the nascent daughter strands. Following EdU labeling, chromatin was isolated and digested by MNase. Then, the EdU labeled DNA was biotin labeled by click chemistry prior to streptavidin affinity capture. Streptavidin-bound DNA was recovered and subjected to next-generation paired-end sequencing on the Illumina

platform (Henikoff et al., 2011; Belsky et al., 2015)

We first generated chromosome-wide coverage plots of the sequencing depth to verify that EdU incorporation was specific and restricted to sequences proximal to early activating origins of DNA replication. As expected from prior genomic experiments labeling early replication intermediates with BrdU (Lengronne et al., 2001), we detected strong peaks of EdU incorporation centered on early activating origins of DNA replication along chromosome IV (Figure 2.1A; middle panel). The sequences surrounding early origins of replication were enriched ∼20-fold relative to late origins (Figure 2.2A).

The innovative aspect of NCOPs is the limited MNase digestion of EdU-enriched chromatin followed by the recovery and sequencing of fragments ∼200 bp and smaller. DNA occupancy by a histone octamer will protect a ∼150 bp fragment while smaller site-specific DNA binding factors (e.g. transcription factors and replication factors) will typically protect fragments smaller than 80 bp (Henikoff et al., 2011; Belsky et al., 2015). To visualize the NCOPs, we plotted the length of the paired-end reads as a function of their chromosomal position; thus, nucleosomes are evident as well-phased clusters of fragments with lengths of approximately 150 bp and smaller DNA binding factors are evident as discrete clusters of fragments with lengths smaller than 80 bp. We recovered and visualized EdU-labeled chromatin in the vicinity of early activating origins of DNA replication. We found that both nucleosomes and DNA-binding factors were readily distinguishable in the NCOPs from EdU-labeled chromatin (Figure 2.1A, bottom). In contrast, no discernable EdU-enrichment or chromatin organization was detected in the origin distal regions (Figure 2.1A, top), demonstrating the specificity of the NCOP assay for EdU-labeled DNA.

We analyzed the aggregate nucleosome distribution surrounding 539 gene promoters that were within 3500 bp of early origins, and found that nucleosome phasing and occupancy was very similar between the NCOP and 'bulk' chromatin (no EdU

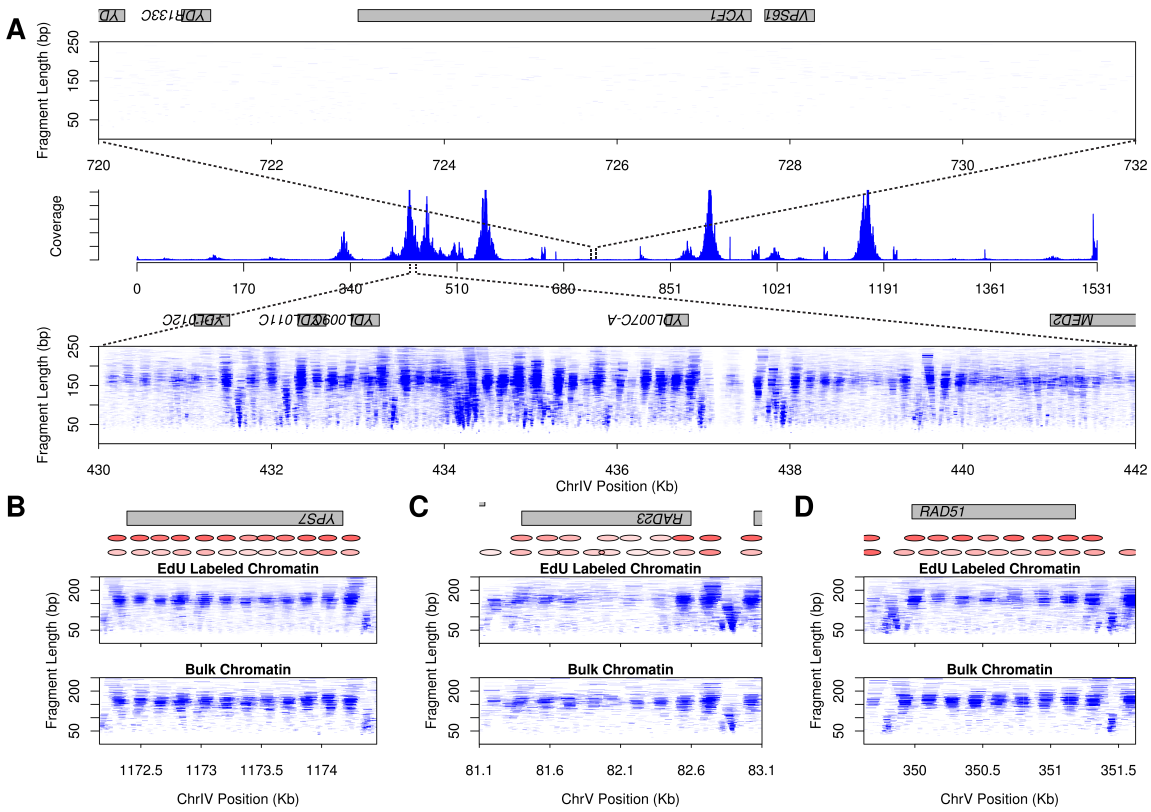

FIGURE 2.1: *Enrichment of EdU labeled chromatin at sequences proximal to early origins.* A. Sequencing coverage across chromosome IV. NCOP at an early replicating origin shows a defined chromatin architecture (bottom panel) compared to a non-replicated region (top panel). B, C and D. Chromatin profiles at genes proximal to an early origin that showed unchanged (B) and changed (C and D) chromatin structure. Nucleosome positions are depicted in red. D. *RAD51* shows recruitment of a transcription factor and downstream nucleosome shift in the EdU pull down experiment compared to total chromatin.

labeling/enrichment) (Figure 2.2B). When examined at the level of individual genes, we found that the recovered EdU-labeled NCOPs largely resemble those prepared from untreated bulk chromatin (Figure 2.1B). However, we did detect a handful of locus-specific alterations in chromatin structure, but these differences were largely attributable to differential transcription of HU-responsive genes. For example, at the RAD23 locus, we observed displaced nucleosomes from the gene body (Figure 2.1C).

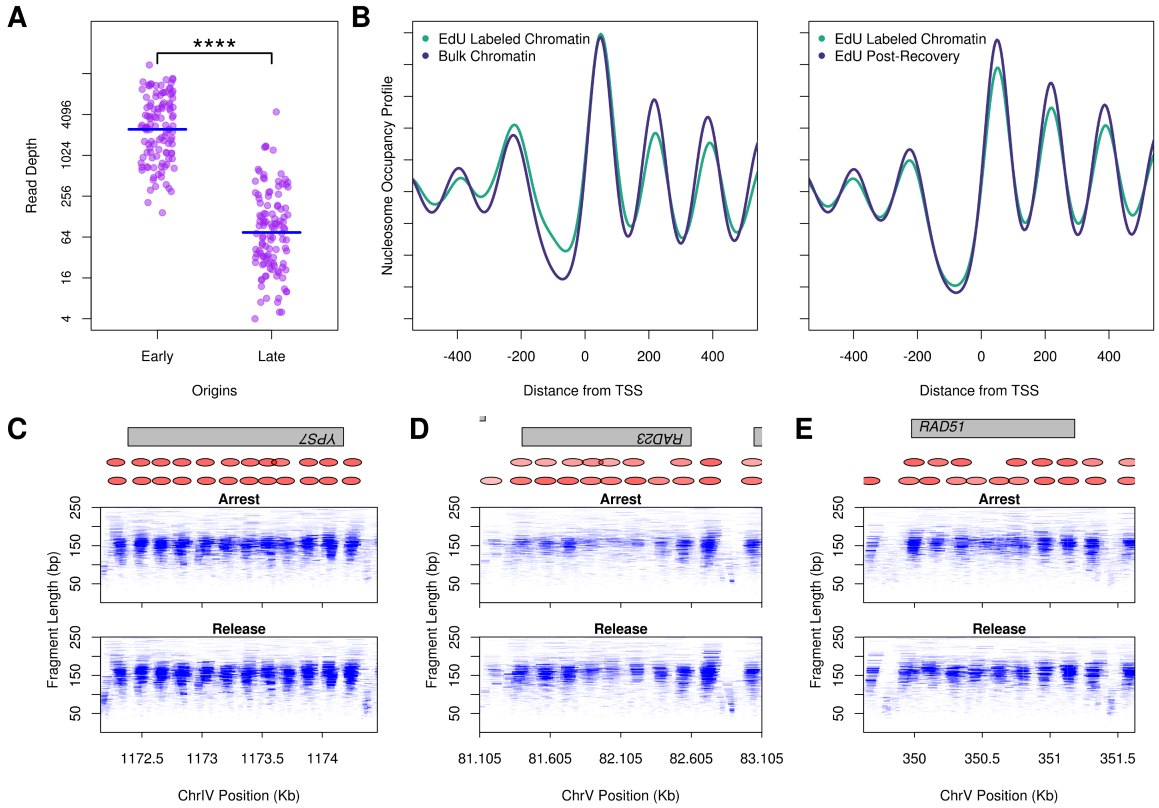

FIGURE 2.2: *EdU-labeling and recovery of chromatin surrounding early origins of DNA replication.* A. Sequencing read depth at 114 early and 114 late replicating origins (Belsky et al., 2015) from the data described in Figure 1A (note the log2 scale). Mean sequencing coverage of early and late origins is 4163.8 and 207.7 reads respectively . T-test **** $p \leqslant 0.0001$. B and C. Nucleosome occupancy profiles for the EdU early origin labeling experiment described in Figure 1 and for a separate experiment where following the HU-arrest, the EdU-labeled cells were allowed to proceed back into the cell cycle. Nucleosome occupancy profiles were calculated from 539 gene promoters located within 3500 bp of an early origin. C - E. NCOPs showing EdU labeled chromatin from arrested cells in HU and following a 2 hour release. The gene locations are the same as described in Figure 2.1B, C and D.

At the RAD51 locus we detected an expansion of the nucleosome free region at the promoter, and the association of an additional DNA-binding factor upstream of the gene in the presence of HU (Figure 2.1D). The recruitment of this factor, which we speculate to be the MBF complex (Leem et al., 1998; Mathiasen and Lisby, 2014), was accompanied by a downstream shift in the phasing of the genic nucleosomes. To confirm that these alterations in chromatin structure were due to the HU arrest and not a consequence of the EdU-labeling and enrichment, we also performed an experiment where cells were HU-arrested in the presence of EdU and then released from the arrest for two hours. We found that HU-dependent chromatin changes detected by the NCOPs were restored following release from HU and re-entry into the cell cycle (Figure 2.2B, right panel, Figure 2.2C-E). Together, these results demonstrate the specificity of NCOPs in detecting EdU labeled chromatin and their sensitivity for detecting local changes in chromatin structure.

2.2.2 Locus specific differences in the re-establishment of chromatin architecture

Chromatin architecture is disrupted and re-established every cell cycle during the course of S-phase. We sought to survey the dynamics of chromatin maturation throughout the genome by pulse-chase labeling replicating cells with EdU. In order to enrich for cells in S-phase, we first synchronized cells in G1 by the addition of α-factor for 2 hours at 24C. The cells were then released into S-phase for 45 minutes and pulsed with EdU for 10 minutes to label nascent chromatin. This was then followed by a 30 minute chase with thymidine to allow the EdU-labeled chromatin to mature (Figure 2.3A). By 45 minutes following α-factor release, the majority of cells were in S-phase with mid-S DNA content (Figure 2.4A) allowing us to EdU label replication forks from both early and late firing origins. Consistent with this, we observed a relatively uniform distribution of EdU incorporation across the genome and there were only modest differences in EdU incorporation between early and late

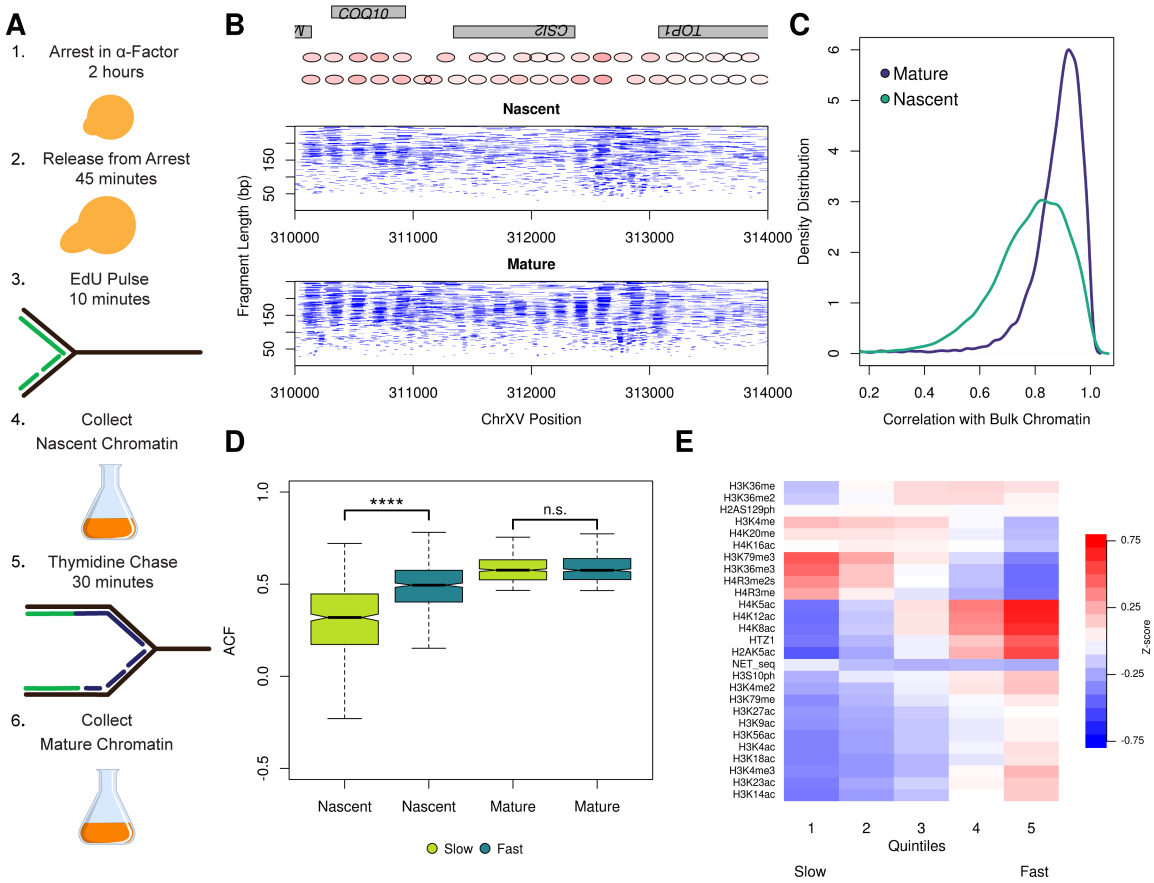

FIGURE 2.3: *Nascent chromatin occupancy profiles.* A. Schematic of experimental design for capturing EdU-labeled nascent and mature chromatin. B. NCOPs reveal the maturation of chromatin behind the DNA replication fork at a representative locus, *CSI2*. C. Nascent chromatin organization in gene bodies is less organized than mature chromatin. Distribution of correlation scores between nascent and bulk (teal) or mature and bulk chromatin (purple). D. Differential chromatin maturation kinetics for genes with well-organized mature chromatin. The autocorrelation function (ACF) was used to identify the top 50% of genes with regularly phased arrays of nucleosomes from mature chromatin. These 2700 genes were then binned into quintiles based on the correlation between nascent and mature chromatin. The first and fifth quintiles represent genes with slow and fast chromatin maturation kinetics. The distribution of ACF values (as a proxy for gene organization) in nascent and mature chromatin are depicted for the genes with slow (light green) and fast (dark green) chromatin maturation kinetics. The difference in ACF values among fast and slow maturing chromatin was very significant (p-value 2.2 x 10^{-16}). E. Heatmap representing average Z-score values for 25 histone post-translational modifications, the histone variant H2A.Z, and NET-seq scores for the individual quintiles of the correlation between nascent and mature chromatin for the 2700 genes described above.

replicating sequences (Figure 2.4B).

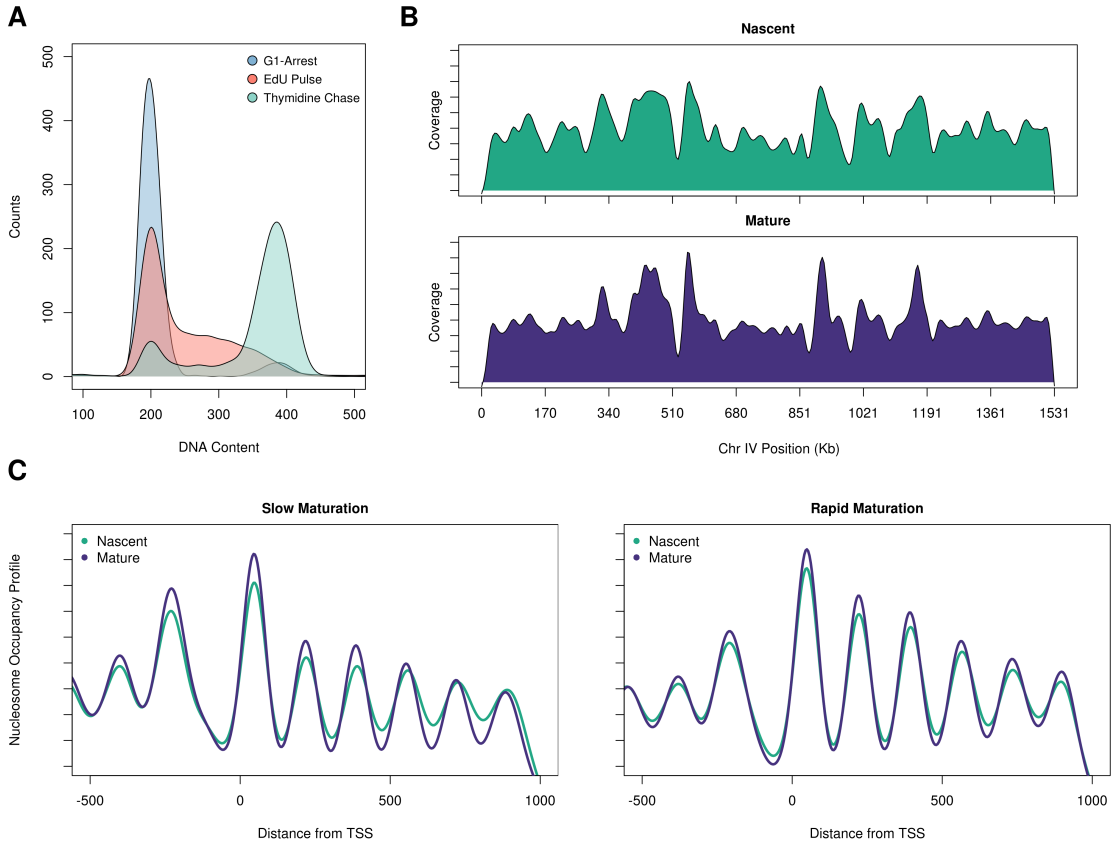

FIGURE 2.4: *Experimental conditions to capture nascent and mature chromatin.* A. A representative flow cytometry plot shows the cell cycle profile of yeast labeled with EdU (red track) and the state of cells following a 30 min thymidine chase (green track). B. Coverage plot of nascent and mature chromatin over chromosome IV shows relatively similar levels of EdU incorporation across the chromosome. C. Nucleosome occupancy profiles of the slow maturing (first quintile) and fast maturing (fifth quintile) genes.

NCOPs at individual loci revealed that nascent chromatin was inherently more disorganized than mature chromatin. For example, at the *CSI2* locus we observed a marked difference in chromatin organization both upstream and in the gene body between the nascent and mature chromatin samples (Figure 2.3B). To more systematically characterize the differences in nucleosome organization between nascent and

mature chromatin, we first used nucleosomal sized reads (140-180 bp) to calculate a nucleosome occupancy profile for each gene in the nascent and mature chromatin state. The nascent and mature nucleosome occupancy profiles were then compared to profiles calculated for bulk chromatin isolated from an asynchronous sample that was not EdU enriched. We found that the structure of mature chromatin at genic regions was highly correlated with bulk chromatin (median R value 0.901); in contrast, nascent chromatin was significantly less correlated (median R value 0.799) and significantly different from the mature chromatin population (p-value 0.73) (Figure 2.3C). We also observed similar patterns from varying the EdU pulse length (5 or 10 min) (Figure 2.5).

Interestingly, the differences in chromatin organization were not uniform across the genome, but instead seemed to be specified on a gene by gene basis. For example, in contrast to *CSI2*, the chromatin at the neighboring gene *COQ10* was well organized in both the nascent and mature chromatin samples.

To explore the features associated with the maturation dynamics of individual genes, we focused on those genes that exhibited an organized chromatin structure in the mature chromatin state. We used the autocorrelation function (ACF) to determine the regularity of nucleosome phasing and organization within gene bodies, and identified 2700 genes above the median (0.460) of the ACF values. By only focusing on those genes that ultimately have an organized structure in mature chromatin, we were able to explore the range of maturation dynamics from the nascent state. The genes with well organized mature chromatin were stratified into quintiles based on the correlation between nascent and mature chromatin. Because we only focused on those genes with well-organized mature chromatin, their organization in nascent chromatin likely reflects their maturation dynamics with the low (first quintile) and high (fifth quintile) extremes of nascent organization representing slow and fast chromatin maturation dynamics, respectively.

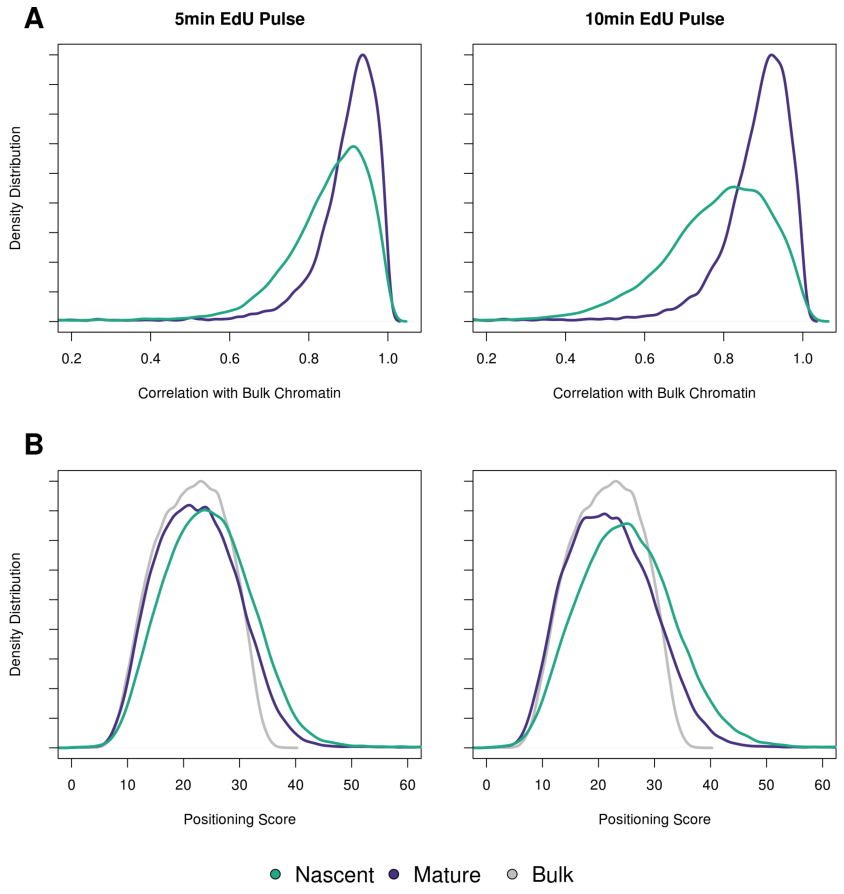

FIGURE 2.5: *Nucleosome organization for nascent and mature chromatin from a 5 or 10 min EdU pulse.* The efficacy of a 5 or 10 minute EdU pulse was assessed in replicate for each experiment. A. Correlation of nascent or mature chromatin with bulk chromatin from either a 5 (left) or 10 (right) min EdU pulse for gene bodies. B. Distribution of the nucleosome positioning scores genome-wide for nascent and mature chromatin from the 5 and 10 min EdU labeling pulse (see Figure 2.6A).

The positioning of the nucleosomes, as revealed by the nucleosome occupancy profiles, was identical between nascent and mature chromatin for those genes in the fifth or fast chromatin assembly quintile. In contrast, the nascent chromatin structure for genes in the first or slow quintile was less organized and readily distinguishable from mature chromatin (Figure 2.4C). We then analyzed the distribution of ACF values to examine the regularity of nucleosome phasing and organization within the genes

that exhibited fast or slow chromatin maturation dynamics. As expected, the mature state from both classes had a high ACF, indicating well-phased and organized nucleosomes. In contrast, the class of genes with slow chromatin maturation kinetics exhibited significantly poorer nucleosome organization and structure (low ACF) in the nascent as compared to mature state (P-value 1.82 x 10^{-36}) (Figure 2.3D). Together, these data reveal locus-specific differences in chromatin maturation for a large subset of gene bodies.

Our observation that individual genes have distinct maturation dynamics led us to hypothesize that gene-specific chromatin features such as histone post-translational modifications or the occupancy of histone variants are predictive of the slow or rapid chromatin maturation observed at individual genes. To test this hypothesis, we obtained data describing the genome-wide distribution and enrichment of post-translational histone tail modifications and the histone variant H2A.Z (Weiner et al., 2015). We calculated Z-scores of enrichment for each of the chromatin modifications and generated a heatmap of Z-score values for each modification across the quintiles of nascent chromatin organization as described above (Figure 2.3E). We found that genes with rapid chromatin maturation kinetics (fifth quintile) were enriched for H2A.Z and acetylation marks, including those in the lysine residues 5, 8, and 12 of histone H4. Interestingly, H3K4me3 was also enriched in this group of fast maturing genes, and this mark along with H4K12Ac has been shown to colocalize with H2A.Z (Chen et al., 2012). There was also a significant decrease in the chromatin marks associated with actively transcribed genes, including H3K36me3 and H3K79me3. Consistent with the relative depletion of marks associated with active transcription, we found that these genes with rapid chromatin maturation exhibited less active transcription as determined by native elongating transcript sequencing (NET-seq) data (Churchman and Weissman, 2011). In contrast, we observed a depletion of the H2A.Z variant and enrichment of transcription marks in the genes with

slow maturation kinetics (first quintile).

2.2.3 Nucleosomes exhibit distinct patterns of positioning and occupancy genome wide

The yeast genome is relatively gene dense compared to metazoan genomes; however, 40% of the genome remains intergenic. Thus, while our prior analysis focused on chromatin maturation in gene bodies, we also sought to explore chromatin maturation at the level of individual nucleosomes. We first identified the position of the nucleosome dyad for ∼70,000 high confidence nucleosomes from bulk chromatin. For every nucleosome sized fragment recovered from either the nascent or mature chromatin, we calculated the distance of the fragment midpoint to the nearest nucleosome dyad in bulk chromatin. The average of these distances for each nucleosome represented a positioning score, with well-positioned nucleosomes having a low score, and poorly positioned nucleosomes having a high score. Genome-wide, we found that individual nucleosomes in nascent chromatin are more poorly positioned as compared to mature or bulk chromatin (Figure 2.6A).

Individual nucleosomes were broadly classified as either intergenic or genic. We found that nucleosomes within intergenic regions have better positioning scores than nucleosomes within gene bodies. To begin to understand the differences in chromatin maturation for intergenic and genic nucleosomes and their relationship to transcription, we identified the nucleosomes associated with the most (top 10%) and least (bottom 10%) expressed genes as determined by prior NET-Seq experiments (Churchman and Weissman, 2011) (Figure 2.6B). A similar number of nucleosomes were sampled from intergenic regions. In each category, we found that the positioning of the nucleosomes was decreased in nascent relative to mature chromatin. Nucleosomes in intergenic and poorly transcribed genes were better positioned than nucleosomes from active genes, consistent with transcription dependent nucleosome

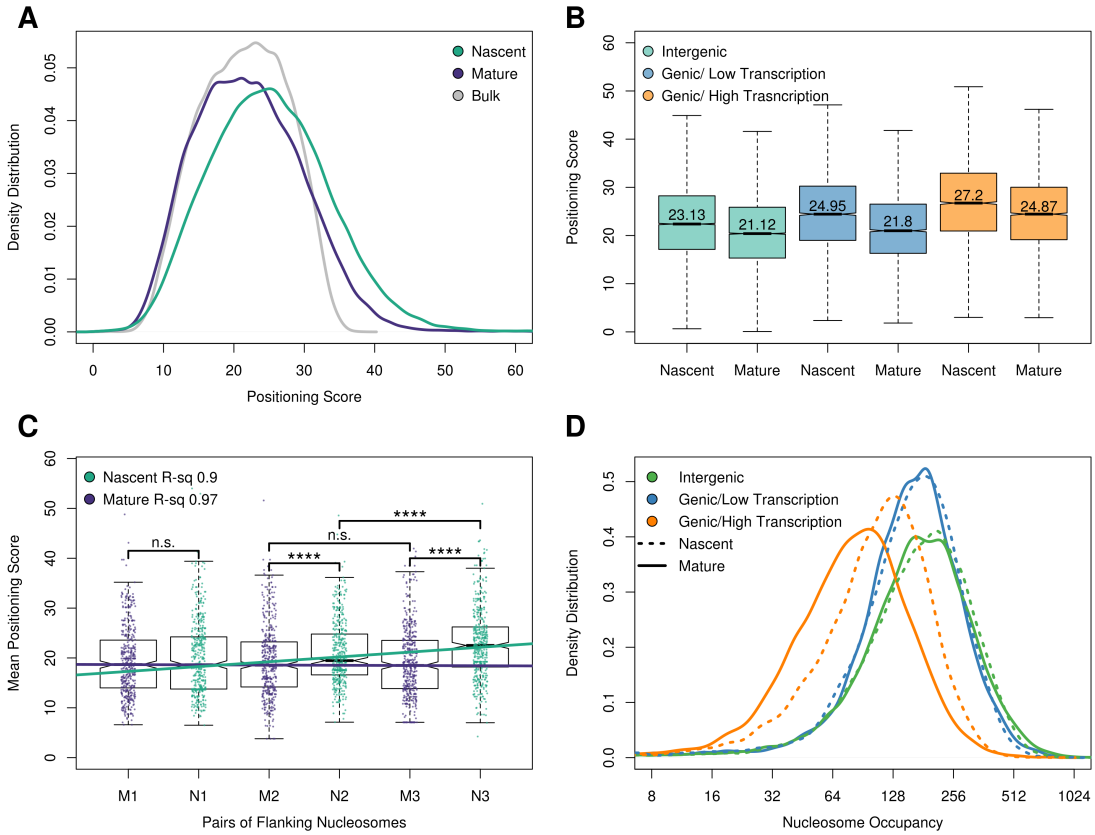

FIGURE 2.6: *Genome-wide nucleosome positioning and occupancy in nascent and mature chromatin.* A. Genome-wide distribution of nascent, mature and bulk nucleosome positioning. ∼70,000 high-confidence nucleosome dyads were identified in bulk chromatin. For each chromatin fraction (nascent, mature and bulk) the distance from the midpoint of each sequencing read to the nearest nucleosome dyad was calculated as a nucleosome positioning score. B. Nascent and mature chromatin organization (nucleosome positioning scores) at intergenic and intragenic nucleosomes. Intragenic nucleosomes were subdivided into high (top 10%) and low (bottom 10%) transcriptional activity. C. Transcription factors influence chromatin maturation kinetics. Positioning scores of the first, second, and third pairs of nucleosomes flanking 436 transcription factors with strong occupancy in mature chromatin. T-test **** $p \leqslant 0.0001$; n.s. $p > 0.05$. D. Active transcription displaces nucleosomes from mature chromatin. Distribution of nucleosome occupancy scores (sequencing reads assigned to individual nucleosomes) for nascent and mature chromatin at intragenic and intergenic sequences. Actively transcribed genes have more nucleosome occupancy in nascent chromatin than in mature chromatin (P-value 4.80 x 10^{-67}).

eviction and remodeling (Lee et al., 2004; Boeger et al., 2004; Bernstein et al., 2004).

Transcription factors such as Abf1p and Rap1p are thought to play a critical role in establishing nucleosome organization and chromatin structure (Yarragudi et al., 2004; Ganapathi et al., 2011). We reasoned that the greater nucleosome organization observed in intergenic regions was due, in part, to the presence of transcription factors. To assess the chromatin maturation dynamics at intergenic regions, we examined nucleosome positioning for the first, second and third pairs of nucleosomes surrounding 436 predicted transcription factor binding sites (MacIsaac et al., 2006) with defined occupancy footprints in mature chromatin (Figure 2.6C). We found no significant difference in nucleosome positioning for the mature nucleosomes. In contrast, we identified a significant distance-dependent increase in nucleosome positioning scores for each successive nucleosome pair in the nascent chromatin. Together, these results underscore the role of transcription factors functioning as barrier elements in establishing nucleosome organization (Zhang et al., 2009) following DNA replication.

To further explore the dynamics of chromatin maturation, we also examined nucleosome occupancy for intergenic and genic regions of the genome. An occupancy score was calculated for individual nascent and mature nucleosomes as the number of fragment midpoints mapping within 70 bp of the high-confidence nucleosome dyads identified from bulk chromatin (see above). We found that nucleosomes in intergenic regions and genes that were not being actively transcribed exhibited similar nucleosome occupancies in both nascent and mature chromatin states (Figure 2.6D). Thus, while nucleosomes are rapidly deposited behind the replication fork, they do not converge on their preferred position until maturation. In contrast to intergenic and non-transcribed regions, we observed significantly more nucleosome occupancy in nascent relative to mature chromatin in actively transcribed genes, suggesting that the newly deposited nucleosomes behind the DNA replication fork are evicted

by active transcription.

2.2.4 Actively and passively replicated origins have distinct maturation dynamics

Replication forks must traverse the entire genome during S-phase but emanate from a select few DNA replication origins. Each licensed origin has its own inherent efficiency of activation during S-phase (Aparicio, 2013; Hawkins et al., 2013). A consequence of this is that highly efficient origins will initiate bidirectional DNA replication every cell cycle whereas the least efficient origins will be passively replicated by forks from neighboring efficient origins. We reasoned that there might be distinct differences in chromatin maturation at active origins that initiate DNA replication versus those origins that are passively replicated. The distribution and strandedness of Okazaki fragments around origins were used as a proxy for origin efficiency (McGuffee et al., 2013). We first examined locus specific NCOPs for *ARS805* and *ARS602*, an efficient early origin and a passively replicating origin, respectively (Figure 2.7A-B).

As previously reported, we observed well-ordered and phased nucleosomes flanking either origin in mature chromatin (Belsky et al., 2015). We also detected smaller fragments (< 80 bp) in the mature chromatin that are indicative of ORC binding in the NFR at the ARS consensus sequence of both origins. In contrast, we observed a difference in nascent chromatin organization between *ARS805* and *ARS602*. Specifically, we found that there was significantly more nascent chromatin organization at the active replication origin as compared to the passively replicated origin.

To more comprehensively examine chromatin maturation dynamics at active and passive origins, we identified 261 origins with an ORC-dependent footprint (Siow et al., 2012; Belsky et al., 2015) and then stratified the origins by their efficiency score to identify the top 100 active and passive replication origins. The mean efficiency for each class of origins was 0.65 (active) versus 0.017 (passive). For each actively

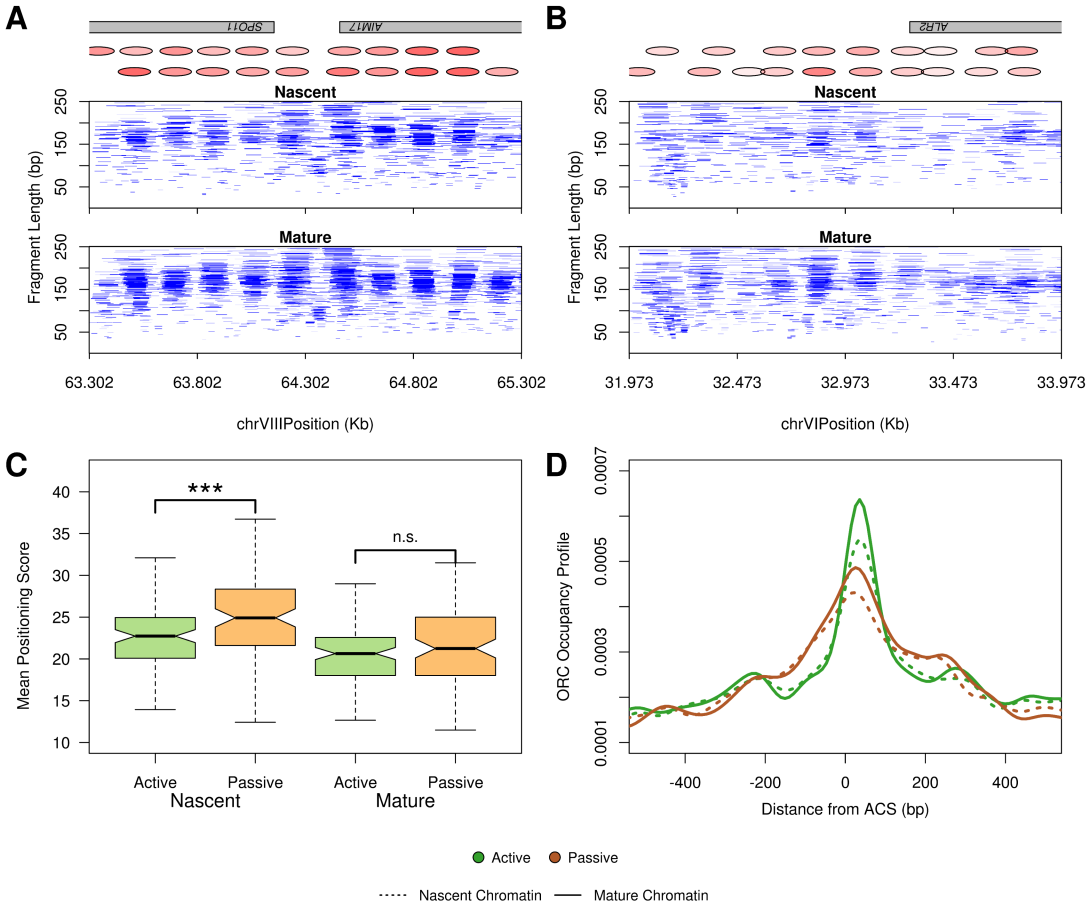

FIGURE 2.7: *Chromatin maturation at actively and passively replicated origins.* A. Chromatin occupancy profile for nascent and mature chromatin at the active origin *ARS805*. B. Chromatin occupancy profile for nascent and mature chromatin at the inactive origin *ARS602*. C. Passively replicated origins have slower chromatin maturation kinetics. Boxplots depicting the distribution of nucleosome positioning scores for nascent and mature chromatin at 100 active and 100 inactive origins. Nascent chromatin surrounding passively replicated origins is more disorganized than at active origins. T-test *** $p \leqslant 0.001$; n.s. $p > 0.05$. D. ORC occupancy is decreased at passively replicated origins. Density distribution of ORC occupancy footprints as determined from the NCOPs.

or passively replicated origin, we examined the nucleosome positioning scores for the first three nucleosomes flanking each origin up and downstream (Figure 2.7C), where increasing chromatin organization is represented by lower nucleosome positioning scores. We found that the nascent chromatin surrounding passive origins was significantly more disorganized than at active origins. In contrast, there were no detectable differences in chromatin organization between active and passive origins in the chromatin that had matured behind the replication fork. Our NCOPs also revealed a decrease in small fragments (<80 bp) at the passively replicated origins, consistent with decreased ORC occupancy (Figure 2.7D). Together, these results suggest a feedback mechanism in place at active origins to promote ORC recruitment and immediately re-establish chromatin architecture for the next cell cycle.

2.2.5 Transient association of DNA binding factors with nascent chromatin

DNA binding proteins, such as transcription factors and ORC, associate with specific primary sequences; however, for any given factor there are many more potential sequence motifs than occupied sites in the genome (Breier et al., 2004; Eaton et al., 2010; Wang et al., 2012; Slattery et al., 2014). Nucleosome occupancy is thought to limit access to many of these potential motif matches, thus defining the regulatory landscape. NCOPs provided an opportunity to comprehensively survey DNA occupancy in a factor agnostic manner throughout the genome in both nascent and mature chromatin. We hypothesized that as nucleosomes are deposited and become organized in nascent chromatin, there may be transient or promiscuous interactions between transcription factors and DNA immediately behind the replication fork.

To identify all potential sites of DNA occupancy that were not protected by a nucleosome, we focused on the paired-end sequencing fragments that were less than 80 bp and identified 6272 loci that were significantly enriched ($p<0.05$) for these size fragments in both nascent and mature chromatin. To evaluate changes in DNA-

binding factor occupancy, we first determined the log2 ratio of normalized occupancy scores for nascent and mature chromatin at each of the 6272 sites and plotted these values as an ordered heatmap (Figure 2.8A). In general, the heatmap revealed three classes of DNA binding profiles which were indicative of their chromatin maturation dynamics: i) slow maturation – sites with greater occupancy in mature chromatin; ii) fast maturation – sites that were equally occupied in both mature and nascent chromatin; and iii) transient occupancy – sites that were enriched in nascent and not mature chromatin.

To better characterize the chromatin maturation of regulatory sites bound by transcription factors and other DNA-binding factors, we identified the extreme deciles of sites (∼625 sites for each decile) representing locations with slow or transient occupancy and an equal number of loci with fast maturing kinetics (Figure 2.8B). As expected, the occupancy of small DNA-binding factors in the sites with slow maturation kinetics was less in nascent than mature chromatin (compare dashed and solid red lines). Despite the different kinetics of maturation, similar occupancy levels were ultimately established in the mature chromatin (compare solid red and grey lines).

We analyzed the nucleosome organization surrounding slow, fast, and transiently maturing sites of DNA-binding factor occupancy (Figure 2.8C). We found that both the slow and fast maturing sites of DNA occupancy were surrounded by well positioned nucleosomes in both nascent and mature chromatin. This suggests that for at least some sites, defined nucleosome positioning may be a requisite for factor occupancy. In contrast, we found that specific pioneer factors (e.g. Abf1p and Reb1p) exhibited occupancy footprints and chromatin organization profiles that were indistinguishable between nascent and mature chromatin, consistent with their immediate deposition behind the DNA replication fork (Figure 2.9).

The sites with transient occupancy only observed in the nascent chromatin suggest that they may represent promiscuous binding sites to accessible motifs in regions

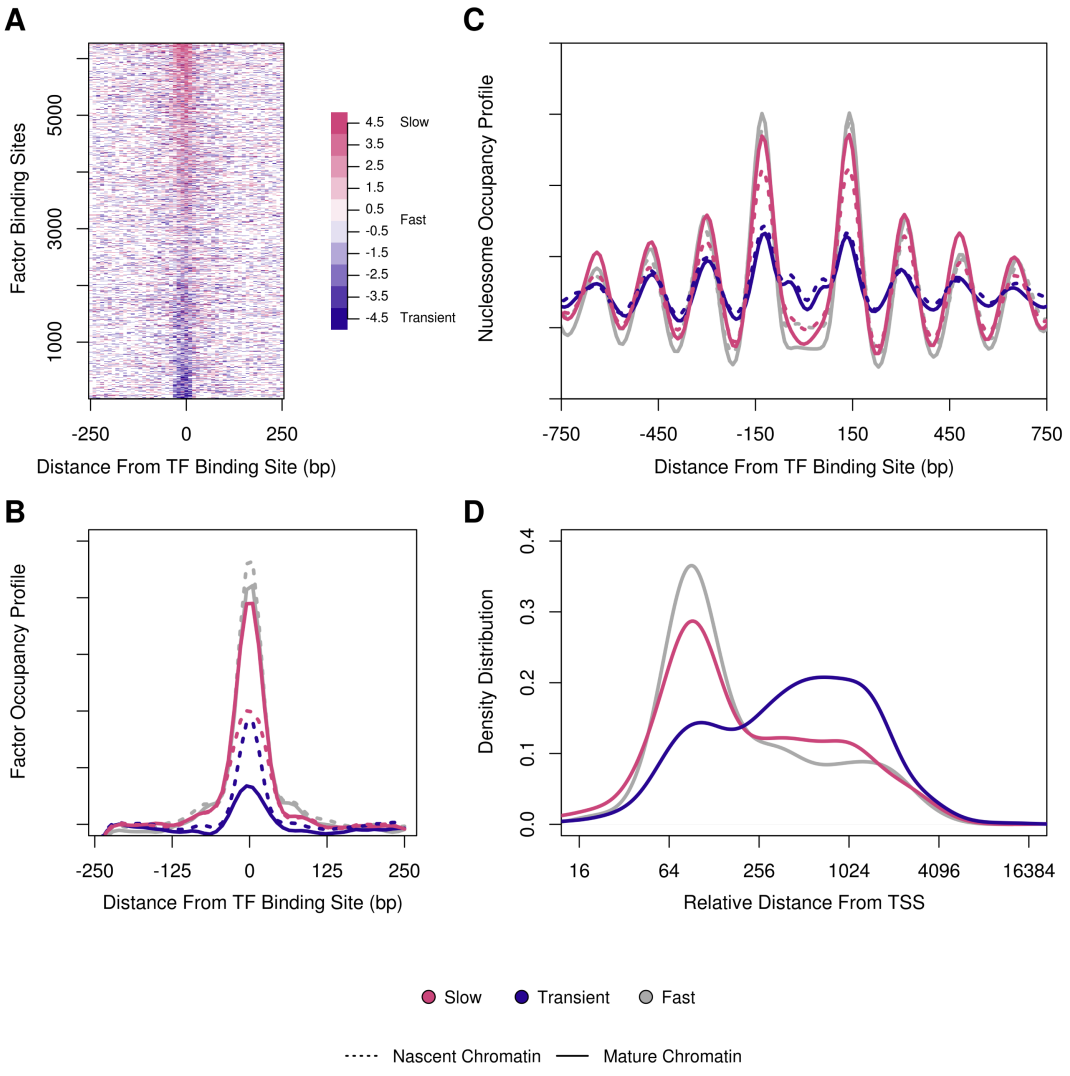

FIGURE 2.8: *Transcription factor association with nascent and mature chromatin.* A. Ratio heatmap log2(mature/nascent) of 6272 high-confidence sites of small DNA binding factor occupancy obtained from DNA fragment lengths between 20 bp and 80 bp in length. B. Identification of three classes of chromatin maturation for DNA binding factor occupancy: fast – equal occupancy in nascent and mature chromatin; slow – greater occupancy in mature than nascent chromatin; and transient – greater in nascent than mature. C. Average plot of nucleosome organization at sites occupied by the factors described in (B). D. Sites of transient occupancy are promiscuously enriched at sites distal from the transcription start site (P-value 2.01 x 10^{-19} between fast and transiently associating factors).

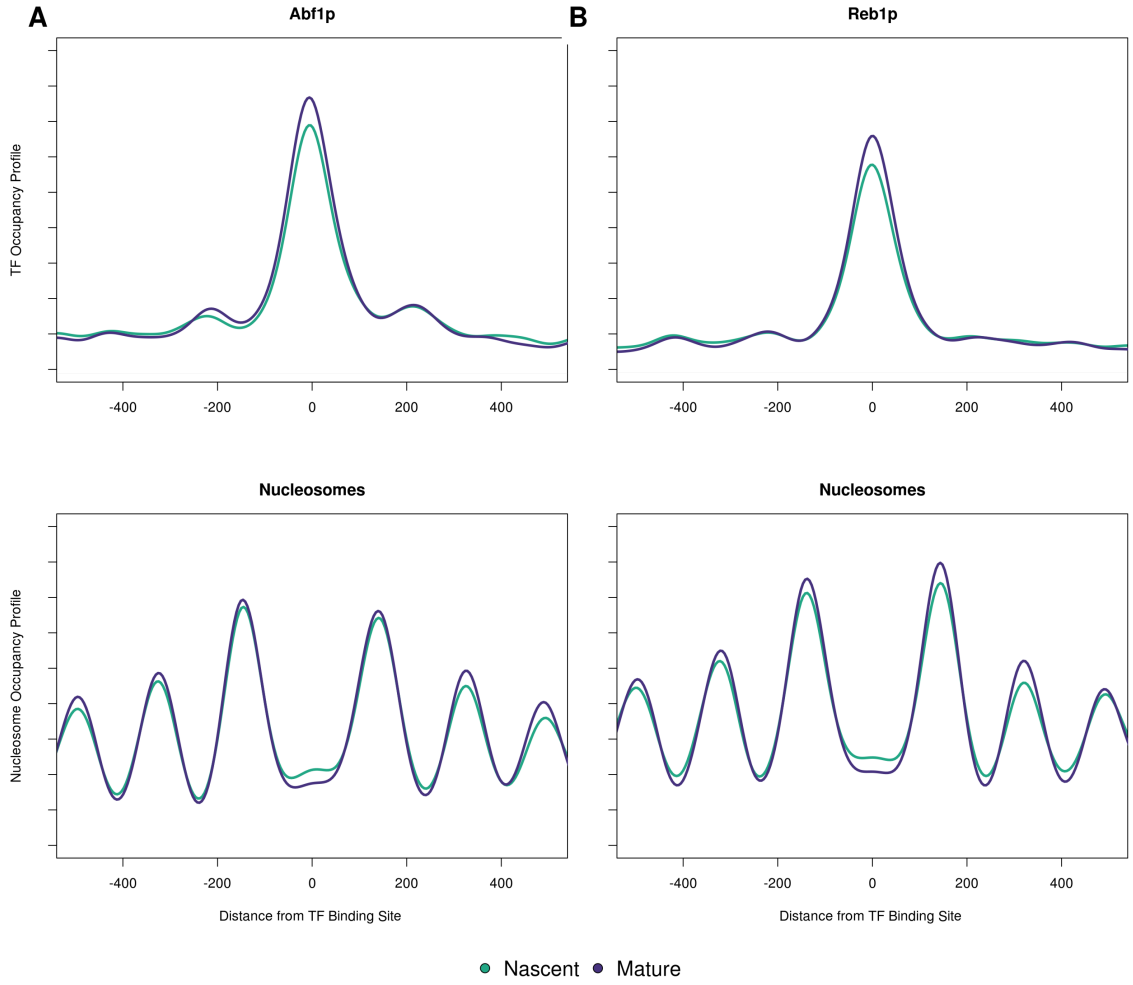

FIGURE 2.9: *Chromatin profiles at general regulatory factor binding sites.* A. Transcription factor occupancy for 151 Abf1p and B. 156 Reb1p binding sites (top two panels) and the corresponding nucleosome occupancy profiles (bottom two panels).

of poor chromatin organization. The compact nature of the yeast genome dictates that most regulatory DNA binding sites are proximal to gene promoters. Thus, we examined the location of each binding site for all three occupancy classes – fast, slow and transient – relative to their gene start site. The DNA binding sites with fast and slow maturation were found proximal to promoters (Figure 2.8D). In contrast, the distribution of transient sites was not limited to promoters, but rather occurred

distally to promoters and likely in gene bodies. Together, these results suggest that potential binding motifs are exposed in poorly organized nascent chromatin and that they are ultimately removed by chromatin maturation or transcription.

2.3 Discussion

In proliferating cells, it is critical that both genetic and epigenetic information be faithfully transmitted between generations. One of the consequences of semi-conservative DNA replication is that the chromatin landscape needs to be disrupted ahead of the replication fork and then re-established in its wake. While genetic and biochemical experiments have elucidated many of the factors and mechanisms regulating the inheritance and assembly of chromatin behind the fork (Smith and Stillman, 1989; Shibahara and Stillman, 1999; Tyler et al., 1999; Li et al., 2008; Sirbu et al., 2013; Alabert et al., 2014), the spatio-temporal dynamics of chromatin assembly across the genome are just starting to be revealed (Fennessy and Owen-Hughes, 2016; Ramachandran and Henikoff, 2016; Vasseur et al., 2016). We have utilized nascent chromatin occupancy profiles (NCOPs), which combine MNase epigenome mapping with EdU-labeling of nascent DNA to provide a factor agnostic view of chromatin maturation at nucleotide resolution across the *S. cerevisiae* genome.

The assembly of chromatin during DNA replication is a rapid process facilitated by replication-dependent histone chaperones and assembly factors (Alabert and Groth, 2012; Serra-Cardona and Zhang, 2018). Despite the rapid deposition of histone octamers behind the replication fork, nascent chromatin exhibits increased nuclease sensitivity (DePamphilis and Wassarman, 1980; Klempnauer et al., 1980; Annunziato and Seale, 1982; Stillman, 1986) suggesting poorly organized nucleosomes relative to mature chromatin. The establishment of chromatin organization is thought to be dependent on primary sequence (Segal et al., 2006; Kaplan et al., 2009), transcription (Weiner et al., 2010), and the positioning of nucleosomes rel-

ative to fixed barrier elements (Bai et al., 2010; Li et al., 2015; Yan et al., 2018). In addition, our NCOPs revealed locus-specific differences in chromatin maturation kinetics at genes and origins of replication suggesting that the maturation process is also influenced by the local chromatin state and origin activity.

We first focused on the chromatin maturation dynamics of genes with well-organized nucleosome architecture in mature chromatin. Among the genes with well-positioned nucleosomes in mature chromatin, we were able to rank them by their nascent chromatin organization and identify those genes that had either poorly organized (slow maturing) or well-organized (fast maturing) chromatin organization. In contrast to prior reports describing transcription-dependent chromatin maturation dynamics in yeast (Vasseur et al., 2016), we found that maturation of nascent chromatin was fastest for genes with low transcriptional activity (Figure 2.3E). The Vasseur study focused on the temporal maturation of nascent chromatin using the correlation with mature chromatin as a proxy for maturation within the gene body. As active transcription frequently results in nucleosome eviction and disorganized phasing in bulk chromatin (Lee et al., 2004; Boeger et al., 2004; Bernstein et al., 2004), they were looking at a de facto transcription-dependent effect. We also note in our studies that we detected the transcription-dependent eviction of nascent histone octamers from actively transcribed gene bodies (Figure 2.6D) consistent with the role of transcription in shaping the mature chromatin landscape.

A long standing model for how nucleosome positioning is achieved proposes that barrier elements such as transcription factors aid in establishing the fixed positions of their flanking nucleosomes (Fedor et al., 1988; Pazin et al., 1997; Mavrich et al., 2008; Li et al., 2015). As a result, this poses further positioning constraints on the subsequent +2 and +3 nucleosomes which thereby stabilizes their positioning. This is because the nucleosomes are packaged within short genomic regions, and the fixed position of one nucleosome influences the organization of adjacent ones in the array.

General regulatory factors (GRFs) like Abf1p and Reb1p are capable of positioning flanking nucleosomes without the need for chromatin remodeling (Yarragudi et al., 2004; Hartley and Madhani, 2009; Ganapathi et al., 2011), and can position nascent nucleosomes following passage of the replication fork (Yadav and Whitehouse, 2016). In agreement with this, we find that the first pair of nucleosomes immediately flanking factors bound to mature chromatin are tightly positioned in both nascent and mature chromatin (Figure 2.6C), indicating that the association of regulatory factors with DNA is important to position nucleosomes behind the fork.

Histone post-translational modifications and histone variants have long been postulated to form the basis of epigenetic memory (Probst et al., 2009; MacAlpine and Almouzni, 2013). The local inheritance of parental H3-H4 histones at the replication fork, in part, contributes to the re-establishment of the parental chromatin state on the newly replicated DNA. As the chromatin maturation dynamics we observed appeared to be specified on a gene by gene basis, we hypothesized that the local parental chromatin state may influence chromatin maturation dynamics on the nascent DNA. To explore this idea, we systematically examined a comprehensive panel of histone post translational modifications and histone variants derived from an asynchronous yeast population (Figure 2.3E) (Weiner et al., 2015). We found that genes with rapid maturation kinetics were poorly expressed and depleted of modifications frequently associated with gene expression (e.g. H3K36me3 and H3K79me3) and instead were enriched for specific histone modifications including H4K12ac, H3K4me3 and the histone variant H2A.Z. In *S. cerevisiae*, H2A.Z is located at the promoters of inactive or poorly transcribed genes, and helps to stabilize their promoter architecture for recruitment of regulatory factors (Guillemette et al., 2005; Li et al., 2005). Deposition of parental H3-H4 tetramers containing H4K12ac and or H3K4me3 behind the fork may help facilitate the recruitment of H2A.Z (htz1p) to inactive promoters, as single molecule studies revealed that Htz1 variant containing nucleosomes are enriched for

H3K4me3 and H4K12Ac (Chen et al., 2012). The recruitment of H2A.Z may rapidly stabilize nascent nucleosome organization by inhibiting ATP-dependent chromatin remodeling activity (Li et al., 2005).

We also investigated the chromatin maturation dynamics surrounding other chromosomal features including origins of DNA replication. The timing and efficiency by which individual origins of DNA replication are activated during S-phase is determined, in part, by primary sequence, rate limiting initiation factors (Mantiero et al., 2011; Tanaka et al., 2011), and the local chromatin structure (Berbenetz et al., 2010; Eaton et al., 2010; Kurat et al., 2017). We found that the maturation dynamics of the chromatin surrounding origins of DNA replication was dependent on whether the origin actively initiated DNA replication or was passively replicated by a replication fork from elsewhere in the genome. Efficient origins that initiate replication in the majority of cell cycles (McGuffee et al., 2013) rapidly reset their chromatin structure and exhibit well positioned nucleosomes flanking the origin immediately after initiation in nascent chromatin. The differential maturation of chromatin between efficient and passive origins likely reflects distinct molecular mechanisms in the reassembly of chromatin following either an initiation event or the disassembly of the pre-replication complex at inefficient origins. It is tempting to speculate that immediately following unwinding of the origin DNA and initiation of DNA replication, an active mechanism promotes the rapid re-association of ORC and precise phasing of nucleosomes which have become a hallmark for eukaryotic origins (Berbenetz et al., 2010; Eaton et al., 2010; Lubelsky et al., 2011; Cayrou et al., 2015; Miotto et al., 2016).

Chromatin maturation is governed by both replication-dependent and independent processes. While the deposition of nascent histone octamers behind the fork is replication-dependent, the re-establishment of the regulatory landscape and binding of transcription factors is dependent on chromatin remodeling, local epigenetic

signatures and recruitment of other trans-acting factors (Allis and Jenuwein, 2016). For most transcription factors, there are many more potential motif matches in the genome than there are sites occupied by the factor. Competition between nucleosome occupancy and transcription factor binding has long been thought to be a determinant of which regulatory motifs are occupied (Wasson and Hartemink, 2009; Li et al., 2015; Ramachandran and Henikoff, 2016). The factor agnostic NCOPs provided insight into how trans-acting factors and the regulatory landscape was established during chromatin maturation. Not surprisingly, we found a range of maturation kinetics for loci occupied by non-nucleosomal DNA binding factors, including those that rapidly associate with nascent chromatin (fast) and those that associate later in mature chromatin (slow). However, we also identified a significant number of sites that were only transiently occupied in nascent chromatin. Unlike the binding sites with slow or fast kinetics which were promoter proximal, the transient sites were frequently located distal to the promoter and in gene bodies. This is consistent with a promiscuous or opportunistic mode of binding in the absence of organized chromatin immediately following DNA replication (Yan et al., 2018). During development in higher eukaryotes, the DNA replication program is characterized by changes in the number of firing origins, the length of S-phase, and the timing of replication (Duronio, 2012; Rhind and Gilbert, 2013). The developmental plasticity in the DNA replication program may lead to promiscuous binding of regulatory factors through the chromatin changes that occur throughout this process, and thus contribute to epigenetic regulation and cell-type specific gene expression programs.

2.4 Acknowledgements

We thank members of the MacAlpine lab for critical comments and suggestions. We also thank Jason Belsky for advice on data analysis. This work was supported by the National Institutes of Health R35 GM127062 DMM and F31-GM115158 MPG.

2.5 Methods

2.5.1 Yeast strains

The yeast strain DMMy218 is in the W303 background and has the genotype MATa, leu2-3,112, BAR1::TRP, can1-100, URA3::BrdU-Inc, ade2-1, his3-11,15.

2.5.2 Chromatin occupancy profiling

To label cells in early S-phase, yeast were grown in rich medium at 30C to an O.D of ∼0.7 and arrested in G1 phase with α-factor (GenWay) at a final concentration of 50 ng/mL for 2 hours. Cells were then washed twice in sterile water, resuspended in fresh medium containing 0.2 M hydroxyurea (Sigma) and 130 μM EdU (Berry & Associates, Inc), and grown for 2 hours. Cells were washed twice with sterile water and the pellets were quick-frozen and stored at -80C.

In order to profile nascent and mature chromatin, yeast cells were grown at 25C to an O.D of ∼0.7 and arrested in G1 phase with -factor at a final concentration of 50 ng/mL for 2 hours. Cells were then washed twice in sterile water, resuspended in fresh medium and allowed to grow for 45 minutes to enter S phase. EdU was then added to a final concentration of 130 μM and allowed to grow for 10 minutes (pulse), after which a sample was taken for nascent chromatin. Cells were washed, resuspended in fresh medium containing 1.3 mM thymidine (Sigma), and allowed to grow for 30 minutes (chase) when a sample was taken for mature chromatin. Cells were washed twice with sterile water and the pellets were quick-frozen and stored at -80C. All experiments were performed using independent biological replicates.

2.5.3 Chromatin preparation

MNase digestions were performed as described in (Belsky et al., 2015).

2.5.4 Click reaction and streptavidin affinity capture

100 μg of MNase-digested DNA were concentrated in a speed vacuum. DNA was incubated in click chemistry reaction buffer as described in (Kliszczak et al., 2011; Sirbu et al., 2012; Leung et al., 2013). CuSO4 and ascorbic acid were replaced with CuBr and TBTA (Sigma). The click reaction proceeded for 1 hour at room temperature with gentle shaking. DNA was recovered by ethanol precipitation and resuspended in 30 μl sterile water.

Biotin conjugated EdU-labeled DNA was enriched using 5 μl streptavidin magnetic beads (New England Biolabs). Beads were resuspended in blocking solution (2 % I-Block (Thermofisher), 25 mM Tris, 150 mM NaCl, and 0.5 % SDS) for 2 hours with gentle shaking at room temperature. Beads were then washed twice with cold binding buffer (Leung et al., 2013). Recovered DNA was added to blocked beads and incubated in 200μl cold binding buffer for 1 hour at 4C. Bead-bound DNA was washed once with binding buffer followed by 3 washes with EB buffer (Qiagen).

2.5.5 Flow cytometry

To analyze yeast cells by flow cytometry, cells were resuspended in 70% ethanol and fixed overnight at 4C. Then, cells were washed, sonicated, and incubated in 50 mM sodium citrate pH7.4 with 0.3 mg/ml RNase A for 2 hours at 50C. 0.6 mg/ml proteinase K was then added and incubated for an additional 2 hours at 50C. Finally, cell pellets were resuspended in 50 mM sodium citrate and 1:5000 sytox green (Invitrogen) and incubated for 1 hour at room temperature. Flow cytometry was performed on a BD FACSCanto analyzer and 30,000 cells were recorded for each sample.

2.5.6 Sequencing library preparation

Illumina sequencing libraries were prepared as previously described (Henikoff et al., 2011; Belsky et al., 2015), with the following modifications: All library preparations were done on bead-bound DNA. After each step, clean up was accomplished by washing beads twice with binding buffer and three times with EB buffer. NEBNext Multiplex Oligos for Illumina kit (New England Biolabs) was used in adapter ligation and PCR steps. PCR reactions were cleaned using Agencourt AMPure XP beads (Beckman).

2.5.7 Data Analysis

Data analysis was performed in R version 3.2.0 or using bash scripts. Data processing scripts are available at https://gitlab.oit.duke.edu/dmm29/Gutierrez_2018

2.5.8 Sequence Alignment

Sequencing reads were aligned in paired-end mode to the sacCer3/R61 version of the *S. cerevisiae* genome using Bowtie 0.12.7 (Langmead et al., 2009).

2.5.9 Analysis of chromatin organization and structure

Biological replicates were merged for all data analyses. Merged files were then sampled to obtain equal read depth. Each merged experiment represents a minimum of 30 million reads. To characterize chromatin structure in nascent and mature chromatin at gene bodies, nucleosome-size reads between 140 bp and 180 bp in length were used to calculate a density curve of nascent, mature, and bulk chromatin across the length of each gene, with a 30 bp bandwidth Gaussian kernel. The pairwise pearson correlation between the density curves at each gene was calculated for nascent and bulk, or mature and bulk chromatin.

Similarly, we obtained nucleosome-size reads overlapping each gene and deter-

mined the midpoint location of each. We then calculated the density distribution of all the read midpoints with a 30 bp bandwidth Gaussian kernel. This output signal was smoothed and the refined predicted values generated a curve where each peak represents a nucleosome dyad and each trough represents linker DNA. We used an autocorrelation function (ACF) to determine if the pattern of positioning for the first four nucleosomes within each gene changed or remained organized. This ACF value was used as a proxy for individual gene structure.

2.5.10 Signal normalization of histone post-translational modifications, H2A.Z variant occupancy, and NET-seq

The accession number GSE61888 was used to locate and download ChIP-seq raw histone post translational modification (PTM) and htz1 histone variant data from (Weiner et al., 2015). Raw NET-seq data was obtained using the accession number GSE25107 (Churchman and Weissman, 2011). Reads were aligned in single end mode to the sacCer3/R61 version of the *S. cerevisiae* genome using Bowtie 0.12.7 (Langmead et al., 2009).

To determine PTM and htz1 occupancy, we calculated all the mapped reads overlapping gene bodies and normalized read counts using RPKM (Reads Per Kilobase of transcript per Million mapped reads). NET-seq data was processed similarly; however, the enrichment of reads was determined for sequences that spanned 100 bp upstream of the gene start to 300 bp downstream of the gene start. Gene orientation was considered during this calculation.

2.5.11 Individual nucleosome positioning and occupancy scores

Nucleosome locations were determined by obtaining nucleosome-size reads between 140bp and 180 bp in length and calculating a density curve across the length of each chromosome with a 30 bp bandwidth Gaussian kernel. Each chromosome-length density calculation was passed through the turnpoints function in the pastecs R

package to determine all the potential peaks from the density curves. These peaks were used to calculate nucleosome coordinates 70 bp upstream and downstream from each peak (140 bp total width).

All the nucleosome-size reads from each chromosome were acquired and their midpoint positions determined. Then, the distance of each midpoint to its nucleosome dyad was calculated. This average was used as the nucleosome positioning score. To ascertain nucleosome occupancy, the total number of normalized sequencing reads overlapping a nucleosome was determined (Figure 2.10).

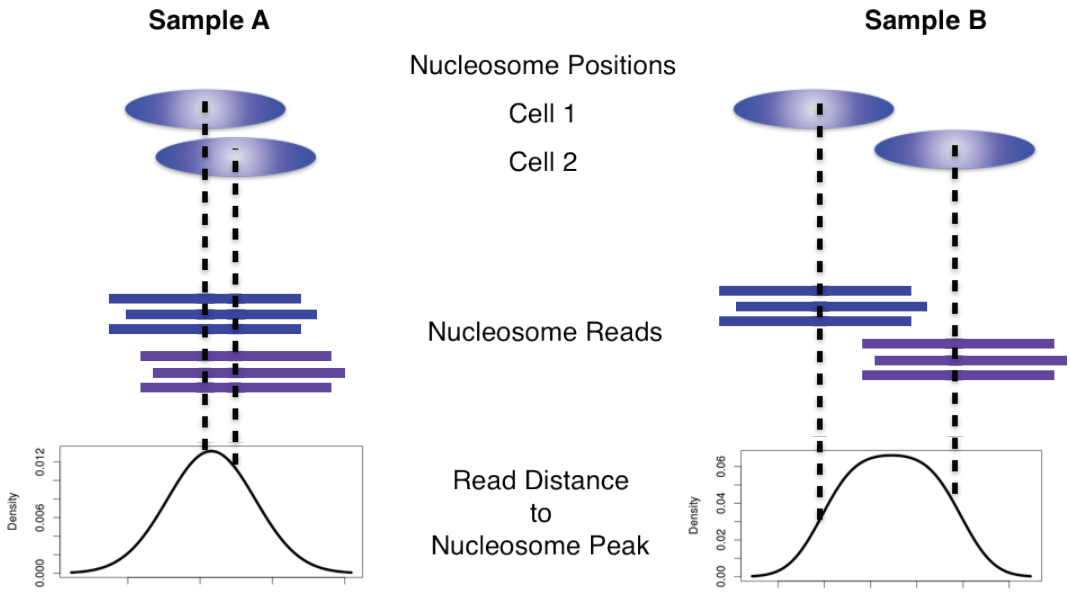

FIGURE 2.10: *Nucleosome positioning and occupancy schematic.* Well positioned nucleosomes will yield overlapping reads centered on the nucleosome dyad (sample A). On the other hand, reads overlapping poorly positioned nucleosomes will have a wider distribution (sample B).

2.5.12 Data Availability

All genomic data is publicly available at NCBI SRA accession SRP158706

3

Chromatin Assembly Activity of Chd1 and CAF-1 Change the Maturation Kinetics Genome-Wide

3.1 Introduction

The role of an ATP-dependent reaction in chromatin assembly was first described by Worcel and colleagues. In their reports, an *in vitro* system using *Xenopus* oocyte extracts showed greater levels of chromatin assembly and DNA supercoiling in the presence of ATP and Mg^{2+} (Glikin et al., 1984). They also uncovered that this ATP-dependent event not only helped in chromatin assembly but contributed to chromatin maturation (Ruberti and Worcel, 1986). Chromatin assembly and maturation behind the DNA replication fork require the orchestrated role of many histone chaperones and ATP-dependent chromatin remodeling enzymes. These enzymes can be divided into four main groups, including the SWI/SNF-type complex, the ISWI (imitation switch)-type complex, the INO80-type complex, and the CHD-type complex (Clapier and Cairns, 2009), and are highly conserved from yeast to humans. While many remodelers share core enzymatic factors, these protein complexes express highly diverse functions. They contribute to nucleosome sliding and eviction,

shaping and controlling the chromatin architecture.

Chromatin remodelers have been implicated in both origin selection and replication fork elongation. *In vitro* studies have shown that ORC and an ATP-dependent chromatin-remodeling complex are necessary and sufficient to establish the precise nucleosome positioning flanking an origin of replication (Eaton et al., 2010). Consistent with the role of chromatin remodeling at origins of replication, it was found that the ISWI chromatin remodeler was specifically enriched at ORC binding sites in the *Drosophila* genome (Eaton et al., 2011). ISWI works in complex with other proteins, and in *Drosophila* it is the key ATPase subunit of NURF (nucleosome-remodeling factor), ACF (ATP-utilizing chromatin assembly and remodeling factor) and CHRAC (chromatin assembly complex). This evidence emphasizes the dynamic relationship between chromatin remodeling and the DNA replication program.

What is the exact role of chromatin remodelers in promoting DNA replication? The chromatin remodelers INO80 and ISW1A enhance leading-strand synthesis in the presence of FACT (Kurat et al., 2017), and INO80 promotes fork progression rather than replication initiation in mouse embryonic fibroblasts (Lee et al., 2014) and budding yeast (Vincent et al., 2008). While these studies have linked these chromatin remodelers with DNA replication, their exact role in promoting replication fork progression still remains unknown. A recent study found that individual and double deletions of the chromatin remodelers Chd1, ISWI1, and the histone chaperone CAF-1 caused a negative chromatin assembly phenotype characterized by decreased nucleosome periodicity (Yadav and Whitehouse, 2016), indicating that chromatin remodeling activity following DNA replication is necessary to propagate a stable chromatin architecture.

Chd1 is a remodeler involved in several chromatin processes including spacing of nucleosome arrays and nucleosome assembly. It has long been studied in the context of gene transcription, where it localizes to actively transcribed genes through interac-

tions with the transcription machinery, prevents histone turnover, and forms spaced nucleosomes (Simic et al., 2003; Lusser et al., 2005; Smolle et al., 2012). Studies aiming to characterize *Drosophila* CHD found that the remodeler was able to assemble nucleosomes in the presence of a histone chaperone and ATP, generating nucleosome arrays with strong periodicity (Lusser et al., 2005). Interestingly, separation of function mutations have shown that the chromatin assembly activity of Chd1 is completely independent from its remodeling behavior (Torigoe et al., 2013), indicating that Chd1 is an important contributor to proper chromatin assembly following DNA replication. Thus, studying the role of Chd1 in chromatin maturation and its long term chromatin remodeling effects on newly synthesized and mature chromatin could shed light into this novel function of Chd1.

The roles of histone chaperones in the process of chromatin assembly have been widely studied in deletion mutants from yeast to humans, and their function in disease phenotypes (e.g. cancer) has emerged in recent years. I aimed to study the effect of abrogating the function of CAF-1 to understand how defects in the chromatin assembly pathway affect the dynamics of chromatin maturation across the genome of *S. cerevisiae*. I observed global chromatin maturation delays in nascent chromatin that did not affect the state of mature chromatin. This was in contrast to my studies in wild type cells (described in chapter 2), which exhibited a range of chromatin maturation kinetics evident by the structure of genes in the nascent state.

I also studied the role of Chd1 in chromatin maturation, and found that Chd1 deletion causes global defects in chromatin assembly. Interestingly, the effects of Chd1 deletion are more extreme in highly transcribed genes, where establishment of positioned nucleosomes was severely impaired. I also observed that in both the CAF-1 and Chd1 mutants, the footprint of small DNA binding proteins was significantly impaired in nascent chromatin compared to experiments in wild type cells. Altogether, my results provide a spatial and temporal view of the chromatin matu-

ration dynamics that occur in the context of CAF-1 and Chd1. This work has not been submitted for peer review and represents a preliminary analysis of experiments currently underway.

3.2 Results

3.2.1 Gene body chromatin maturation is impaired in chd1Δ yeast

In my analyses of wild type (WT) yeast, I identified two groups of genes characterized by fast and slow chromatin maturation. These kinetics were evident in the structure of nascent chromatin, where NCOPs allowed me to identify ∼2700 genes that varied in their nascent chromatin structure but were highly organized in mature chromatin. Fast maturing genes were characterized by well organized nascent nucleosomes, forming an array that highly correlates with the total bulk of chromatin. This is in contrast to genes with slow maturation kinetics, which had poor nascent chromatin architecture and low correlation values with total bulk chromatin. I generated NCOPs across the genomes of *chd1*Δ and *cac1*Δ. Cac1 is the largest subunit of CAF-1, and deletion of this gene is sufficient to completely eliminate the histone chaperone activity (Yadav and Whitehouse, 2016). I observed a general trend of poorly organized nascent nucleosome arrays in *cac1*Δ, including at genes that were described with rapid maturation kinetics in WT cells (Figure 3.1A, left panel *COQ10* gene). *chd1*Δ cells also exhibited chromatin disorganization at these genes, although the effect of *chd1*Δ appears to have a milder effect on nucleosome structure than *cac1*Δ (Figure 3.1A, right panel *COQ10* gene).

To assess the role of Chd1 and CAF-1 in the maturation kinetics of these genes, I used the autocorrelation function (ACF) as described in chapter 2 and compared the distribution of ACF values for *chd1*Δ,*cac1*Δ, and WT. The ACF values were plotted in correspondence with the first and fifth quintiles of nascent chromatin organization (slow and fast maturing genes, respectively) described in my characterization of

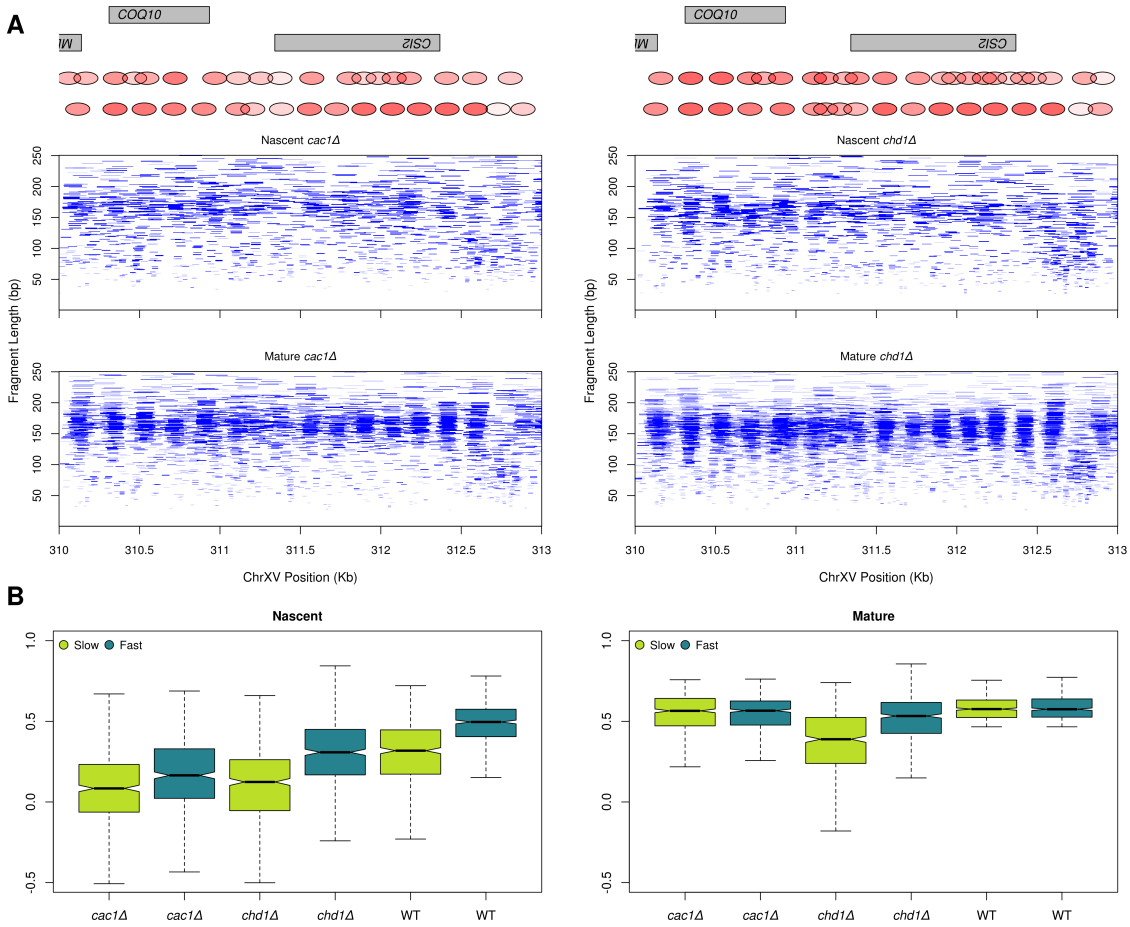

FIGURE 3.1: *Nascent chromatin occupancy profiles in chd1Δ and cac1Δ.* A. NCOPs reveal the maturation of chromatin behind the DNA replication fork in *cac1Δ* (left panel) and *chd1Δ* (right panel) at the genes *COQ10* and *CSI2*. B. Chromatin maturation kinetics for genes with well-organized wild type mature chromatin as described in Figure 2.3. The distribution of ACF values, as an indication of gene chromatin structure, show that the nascent chromatin organization at fast and slow maturing genes is impaired in both *chd1Δ* and *cac1Δ* (left panel). The delay in chromatin maturation in *cac1Δ* does not affect the structure of mature chromatin; however, many genes in *chd1Δ* cells failed to fully recover their chromatin architecture (right panel).

chromatin maturation in chapter 2. I found that in the two mutant backgrounds, the nascent gene structures were significantly more disorganized compared to WT cells in both quintiles (Figure 3.1B, left panel). Surprisingly, the architecture of the mature chromatin was severely impacted in *chd1*Δ cells (Figure 3.1B, right panel), and genes with slow maturation kinetics failed to fully recover their chromatin in the mature state. In contrast, the delay seen in *cac1*Δ cells did not prevent chromatin maturation and genes reached the structure of parental chromatin (Figure 3.1B, right panel).

Density plots of nucleosome architecture reflect this maturation kinetics at both *chd1*Δ and *cac1*Δ. Figure 3.2A shows the deteriorated nascent chromatin in both *chd1*Δ and *cac1*Δ mutants. This defect in establishing a chromatin array affects mostly genes with slow kinetics of maturation. While the chromatin structure of the *cac1*Δ cells is reconstituted in the mature state of both fast and slow maturing genes, the *chd1*Δ mutants are unable to fully restore their nucleosome organization (Figure 3.2B). This feature of *chd1*Δ is more pronounced at genes in the first quintile representing slow maturation kinetics. Slow maturing genes are characterized by marks of active transcription (Figure 2.3E), and it is possible that the organization in the mature chromatin of genes in *chd1*Δ cells is a reflection of a chromatin remodeling defect rather than deficiency in chromatin assembly. It is important to note that coverage plots of the nascent and mature chromatin samples in both *chd1*Δ and *cac1*Δ show the profile of cells labeled primarily in early S-phase due to slow transition from the G1 α-factor arrest (Figure 3.2C). In spite of this, there was enough genome-wide coverage to perform these analyses, although they need to be carefully interpreted.

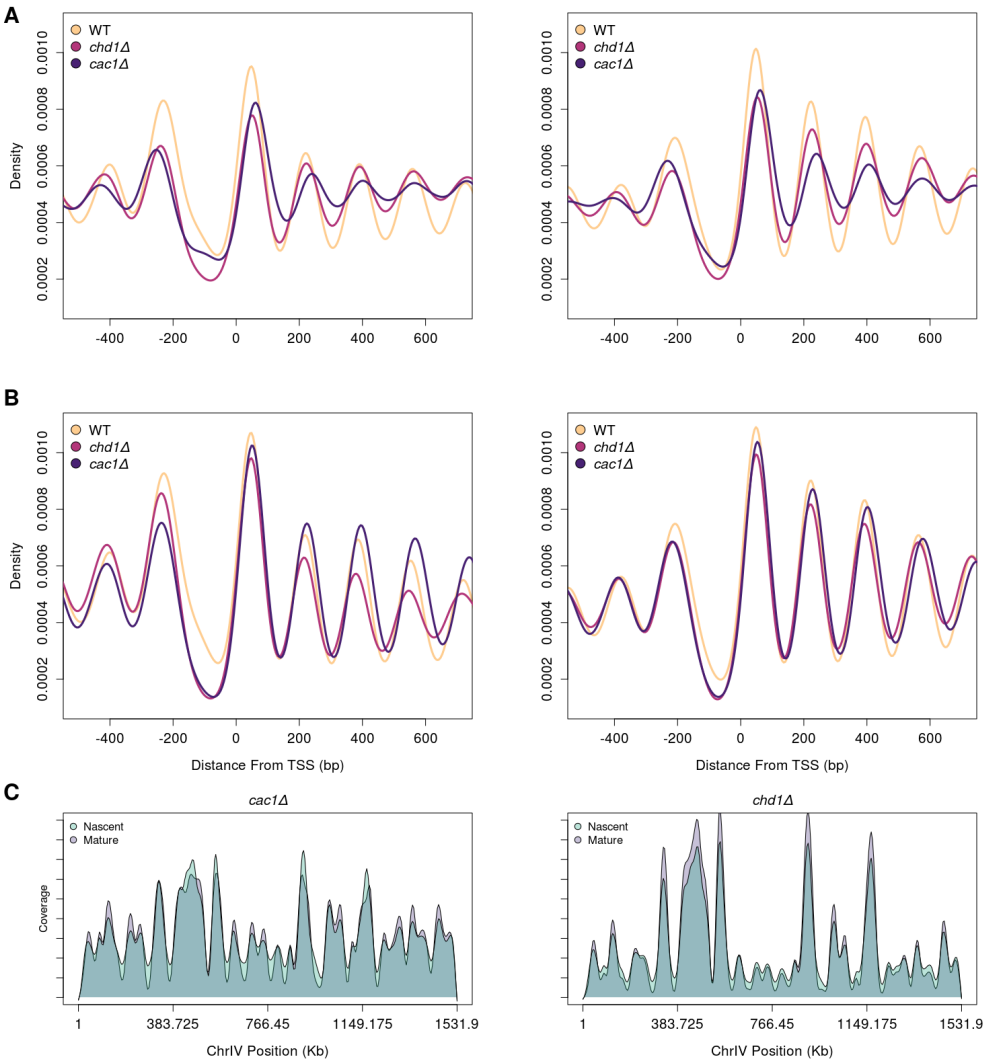

FIGURE 3.2: *Chromatin structure of cac1Δ and chd1Δ cells at fast and slow maturing genes.* A. Nascent chromatin architecture in the slow (left panel) and fast (right panel) maturing genes. B. Mature chromatin architecture as in A. C. Coverage plots of nascent and mature chromatin over chromosome IV show relative EdU incorporation in *cac1*Δ (left panel) and *chd1*Δ (right panel).

3.2.2 Chromatin assembly and chromatin remodeling activities of Chd1 at gene structures

In my previous analyses of chromatin maturation in WT cells, I described that slow maturing genes were enriched for histone marks associated with active transcription (H3K36me3 and H3K79me3). The *chd1*Δ chromatin disorganization observed at these genes led me to hypothesize that Chd1 deletion would severely impair chromatin maturation at highly transcribed genes. To further analyze the chromatin architecture differences at genes based on their levels of transcription, I ranked 5173 genes by their NET-seq scores and generated nucleosome profiles for the 20th percentile of genes with the highest and lowest expression values. Both *chd1*Δ and *cac1*Δ showed higher disorganization compared to WT cells in density plots of nascent chromatin at both active and non-transcribed genes (Figure 3.3A). This further supports the idea that Chd1 has an important chromatin assembly role that warrants further investigation and characterization.

In contrast to the highly disorganized nascent chromatin structure across all gene bodies, the mature nucleosome array of active and non-transcribed genes varies in structure. The architecture of the most actively transcribed genes is significantly disorganized in *chd1*Δ compared to *cac1*Δ or WT cells (Figure 3.3B, right panel). In contrast, the mature chromatin structure of poorly transcribed genes is identical to WT in both mutant populations (Figure 3.3B, left panel). These results imply that the differences in the organization of mature chromatin between active and inactive genes might represent a remodeling dependent event rather than a chromatin assembly effect. This indicates that the assay can help elucidate this separate functions of the Chd1 chromatin remodeler, and suggest a model in which Chd1 is both essential at the fork during chromatin assembly and required during transcription to maintain the structure of genes after passage of the transcription machinery.

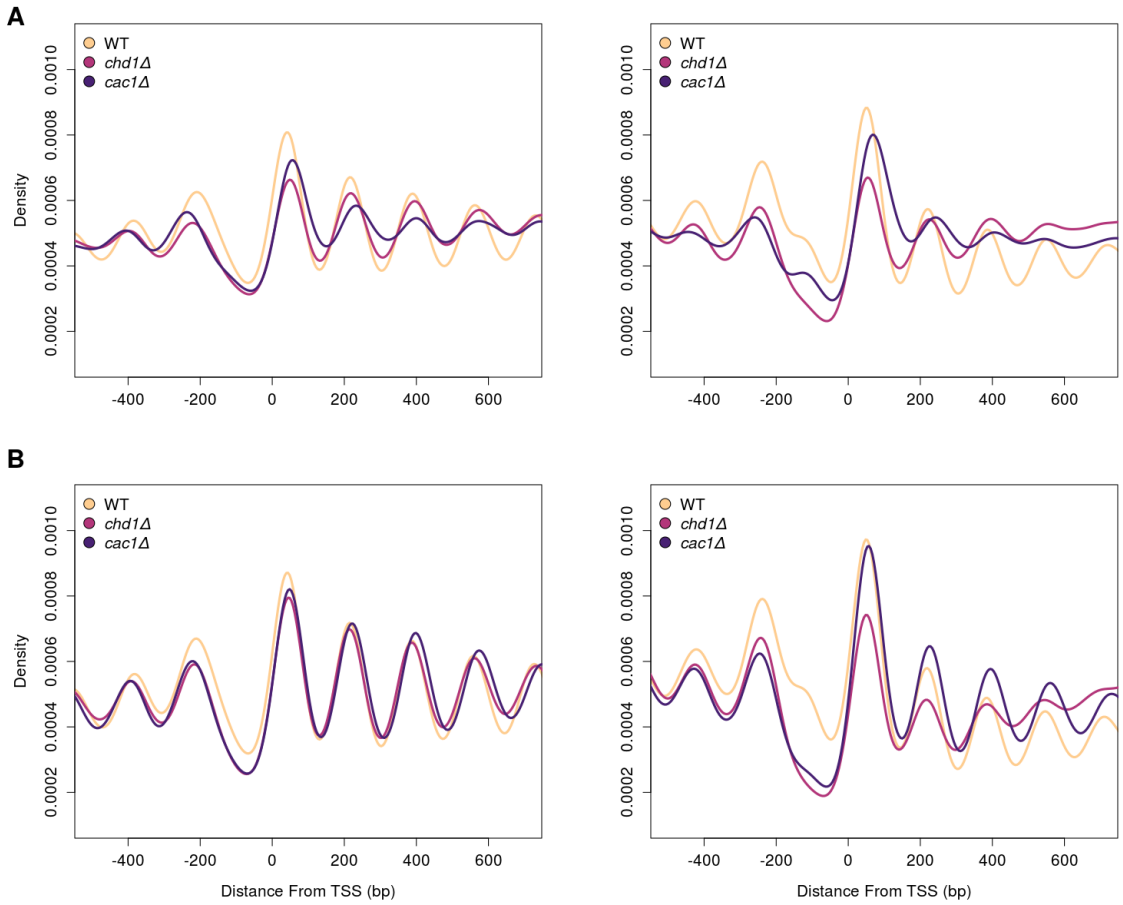

FIGURE 3.3: *Chromatin structure of cac1Δ and chd1Δ cells at highly and poorly expressed genes.* A. Nascent chromatin structure at 1034 poorly (left panel) and 1034 highly (right panel) transcribed genes. B. Mature chromatin architecture as in A.

3.2.3 chromatin assembly at origins of replication is severely impaired in cac1Δ cells

There were two important observations from the gene body chromatin analyses. First, CAF-1 causes a global delay in chromatin assembly that affects both fast and slow maturing genes similarly, but does not affect their maturation kinetics. Secondly, Chd1 has a clear role in chromatin assembly since *chd1Δ* cells had impaired nascent chromatin organization comparable to CAF-1, but *chd1Δ* cells were deficient in organizing the mature chromatin of actively transcribed genes. To test if these

effects translated to other genomic regions, I analyzed the structure of chromatin surrounding origins of replication. I generated NCOPs at two active origins (*ARS805* and *ARS1*) to demonstrate the effect of *chd1*Δ and *cac1*Δ in re-establishing chromatin architecture at these sites (Figure 3.4). Visual inspection showed that, like the gene body phenotypes, nascent origin architecture appears to be severely affected by deficient chromatin assembly in *cac1*Δ cells (Figure 3.4A). *chd1*Δ were capable of organizing origins with better nucleosome arrays than *cac1*Δ (Figure 3.4B), but do not appear to be as efficient as WT (Figure 3.4C).

To quantify these observations, I stratified origins by their activation efficiency and classified them as actively replicating or passively replicated origins. Using the positioning score metric described in chapter 2, I plotted the average nucleosome organization scores for the first three pairs of nucleosomes flanking each origin. In all three cases, actively replicating origins exhibited higher degree of organization compared to passively replicated origins in nascent chromatin (Figure 3.5A, left panel). However, the overall degree of nascent chromatin architecture decreased from WT to *chd1*Δ, with *cac1*Δ having the most disorganized nascent chromatin. In contrast, the structure of mature chromatin was restored across all origins in both *chd1*Δ and *cac1*Δ (Figure 3.5A, right panel).

I then followed up on my previous analyses, and evaluated the effect of these mutations in the ORC footprint. Surprisingly, the ORC footprint was significantly decreased in *chd1*Δ cells compared to *cac1*Δ at both active and passively replicated origins (Figure 3.5B). This decrease was even more apparent at passively replicated origins, where the footprint was completely absent (Figure 3.5B, right panel). I cannot rule out that the differences in the footprint at passively replicated origins are an experimental artifact due to the inefficient α-factor release (Figure 3.2C). Furthermore, I note that overall differences in the footprint at both types of origins could be attributed to variability of the MNase digestion. However, chromatin plots

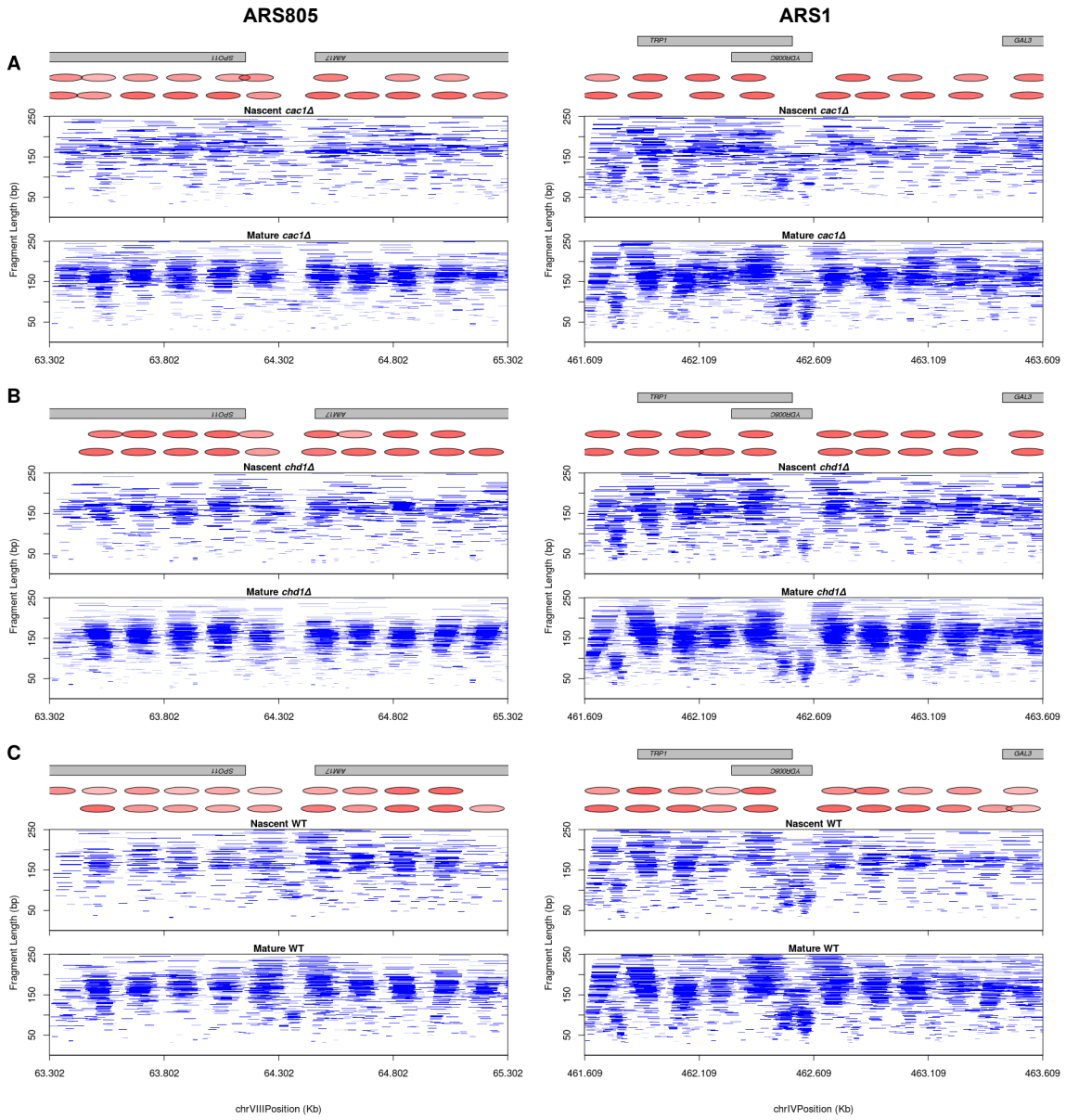

FIGURE 3.4: *NCOPs at the efficient origins ARS805 and ARS1.* NCOPs of *ARS805* (left panel) and *ARS1* (right panel) for A. *cac1*Δ, B. *chd1*Δ, and C. WT cells.

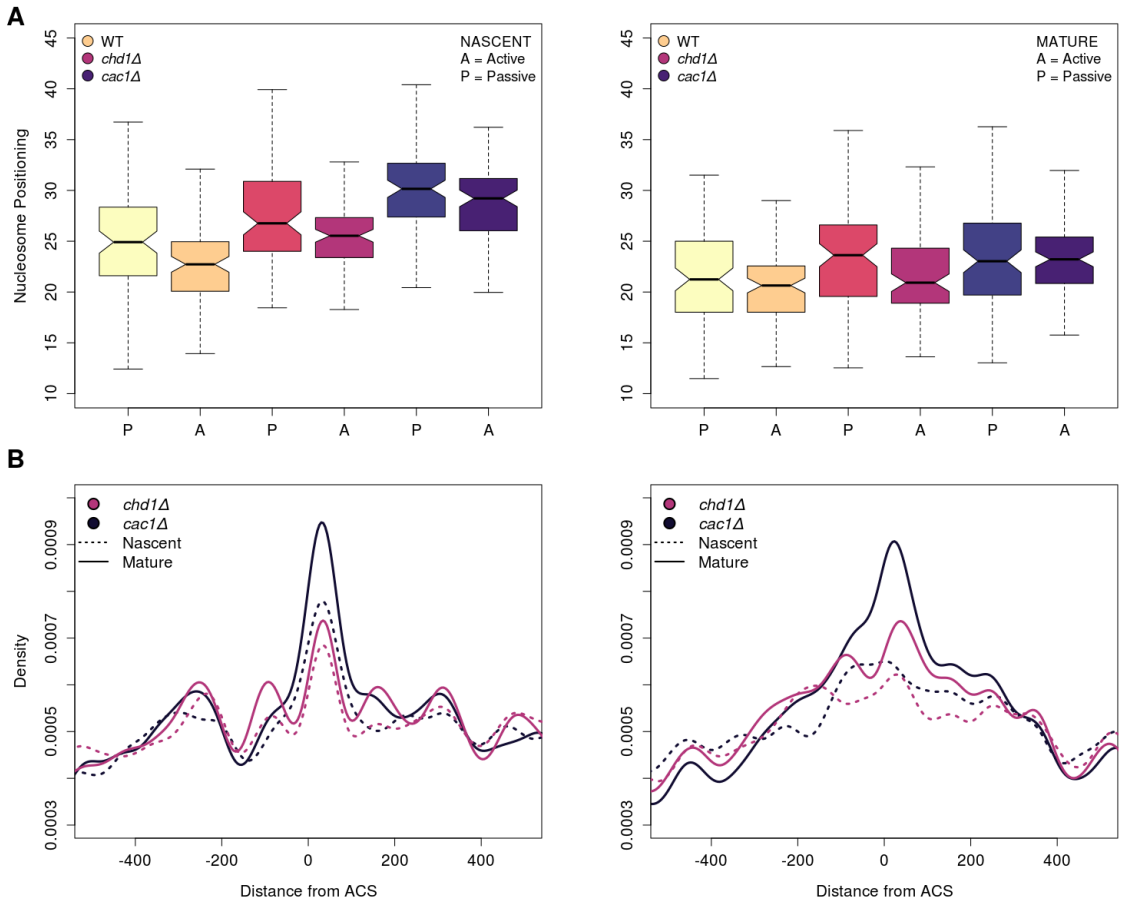

FIGURE 3.5: *Nucleosome positioning of cac1Δ and chd1Δ cells at actively and passively replicated origins.* A. Positioning score distributions for the first three pairs of nascent (left panel) and mature (right panel) nucleosomes flanking actively and passively replicated origins. B. ORC footprints at actively replicating (left panel) and passively replicated (right panel) origins.

show footprints of small DNA-binding proteins in the vicinity of ORC binding sites (Figure 3.4), arguing against a digestion phenotype and supporting the idea that the association of ORC with DNA is affected in the *chd1Δ* cells.

3.2.4 Proper chromatin structure is necessary for transcription factor re-association with chromatin

My analyses of ORC and its association with chromatin led me to explore the footprints of other small-DNA binding proteins. To determine if the effects on the ORC

footprint also impacted other binding factors, I generated heatmaps centered on the binding sites of 1078 previously identified factors (MacIsaac et al., 2006) with strong footprints in the mature chromatin of wild type cells (Figure 3.6A-C). The factors were then ranked by the strength of the footprint in the mature state of each mutant and WT data set. I observed a decrease in the overall occupancy of factors when heatmaps of *chd1*Δ and *cac1*Δ were compared to WT. This difference in occupancy was even more dramatic when evaluating *cac1*Δ, given that there was a severe decrease in factor occupancy in the nascent state of *cac1*Δ cells compared to its mature state (Figure 3.6A). This led me to hypothesize that defects in the structure of chromatin as a result of faulty chromatin assembly inhibited association of these factors with chromatin.

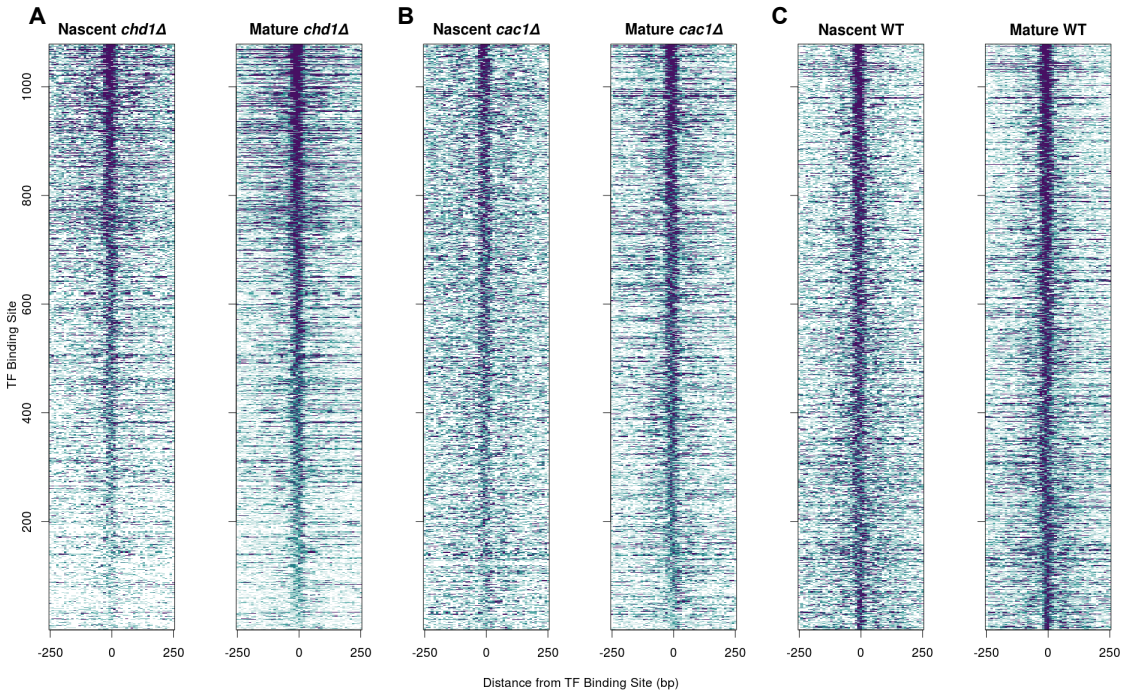

FIGURE 3.6: *Transcription factor footprint in chd1*Δ *and cac1*Δ *cells.* Heatmaps of nascent and mature transcription factor (TF) occupancy in A. *chd1*Δ, B. *cac1*Δ and C. WT cells. 1078 TF binding sites were selected that had a strong footprint in mature WT cells.

To assess the structure of chromatin surrounding these factor binding sites, I took to the nucleosome positioning scores described in chapter 2. I plotted the distribution of positioning scores for the first 3 pairs of nucleosomes flanking a factor binding site in their nascent and mature state to assess nucleosome organization (Figure 3.7A). I found that nascent nucleosomes in both *chd1*Δ and *cac1*Δ cells were significantly less organized, and this disorganization was even more dramatic in *cac1*Δ compared to *chd1*Δ (Figure 3.7A, left panel). However, both mutants are capable of re-establishing the chromatin architecture by positioning nucleosomes close to WT levels (Figure 3.7A, right panel). This suggests that the higher nucleosome organization and positioning in mature chromatin correlates with preferential factor binding.

Pioneer transcription factor binding sites are characterized by well positioned flanking nucleosomes and a strong factor footprint (Figure 2.9). It is currently not known whether this chromatin organization is a consequence of transcription factor binding, or whether this well positioned nucleosomes promote factor association with its binding site. The results in Fig 3.5 led me to hypothesize that well organized nucleosomes are necessary for transcription factor re-association with chromatin. To assess this, I first generated density estimates of the pioneer factor footprints Abf1p and Reb1p (Figure 3.7B). I found that the factor occupancy in nascent chromatin was decreased in *chd1*Δ and more severely in *cac1*Δ cells. The corresponding nucleosome profiles showed that nascent nucleosomes were dramatically more disorganized in *cac1*Δ compared to *chd1*Δ (Figure 3.7C). Nucleosome architecture was re-established following maturation in both mutants. Given that the phenotypes were a consequence of deficient nucleosome assembly, it is likely that the decrease in transcription factor footprint in nascent chromatin was a result of poor nucleosome organization that was not permissive of transcription factor binding.

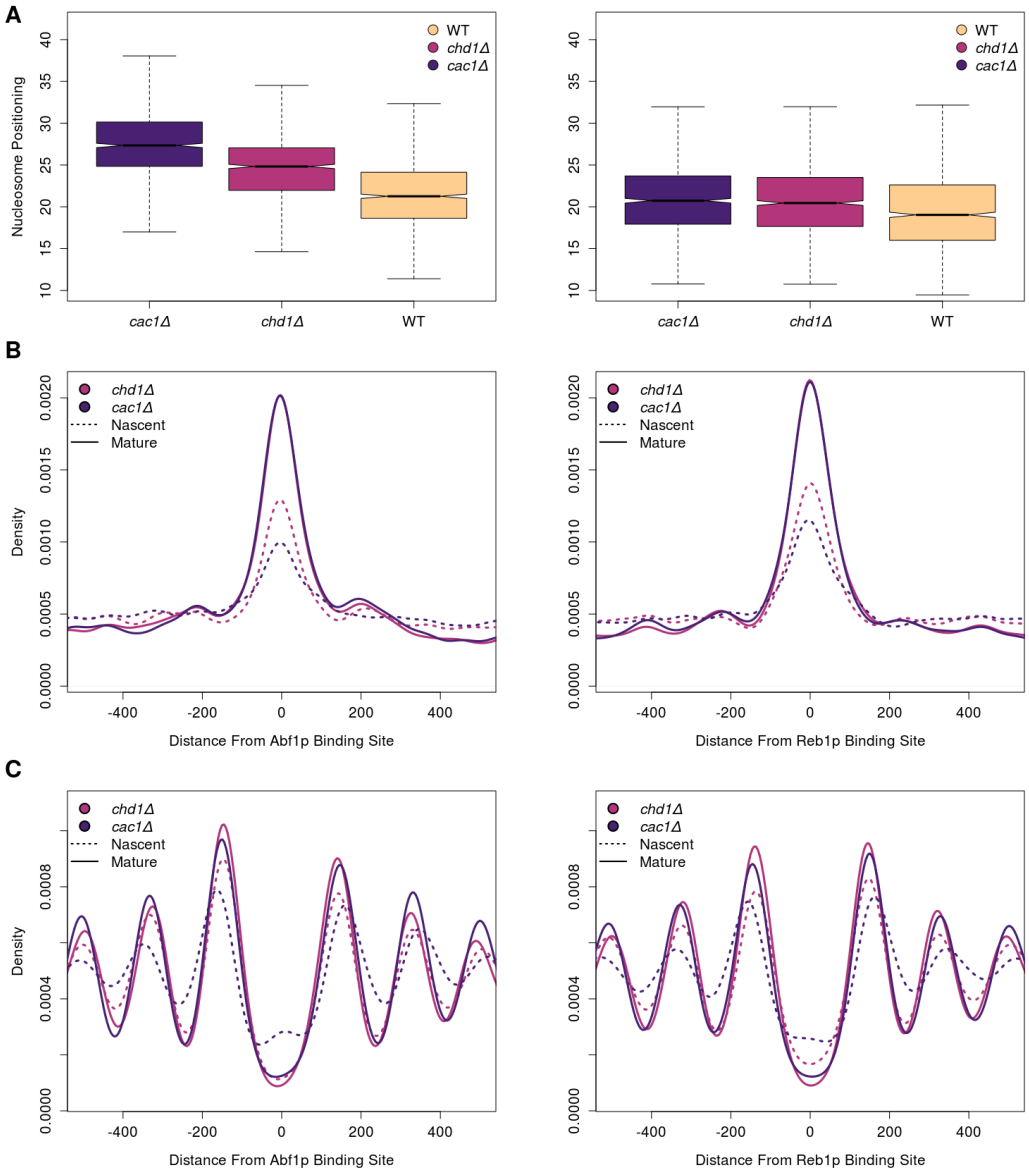

FIGURE 3.7: *Chromatin maturation of cac1Δ and chd1Δ cells at Abf1p and Reb1p binding sites.* Positioning score distributions for the first three pairs of nascent (left panel) and mature (right panel) nucleosomes flanking the TF binding sites described in Figure 3.6. B. Occupancy profiles of Abf1p (left panel) and Reb1p (right panel). C. The corresponding nucleosome profiles.

3.3 Discussion

Through the process of DNA replication, the cell is challenged with replicating its genetic material with high fidelity. Similarly, the cell must also replicate the structure of chromatin to ensure proper epigenetic inheritance in the daughter strands. In this context, chromatin presents multiple challenges during DNA replication. Before replication can proceed, the chromatin environment ahead of the fork needs to be partially dis-assembled to facilitate passage of the replication fork, and then it must be re-established behind the fork to replicate the epigenetic state of the mother cell. As a consequence, histone chaperones and chromatin remodelers are necessary to perform these many chromatin functions during replication. Chromatin remodelers such as INO80 and ISWI have been implicated in promoting proper DNA replication (Vincent et al., 2008; Papamichos-Chronakis and Peterson, 2008; MacAlpine and Almouzni, 2013), and *in vitro* reconstitution of chromatin showed that these remodelers along with FACT promote nucleosome formation in replication substrates (Kurat et al., 2017).

The role of histone chaperones has been widely studied and our understanding of their function in the deposition of histones on newly synthesized DNA has been uncovered as a highly conserved process from yeast to humans (Alabert and Groth, 2012; Serra-Cardona and Zhang, 2018). However, the exact role of chromatin remodelers during replication is less clear. While they are necessary for replication fork progression, it is not known what role these remodelers play at the fork. It is likely that they are necessary to remodel and evict nucleosomes ahead of the fork to facilitate progression of the replication machinery, but they are probably just as important to position nucleosomes on newly synthesized DNA and aid in the chromatin maturation process. *In vitro* and *in vivo* experiments have yet to disentangle this phenomenon; thus, I aimed to use NCOPs to study the effects of key chromatin

remodelers and known histone chaperones in genome-wide chromatin assembly and maturation.

I applied NCOP to reveal chromatin assembly in the background of cells lacking the chromatin remodeler Chd1 or the histone chaperone CAF-1. Following up on the analyses of chapter 2, I determined the maturation dynamics at fast and slow maturing genes. I found a significant decrease in chromatin architecture within the gene bodies of both groups of genes in *chd1*Δ and *cac1*Δ compared to WT cells. The reduced nascent chromatin structure in the fast maturing group of genes was expected in *cac1*Δ given its critical role in chaperoning histones behind the fork, but the fact that *chd1*Δ also displays this severe phenotype indicates that Chd1 plays an important role in chromatin assembly. When the structure of mature chromatin was evaluated, there were drastic differences in the organization of genes in *chd1*Δ cells. *cac1*Δ cells were able to restore gene architecture close to parental levels; however, *chd1*Δ cells were deficient in gene chromatin maturation, and this phenotype was even more dramatic in the group of slow maturing genes known to be enriched for active transcription marks. The similarity in the nascent chromatin phenotype between *chd1*Δ and *cac1*Δ cells implies that Chd1 is involved in the DNA replication dependent chromatin assembly pathway, but this does not fully explain the apparent delay in gene body chromatin maturation observed in *chd1*Δ cells.

These puzzling results led me to hypothesize that the defect in maturation observed in both fast and slow maturing genes in *chd1*Δ cells was a consequence of deficient chromatin remodeling and independent from the chromatin assembly role of Chd1. Chd1 associates with elongating RNA pol II (Simic et al., 2003) and prevents histone turnover in the wake of the transcription machinery (Smolle et al., 2012). Thus, it is likely that the defects in the gene structure of mature chromatin demonstrate deficient chromatin remodeling in the absence of Chd1. Indeed, I found that the poor organization of nascent nucleosomes was restored to WT levels at

low transcribed genes in both *chd1*Δ and *cac1*Δ cells. However, the mature structure of highly transcribed genes remained severely disorganized in *chd1*Δ. In all, my observations between the nascent and mature states of *chd1*Δ cells portray two independent roles of this enzyme. It is clear that the nascent chromatin disorganization is related to the chromatin assembly role of Chd1, while the results at actively transcribed genes in their mature state point to its chromatin remodeling activity. Thus, my observations are capable of resolving the effects of two independent roles of Chd1, chromatin assembly and chromatin remodeling, that have been previously described (Torigoe et al., 2013).

Following the observation that Chd1 contributes to chromatin assembly in a manner that is parallel to that of CAF-1, I assessed the implications of Chd1 chromatin assembly at origins of replication. Similar to the WT results, passively replicated origins remain poorly organized compared to active origins. However, there was gradual decrease in nascent chromatin architecture from *chd1*Δ to *cac1*Δ that affected all origins. In contrast to the gene body structures, nucleosomes flanking origins mature to similar levels in *chd1*Δ and *cac1*Δ. This further emphasizes that Chd1 has a chromatin assembly role that is not only relevant at gene bodies, but critical for nucleosome formation genome-wide. Interestingly, the ORC footprint was severely impacted in these mutants, particularly in the *chd1*Δ cells. It appears as if ORC is unable to re-associate with chromatin, or does so at a limited rate. This phenomenon is even more exaggerated at passively replicated origins, where the nascent ORC footprint is completely absent, and it only recovers in the *cac1*Δ cells. This difference in the ORC association can be attributed to decreased chromatin architecture, but it can not be fully explained in the context of *chd1*Δ, which appears to have greater nascent nucleosome organization than *cac1*Δ. These results need to be interpreted with caution as artifacts and MNase digestion can be confounding these results. Further experiments and biological replicates should be able to address and

corroborate this phenomenon.

Compared to WT, chromatin mutants were much susceptible to defects in factor re-association with chromatin, indicating that lack of chromatin architecture is not permissive of transcription factor binding. I observed an overall decreased in factor binding in both mutants when comparing nascent to their respective mature profiles. This is in contrast to WT, were the footprints remain strong in both stages. Quantification of the nucleosome organization at these sites demonstrated a similar profile to the one observed at origins; *cac1*Δ cells exhibited the most severe defect, but overall the outcome of chromatin maturation is not changed. In all, I provide evidence that proper nucleosome organization is necessary for pioneer factor association with chromatin, as the footprint is not recovered until the chromatin dynamics are re-established in mature chromatin. Further work with other histone chaperones and chromatin remodelers should reveal the role of chromatin in promoting recruitment of regulatory proteins to their binding sites.

3.4 Methods

All experimental methods and data analyses were performed as described in the Methods section of chapter 2.

3.4.1 Yeast stains

The yeast strain DMMy505 is in the W303 background and has the genotype MATa, RLF2::HIS, leu2-3,112, BAR1::TRP, can1-100, URA3::BrdU-Inc, ade2-1, his3-11,15
The yeast strain DMMy155 is in the W303 background and has the genotype MATa, CHD1::KAN, trp1-1, can1-100, URA3::BrdU-Inc, ade2-1, his3-11,15

4

Conclusions

DNA replication plays an integral role in propagating the parental epigenetic state to newly copied sequences. The chromatin environment is dynamic with locus-specific epigenetic states associated with distinct cell types and developmental stages. This plasticity in the chromatin environment likely contributes to the cell type-specific patterns of origin selection and usage found in higher eukaryotes. This means that the re-establishment of the chromatin architecture following DNA replication might be affected by the local chromatin architecture of the parental state. Histone post-translational modifications, DNA binding factors, nucleosome positioning, and histone variants all contribute to the epigenetic landscape and have the capacity to regulate chromatin maturation.

This work is based on my interest in understanding the mechanisms by which chromatin is re-established following DNA replication, a process that disrupts the entire chromatin landscape of the cell. To address the question of how nascent chromatin matures into parental chromatin, I developed a method that builds from previous techniques (MNase-seq and EdU labeling of nascent DNA) to generate Nascent Chromatin Occupancy Profiles (NCOPs). NCOPs allow us to study the dynamics of

chromatin assembly following passage of the DNA replication fork at a genome-wide level and at single base-pair resolution. NCOPs capture nascent chromatin, facilitating the analysis of chromatin maturation across the entire genome. Currently, there has been very little description of the dynamics of chromatin maturation in space and time. To understand how the epigenome is re-established following DNA replication, we need tools to characterize the chromatin maturation dynamics across different genomic locations. Furthermore, while most of our understanding of chromatin maturation comes from analyses of nucleosome assembly behind the fork, we do not know the kinetics of re-association with chromatin for individual transcription factors and other DNA binding proteins.

To study the dynamics of chromatin maturation genome-wide, I also developed bioinformatic strategies to reveal the positioning and occupancy of each nucleosome in the genome. These approaches allowed me to study the nucleosome arrangement in nascent and mature chromatin, and to identify the differences between the two chromatin states. I was interested in exploring the maturation kinetics of intergenic nucleosomes in comparison to gene body structures, which are highly characterized. The main motivation for this is that many regulatory elements are located in non-coding regions and are, thus, very important for genome function and stability. I determined the nucleosome positioning and occupancy within each of the intergenic and intragenic regions, and was able to conclude that intergenic nucleosomes and poorly transcribed genes have the greatest level of organization compared to nucleosomes within actively transcribed genes. Furthermore, I studied the dynamics of chromatin assembly at DNA replication origins, and found that passively replicated origins display slower maturation kinetics compared to active origins. Our analyses of intergenic regions and the features within is a significant step toward understanding the dynamics of chromatin maturation throughout the genome.

An advantage of the NCOP approach is the ability to look beyond nucleosomes to

smaller DNA binding proteins. Using NCOPs, I determined the occupancy of small DNA-binding proteins in the nascent and mature states and provided a first-time look into the dynamics of re-association of small DNA binding factors with DNA. The evidence that factors can transiently associate with nascent chromatin suggests that DNA replication offers a window of opportunity to alter the epigenetic state of the cell. It has been shown that changes in the dynamics of chromatin assembly cause somatic cell reprogramming by loosening nucleosome organization at genes and pluripotency-specific enhancers (Cheloufi et al., 2015), indicating that altering the dynamics of histone deposition lead to gene accessibility and transcription factor binding capable of changing cell identity and behavior. In the same manner, the transcriptional profile of mouse embryonic stem cells depleted of CAF-1 resembled that of totipotent-like cells as a consequences of changes in the chromatin accessibility at these sites (Ishiuchi et al., 2015). These results indicate that developing cells adapt the chromatin assembly program to change gene expression patterns permissive of reprogramming and organismal development. Similarly, changes to the replication program such as those that occur in the fast replicating cancerous cells may cause chromatin assembly errors that have the potential to facilitate non-specific binding of factors and lead to unprogrammed changes in gene expression. The histone chaperones CAF-1 and ASF1 have been proposed as proliferative markers of breast, colon, and prostate cancer (Polo et al., 2004; Staibano et al., 2009); thus, it is likely that their over expression in these cells is linked to defects in chromatin assembly and the subsequent gene expression patterns characteristic of cancer cells.

There has been a decades-long interest in understanding how chromatin is reconstituted following DNA replication. The findings of this work provide new tools to assess the consequences of DNA replication in modifying the chromatin environment and changing the epigenome, as defects in the DNA replication program could impair proper chromatin structure and epigenetic inheritance. For instance, patients with

Meier-Gorlin Syndrome, a rare autosomal recessive disorder characterized by prenatal and postnatal growth retardation (Guernsey et al., 2011; Shen, 2013), have been identified with mutations in components of the pre-RC. A mutation in the MCM5 subunit of the replicative helicase found in a patient with Meier-Gorlin decreased MCM2-7 complex association with chromatin (Vetro et al., 2017). Given that the MCM2 subunit is relevant for parental histone recycling, defects in pre-RC assembly and replication initiation could subsequently lead to faulty chromatin assembly and/or histone recycling. Furthermore, the patient's cells exhibited slow S-phase progression (Vetro et al., 2017), and it will be interesting to uncover the effects of S-phase progression in chromatin assembly dynamics. Mutations in ORC and other pre-RC components have also been linked to Meier-Gorlin syndrome and to a series of diseases collectively referred to as the chromosome instability syndrome (Masai et al., 2010). Together, these disease phenotypes highlight the impact of DNA replication not only during development but in establishing a chromatin environment characterized by appropriate nucleosome phasing that leads to gene expression patterns and optimal organismal development. The tools developed here would allow the study of these mutations and their effects in DNA replication and the establishment of chromatin behind the fork in the context of these disease mutations.

One of the main contributions of this work is the ability to study the effects of histone chaperones and chromatin remodelers in shaping the structure of chromatin following DNA replication. The effect of these mutants on the replication program and chromatin assembly has been analyzed in aggregate; as expected, the reconstitution of chromatin is impaired when these key factors are deleted. However, our understanding of how chromatin remodelers shape the replication program and subsequent chromatin maturation is very superficial compared to our knowledge of the histone assembly pathway. I aimed to explore chromatin assembly and maturation in the context of remodelers and histone chaperones in order to deconstruct their

role in positioning nucleosomes following passage of the replication fork.

My preliminary experiments with the chromatin remodeler Chd1 and the histone chaperone CAF-1 provided new insights into the role of these factors during chromatin assembly. Most surprisingly was the chromatin phenotype observed in *chd1*Δ cells as I was able to identify that Chd1 is an important factor of the DNA replication-dependent chromatin assembly pathway, and this assembly behavior appears to be independent of its known chromatin remodeling role during transcription. Indeed, work *in vitro* has shown that Chd1 is capable of generating arrays of regularly spaced nucleosomes in a chromatin assembly assay (Lusser et al., 2005), and separation of function studies demonstrated that Chd1 can assemble nucleosomes in the absence of its ATP-dependent chromatin remodeling activity (Torigoe et al., 2013). Furthermore, I uncovered that transcription factors have delayed re-association dynamics with chromatin in both *chd1*Δ and *cac1*Δ cells. This behavior included pioneer factors with strong binding affinity to DNA like Abf1p and Reb1p, showing decreased nascent chromatin association as a result of poor chromatin structure surrounding their binding sites. It is important to note that transcription factors and small DNA binding proteins are particularly sensitive to differences in MNase digestion, and high concentrations of the enzyme or longer incubation times could decrease the factor footprint. Abf1p and Reb1p have strong footprints compared to most transcription factors even across short and long MNase incubation times (Henikoff et al., 2011), supporting the idea that the differences in transcription factor occupancy observed in analyses of *chd1*Δ and *cac1*Δ cells are a result of the local chromatin environment and not an experimental artifact. In all, it is possible to conclude that this experimental design has the potential to uncover the behavior of novel factors with unknown dynamics during DNA replication and chromatin maturation. It also opens the door to explore the genome-wide and locus specific effects of known factors like CAF-1 in the chromatin maturation process and re-establishment of the epigenetic

state.

Altogether, the results of this work have implications in the field of chromatin biology in multiple regards. First, there is a need for innovative techniques capable of assaying the genome with the resolution to provide locus specific and temporal dynamics. The pairing of MNase-seq with nucleoside labeling of nascent DNA to probe the site specific chromatin changes that occur following DNA replication can be expanded to a variety of applications. In the proof of principle studies, I was able to label, enrich for, and profile the chromatin of early replicating origins in cells under replicative stress. I then profiled their recovery from the stress after passage through the cell cycle, and was able to track the chromatin changes that occurred at DNA damage response genes. An interesting observation came from the array of chromatin responses at these genes, characterized by recruitment of transcription factors that caused a downstream shift in nucleosome positioning or overall disruption of the nucleosome organization at the gene bodies.

These different changes in response to the same stressors highlight a big gap in the field of chromatin biology as we have yet to understand the distinct chromatin changes that occur at individual locations, which are often masked in bulk and aggregate analyses of chromatin structure. Furthermore, bioinformatics would make it possible to track the locations of stalled replication forks and characterize the chromatin dynamics that occur at these sites, which could help us elucidate the chromatin steps of replication fork recovery. Our experimental and bioinformatic approaches have taken the first steps to address these important questions.

Because the methods developed during my thesis work take advantage of the labeling of nascent DNA during replication, they can be easily applied to the study of chromatin dynamics during DNA repair. Repairing broken DNA requires the recruitment of the replication-dependent chromatin assembly factors ASF1 and CAF-1, and employs a similar assembly pathway to reconstitute nucleosomes at the break

site (Dabin et al., 2016). In this regard, it is likely that our assay can probe chromatin maturation following DNA repair. It also has the potential to elucidate how DNA repair and chromatin restoration are affected by the local chromatin environment.

4.1 Future directions

Our results demonstrate a clear effect of the transcription program in shaping the structure of chromatin following replication. Specifically, I found that the occupancy of nucleosomes in highly transcribed genes decreases in mature chromatin, indicating that our NCOPs can capture the chromatin architecture of genes prior to transcription. These effects would be masked otherwise in bulk analyses, and our tools have the potential to study the effects of the transcription program in shaping the structure of genes. An interesting experiment would change the transcription profile of active and inactive genes to observe their dynamics of maturation following passage of the replication fork. For instance, preventing expression of an actively transcribed gene should enhanced its maturation kinetics and increase nucleosome occupancy in the mature state. This would shed light into the interconnection between these two processes. However, many parameters need to be considered including the directionality of the replication fork and the transcription machinery. This is particularly important in the case of increasing the expression profiles of silent genes. Head-on collisions between the two machineries could cause replication fork stalling and lead to genome instability. This in itself would be an interesting question to address; how is the chromatin environment shaped prior, during and following these collisions? The tools developed here would be able to resolve these structures and provide information on the chromatin changes that occur during these steps.

These tools also allowed me to study the chromatin assembly and the chromatin remodeling roles of Chd1. My conclusions were based on the ability to study protein occupancy genome-wide at a base-pair resolution, which many techniques currently

lack. Thus, our approaches could uncover the role of chromatin remodelers in shaping the chromatin maturation process. For instance, using the separation of function mutants that have been previously described for Chd1 would further disentangle the chromatin assembly role of this remodeler. Similarly, it would be relevant to study the role of INO80 and ISW1A. These remodelers increased nucleosome formation in the presence of FACT as well as the length of the leading-strand products *in vitro*, resulting in a clear distinction between leading and lagging-strand assembly (Kurat et al., 2017). It would be highly informative to determine whether the chromatin remodeling events occur independently of their chromatin maturation function, and their role in promoting symmetrical chromatin assembly in the two daughter strands.

This work also leaves many questions ready for exploration in the areas of epigenetic inheritance. The evidence that genes are marked by an epigenetic footprint characterizing fast or slow maturation kinetics poses an interesting idea for how the epigenome is conserved following replication. This would mean that changing the epigenetic footprints could also alter the maturation dynamics at these sites. Fast maturing genes were characterized by acetylation marks whereas slow maturing genes were enriched in methylation marks associated with transcription. In both cases, it will be ideal to modulate the levels of acetylation and methylation to assess the degree by which these marks change the dynamics of chromatin maturation. Candidate lysine residues can be altered such that they are not modified, or the enzymes responsible for depositing these histone marks (methyltransferases and acetyltransferases) can be transiently removed. NCOPs can then allow us to track the maturation dynamics when the parental epigenetic footprint has changed. This could prove very useful in determining the role of parental chromatin in re-establishing the chromatin state, expanding our understanding of epigenetic inheritance.

Similarly, one key question that follows this work is the reconstitution of chromatin in the leading and lagging strands and the deposition of parental histones.

Previous techniques like eSPAN (enrichment and sequencing of protein-associated nascent strand DNA) have taken advantage of the strand labeling of nascent DNA with nucleoside analogs to probe the enrichment of proteins on either strand of DNA following replication (Yu et al., 2014; Gan et al., 2018; Yu et al., 2018). The principles of our NCOP approach follow the same labeling principles of eSPAN. Thus, in technical terms, it is feasible to study the dynamics of strand specific chromatin maturation at a genome-wide level and base pair resolution. A possible hypothesis is that the chromatin dynamics at the leading and lagging strands follow different kinetics of nucleosome formation. NCOPs would allow us to test this hypothesis through the visualization and quantification of the levels of nucleosome positioning and occupancy in the two daughter strands.

In light of this, my work has the potential to deconstruct the unknown paradigm of parental histone deposition behind the replication fork. Recent studies tracked the parental and new histone deposition on the two daughter strands and found that histone chaperones have a critical role in ensuring symmetric deposition of parental histones behind the fork (Gan et al., 2018; Petryk et al., 2018; Yu et al., 2018). They also provided new evidence suggesting a strand bias of parental histone deposition. In this regard, NCOPs can provide a spatio-temporal view of the chromatin changes that occur in the lack of proper histone recycling, leading to a higher mechanistic understanding of epigenetic inheritance. The NCOP approach can also be paired with ChIP-seq to fully match the structure of chromatin with the occupancy of new and old histones on the two daughter strands.

Yeast is an excellent model system to address these previous questions due to its ease of genetic manipulation and smaller genome, which facilitate data analyses. Given that DNA replication and chromatin assembly are highly conserved processes, these results can be generalized to higher eukaryotes. However, one of the most important contributions of this work is the potential for application in higher

eukaryotes. *Drosophila* is an excellent model system to study the dynamics of chromatin maturation at different developmental stages. While recent work applied a similar technique in *Drosophila* cell lines (Ramachandran and Henikoff, 2016), the NCOP experimental design and bioinformatic approaches can provide additional details masked by this approach, including the precise positioning and occupancy of nucleosomes across regulatory, intergenic, and intragenic regions. In all, the method developed during my thesis work have the potential to increase our knowledge of the maturation dynamics across different genomic locations and chromatin stages, as well as developmentally in cells lines originating from different growth states.

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Biography

Mónica Paola Gutiérrez was born and raised in the city of Manizales in Colombia, and came to the United States after completing high school to pursue a career in science. She obtained an Associate in Arts degree in life sciences from St. Petersburg College in Clearwater, FL. She then completed her undergraduate training in Biology at the University of South Florida in Tampa, where her research focused on mitochondrial dysfunction and Alzheimer's disease using *D. melanogaster* as a model system. At Duke University, Mónica was accepted to the University Program in Genetics and Genomics where she joined the laboratory of David MacAlpine. Mónica's dissertation focuses on the dynamics of chromatin assembly and maturation behind the DNA replication fork.

Publications

1. Gutiérrez MP, MacAlpine HK, MacAlpine DM. Nascent chromatin occupancy profiling reveals locus and factor specific chromatin maturation dynamics behind the DNA replication fork. Manuscript under review in Genome Research. https://www.biorxiv.org/content/biorxiv/early/2018/08/24/398438.full.pdf

2. Mónica P. Gutiérrez and David M. MacAlpine. Chromatin Determinants of Origin Selection and Activation. The Initiation of DNA Replication in Eukaryotes. Ed. Daniel L. Kaplan. Springer, 2016. 87-104. Print.

Grants, Awards, and Honors

1. F31 GM115158 Predoctoral Ruth L. Kirschstein National Research Service Award (NRSA) Fellowship to Promote Diversity in Health-Related Research. National Institute of General Medical Sciences, National Institutes of Health.

Defining chromatin architecture and maturation at sites of DNA replication in the Drosophila melanogaster genome. 4/2015-4/2018.

2. 5T32 GM007754 NIH T32 Training Grant, University Program in Genetics and Genomics Trainee. Duke University. 2012-2014. Durham, NC

3. Summer Leadership Certificate of Excellence. Duke University/ University of North Carolina at Chapel Hill jointly sponsored Summer Leadership Series. Aug 2017. Chapel Hill, NC.

4. IRI Life Sciences, Humboldt-Universitat zu Berlin Travel Award, Jul 2017. Berlin, Germany.

5. Duke University Graduate School Professional Development Grant. 2015 - 2016. Durham, NC

6. The National Institutes of Health, Bridges to the Professoriate NIGMS scholar, 2015.

7. Fitzgerald Poster Award. Department of Pharmacology and Cancer Biology annual retreat, Duke University. Sep 18, 2015. Durham, NC

8. Duke University Graduate School Travel Award. Aug 2015. Durham, NC.

9. Biomedical Graduate Fellowship. Duke University School of Medicine. 2012-2013 & 2013-2014. Durham, NC

10. Deans list of Scholars, University of South Florida. Spring 2011. Tampa, FL

11. Mentor Award, Student Support Services at St. Petersburg College. Apr 2, 2009. St. Petersburg, FL.

12. Certificate of Academic Excellence, Student Support Services at St. Petersburg College. Apr 02, 2009. St. Petersburg, FL.