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CHROMATIN REMODELERS AND THEIR ROLES IN CHROMATIN ORGANIZATION

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"*If we knew what we were doing, it would not be called research, would it?"*

- Albert Einstein (1879-1955)

ABSTRACT

The DNA in the eukaryotic nucleus is organized into a complex DNA-protein structure called chromatin. The basic repeating unit of chromatin is the nucleosome, which consists of 147 bp of DNA wrapped around a histone protein octamer. The nucleosomes form a "beads on a string" structure, which can be folded into higher-order structures that allow an extensive degree of DNA compaction. This compaction is so effective that 2 meters of DNA can fit into the human cell nucleus with a diameter of only 10 μm. Hence, nucleosomes condense and organize the genome, but at the same time they occlude many regulatory elements essential for transcription, replication, repair and recombination. To ensure dynamic access to packaged DNA, cells have evolved a set of proteins called chromatin remodeling complexes, which actively restructure chromatin. These enzymes use the energy from ATP hydrolysis to unwrap, slide, and eject nucleosomes.

This thesis describes the roles of two families of ATP-dependent chromatin remodeling factors in chromatin regulation and organization in the model organism *Schizosaccharomyces pombe* (fission yeast).

We show that the CHD remodeling factor, Hrp1, promotes incorporation of the H3 histone variant CENP-A^{Cnp1} at centromeres and at a set of gene promoters. We suggest that Hrp1 participates in a remodeling process that evicts H3 from promoters, both in euchromatin and centromeric chromatin, which then facilitates CENP-A^{Cnp1} incorporation.

Furthermore, we demonstrate that the Fun30 remodeling factor, Fft3, regulates the chromatin structure over insulator elements and tethers them to the inner nuclear membrane close to nuclear pores. This organizes the chromatin into different domains and ensures correct chromatin structure and gene expression at silent domains.

Additionally, we have generated the first genome-wide map of nucleosome positions in *S. pombe*. This map revealed important differences from the related yeast *Saccharomyces cerevisiae*. The two yeasts showed differences in nucleosome spacing, the roles of DNA sequence features and in the regular nucleosome arrays. This argues against the existence of an evolutionarily conserved genomic code for nucleosome positioning. Instead, species-specific nucleosome positioning factors (e.g. chromatin remodeling complexes) appear to override the biophysical properties of the DNA sequence.

LIST OF PUBLICATIONS

I. Lantermann A, Straub T, **Strålfors A**, Yuan GC, Ekwall K, Korber P. Schizosaccharomyces pombe genome-wide nucleosome mapping reveals positioning mechanisms distinct from those of Saccharomyces cerevisiae. *Nat Struct Mol Biol*. 2010 Feb;17(2):251-7.

II. Choi ES, **Strålfors A**, Castillo AG, Durand-Dubief M, Ekwall K, Allshire RC. Identification of noncoding transcripts from within CENP-A chromatin at fission yeast centromeres. *J Biol Chem*. 2011 Jul 1;286(26):23600-7.

III. **Strålfors A**, Walfridsson J, Bhuiyan H, Ekwall K. The FUN30 chromatin remodeler, Fft3, protects centromeric and subtelomeric domains from euchromatin formation. *PLoS Genet*. 2011 Mar;7(3):e1001334.

IV. **Strålfors A**, Babett Steglich, Olga Khorosjutina & Karl Ekwall. The Fun30 chromatin remodeler Fft3 controls the spatial organization of Pol III genes and subtelomeres in the fission yeast nucleus. *Manuscript in preparation.*

RELATED PUBLICATIONS

Strålfors A, Ekwall K. Heterochromatin and Euchromatin - Organization, Boundaries, and Gene Regulation. In *Epigenetic Regulation and Epigenomics: Advances in Molecular Biology and Medicin*, R. Meyers, ed. (Wiley-VCH Verlag GmbH & Co. KGaA), 2012 pp. 171-187.

Pointner J, Persson J, Prasad P, Norman-Axelsson U, **Strålfors A**, Khorosjutina O, Krietenstein N, Svensson J P, Ekwall K, Korber P. CHD1 remodelers regulate nucleosome spacing *in vitro* and align nucleosomal arrays over gene coding regions in *S. pombe. The EMBO Journal* 2012 Published online: 26 October 2012

Choi ES, **Strålfors A**, Catania S, Castillo AG, Svensson JP, Pidoux AL, Ekwall K, Allshire RC. Factors That Promote H3 Chromatin Integrity during Transcription Prevent Promiscuous Deposition of CENP-A(Cnp1) in Fission Yeast. *PLoS Genet*. 2012 Sep;8(9):e1002985.

Sadeghi L, Bonilla C, **Strålfors A**, Ekwall K, Svensson JP. Podbat: a novel genomic tool reveals Swr1-independent H2A.Z incorporation at gene coding sequences through epigenetic meta-analysis. *PLoS Comput Biol*. 2011 Aug;7(8):e1002163.

Lantermann A, **Strålfors A**, Fagerström-Billai F, Korber P, Ekwall K. Genome-wide mapping of nucleosome positions in Schizosaccharomyces pombe. *Methods*. 2009 Jul;48(3):218-25.

Buchanan L, Durand-Dubief M, Roguev A, Sakalar C, Wilhelm B, **Strålfors A**, Shevchenko A, Aasland R, Shevchenko A, Ekwall K, Francis Stewart A. The Schizosaccharomyces pombe JmjC-protein, Msc1, prevents H2A.Z localization in centromeric and subtelomeric chromatin domains. *PLoS Genet*. 2009 Nov;5(11):e1000726.

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LIST OF ABBREVIATIONS

A	Adenine
Abf1	Autonomously replicating sequence (ARS)-Binding Factor 1
ADP	Adenosine DiPhosphate
Ams2	CENP-A Multicopy Suppressor 2
Arg	Arginine
ATP	Adenosine TriPhosphate
Bbd	Bar-Body Deficient
bp	Base Pair
C	Cytosine
cAMP	Cyclic Adenosine MonoPhosphate
CATD	CENP-A Targeting Domain
CCAN	Constitutive Centromere-Associated Network
cDNA	Complementary DNA
cen	CENtromere
CenH3	Centromeric histone H3
CENP	CENtromere Protein
CHD	Chromatin-Helicase-DNA binding
ChIP	CHromatin ImmunoPrecipitation
Chz1	CHaperone for H2AZ-H2B
Clr6	Cryptic Loci Regulator
Cnp	CeNtromere Protein
cnt	CeNTral core of centromeres
COC	Chromosome-Organizing Clamps
CoIP	CO-ImmunoPrecipitation
CREB	cAMP Response Element-Binding
CREBBP	CREB Binding Protein
Cse	defects in mitotic Chromosome SEgregation
CTCF	CCCTC-binding Factor
DamID	DNA Adenine Methyltransferase IDentification
Dg	Dogentai
Dhp1	DNA strand transferase 2 (Dst2) Homolog in *S. Pombe*
Dis3	Defective In Sister chromatid disjoining
DNA	DeoxyriboNucleic Acid
Dpn1	restriction enzyme from Diplococcus PNeumoniae G41.
EP400	adenovirus early region 1A (E1A) binding 400 kDa protein (p400)
ETC	Extra-TFIIIC
Etll1	Enhancer Trap Locus 1
FACT	Facilitates chromatin transcription
Fft3	Fission yeast Fun Thirty 3
FOA	5-FluoroOrotic Acid
Fun30	Function UNknown 30
G	Guanine
GTF	General -Transcription Factor
GTP	Guanosie TriPhosphate
H2BFWT	H2B histone Family member W Testis-specific

HAT	Histone AcetylTransferase
HDAC	Histone DeACetylase
HDM	Histone DeMethylases
HJURP	Holliday JUnction-Recognizing Protein
HMR	Hidden MAT Right
HMT	Histone MethylTransferases
HP1	Heterochromatin Protein 1
Hrp	Helicase Related Protein
Hs	*Homo sapiens*
Htz1	Histone Two A Z 1
IGB	Integrated Genome Browser
imr	InnerMost Repeats
INM	Inner Nuclear Membrane
Ino80	INOsitol biosynthesis genes 80
ISWI	Imitation SWI
Kb	Kilo Basepair
KisL	KISmet Long
L	Left
LSD	Lysine Specific Demethylase 1
LTR	Long Terminal Repeat
MAT	Mating Type Locus
MBD2	Methyl-CpG Binding Domain protein 2
Mis	Minichromosome InStability
Mit1	Mi2-like protein Interacting with Clr Three 1
MNase	Micrococcal NucleASE
Nap1	Nucleosome Assembly Protein 1
NDR	Nucleosome-Depleted Region
NFR	Nucleosome Free Region
Nup	NUcleoPorin
NuRD	NUcleosome Remodeling and histone Deacetylation
otr	OuTermost Repeats
PARylation	Poly(ADP-ribosyl)ation
PCR	Polymerase Chain Reaction
PHD	Plant Homeo Domain
Pht1	Pombe Histone Two A Z 1
Pol	POLymerase
Psf2	Polyadenylation factor I subunit 2
R	Right
RACE	Rapid Amplification of cDNA Ends
Reb1	RNA polymerase I Enhancer Binding protein
RNA	RiboNucleic Acid
RNAi	RNA Interference
RNAPII	RNA Polymerase II
Rpd3S	Reduced Potassium Dependency 3 Small complex
rRNA	Ribosomal RNA
RSC	Remodel the Structure of Chromatin
RSF	Remodeling and Spacing Facor
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction

Rvb	RuVB-like
Sc	*Saccharomyces cerevisiae*
S. cerevisiae	*Saccharomyces cerevisiae*
Sp	*Schizosaccharomyces pombe*
S. pombe	*Schizosaccharomyces pombe*
Scm3	Suppressor of Chromosome Missegregation
SHREC	Snf2, HDAC-containing REpressor Complex
Sim	Silencing In the Middle of the centromere
siRNA	Short Intefering RNA
SMARCAD1	SWI/SNF-related, Matrix-associated, Actin-dependent Regulator of Chromatin, subfamily A, containing DEAD/H box 1
SNF	Sucrose NonFermenting
SPT16	SuPpressor of Ty
SRCAP	Snf2-Related CREBBP Activator Protein
Ssn6	Suppressor of SNf1 protein
SSRP1	Structure Specific Recognition Protein 1
SUMO	Small Ubiquitin-related MOdifier
Swi	SWItching defective
Swr1	SWI/SNF Related protein
T	Thymine
TAP	Tandem Affinity Purification
TAS	Trancription Analysis Software
tel	TELomere
TFII	Transcription Factor for polymerase II
TFIII	Transcription Factor for polymerase III
TH2B	Testis specific H2B
tRNA	Transfer RNA
TSS	Transcriptional Start Site
TUK	Transcription from Under Kinetochore
Tup	Tymidine Uptake Protein
ura	Uracil

1 INTRODUCTION TO CHROMATIN

Chromosomes each consist of a single, enormously long DNA molecule that must be contained within the very small space of the nucleus. Every human cell, for example, contains about 2 meters of DNA, whereas the diameter of the cell nucleus is only 5 to 20 µm. This creates a serious packaging problem. The solution is provided by small basic proteins, called histones, which fold and pack the fine DNA thread into a more compact structure (Figure 1). By neutralizing the negative charge and wrapping the DNA, the histone complex allows the DNA to be condensed about 10 000-fold. The resultant complex of DNA and proteins is known as chromatin.

Although cells utilize this compaction as a convenient way to store a large amount of DNA, the DNA must still be accessible for critical cellular processes such as transcription, replication, recombination and repair. There must be a dynamic balance between packaging and genome access. Therefore, cells have developed several strategies to control packaging and unpackaging of chromatin. These strategies mainly involve two classes of enzymes: histone modifying enzymes and ATP-dependent chromatin remodelers. The histone modifying enzymes add or remove chemical groups on the histone proteins. These chemical groups can change the physical properties of the histones and thereby influence the chromatin structure or they can provide a binding platform that promotes or hinders the recruitment of regulatory proteins. ATP-dependent chromatin remodelers are enzymes that through ATP hydrolysis unwrap, slide or eject nucleosomes. Together these sets of enzymes facilitate or prevent access for DNA-utilizing proteins.

In this manner, chromatin structure not only provides an excellent packaging solution, but also a sophisticated apparatus for regulating gene expression and all other DNA related processes.

Figure 1. Packaging of DNA into chromatin. DNA is wrapped around histone octamer to form nucleosomes. The nucleosomes are folded into a higher-order structure that condense the DNA 10 000-fold.

2 THE NUCLEOSOME

The basic repeating unit of chromatin is the nucleosome, which consists of 147 bp of DNA wrapped around a histone protein octamer. The octamer contains two copies of each of the four canonical histones (H2A, H2B, H3 and H4) (Luger et al., 1997). The nucleosomes are connected by short stretches of linker DNA, to which in some organisms a fifth type of histone protein, H1, binds. The canonical histones are highly basic proteins that are composed of a globular domain and flexible "tails" that protrude from the nucleosome surface.

The histone proteins are not only packaging proteins that fold the fine DNA thread into a more compact structure, but are also recognized as being the main regulators of chromatin dynamics. Individual nucleosomes can regulate the exposure and occlusion of the DNA. This is achieved by histone variants that can take the place of canonical histones and by post-translational modifications that change the physical properties of the nucleosome or affect the recruitment of regulatory proteins. Moreover, nucleosomes do not bind DNA randomly and the correct and exact positioning of nucleosomes is critical for proper genome functioning. Together these mechanisms create variations in the chromatin polymer that facilitate or prevent access for DNA-utilizing proteins to their substrate. Consequently, nucleosomes regulate all DNA-related processes in the cell.

2.1 HISTONE VARIANTS

In addition to the canonical histones all eukaryotes have histone variants that replace the core histones at certain locations of the chromosomes. The variants have a different amino acid sequence than the core histones and thereby change the physical properties of the nucleosomes when being incorporated into chromain. These unique properties make it possible for the variants to be implicated in highly specific functions, such as chromosome segregation and DNA repair. The incorporation of variants is performed by specific ATP-dependent remodeling complexes and/or specialized histone chaperones.

2.1.1 H2A variants

Histone H2A has the largest number of variants and their incorporation into chromatin is linked to a variety of cellular activities, such as activation of transcription, DNA repair, heterochromatin formation and mammalian X-chromosome inactivation. H2A.Z and H2A.X have been found in most eukaryotic lineages, whereas the macroH2A and H2ABbd (for Bar-body deficient) seem to be vertebrate-specific (Table 1).

Table 1. Histone H3 and H2A variants.

	Name in mammals	Name in yeast	Distribution	Proposed function	Reviewed in
H3	CENP-A (CenH3)	*Sp*: Cnp1 *Sc*: Cse4	Centromeres	Centromere identity, kinetochore assembly	(Quenet and Dalal, 2012)
	H3.3	Canonical histone H3	Active chromatin, telomeres, centromeres	Compensate for evicted nucleosomes after transcription, Epigenetic memory?	(Szenker et al., 2011)
H2A	H2A.Z	*Sp:* Pht1 *Sc:* Htz1	Intergenic regions	Transcription, genome integrity, heterochromatin silencing etc.	(Billon and Cote, 2012)
	H2A.X	Canonical histone H2A	Evenly distributed throughout the genome	DNA repair	(Ismail and Hendzel, 2008)
	macroH2A	-	Inactive X chromosome, silenced genes	Gene silencing, X chromosome inactivation	(Gamble and Kraus, 2010)
	H2ABbd	-	Transcriptionally active regions	Gene activation	(Gonzalez-Romero et al., 2008)

H2A.Z is essential for development and viability in many organisms (e.g. in mice, fly, and frog) (Billon and Cote, 2012). It is not essential in budding and fission yeast, but loss of the protein leads to slow growth and defects in transcription and genome stability. H2A.Z has been reported to have important roles in various processes such as gene transcription, chromosome segregation, DNA repair, heterochromatin silencing and progression through the different phases of the cell cycle (Billon and Cote, 2012).

H2A.Z differs from H2A mainly in the C-terminus, where H2A interacts extensively with the H3−H4 dimer, and in loop 1, where the two H2A (and H2A.Z) molecules contact each other (Malik and Henikoff, 2003). These differences have been shown to modify the properties of the nucleosome, although the outcome is still debated. Some studies observe a fragility of the H2A.Z-containing nucleosome, when compared to the canonical nucleosome, while others observe stabilization. These contradictory results

might be explained by different methodology or, more importantly, by post-translational modifications of H2A.Z (Billon and Cote, 2012).

Like other histone variants, H2A.Z gets incorporated into chromatin by histone chaperones and ATP-dependent chromatin remodeling complexes. The Nap1 chaperone imports the H2A.Z-H2A dimer into the nucleus (Straube et al., 2010). In the nucleus, the Chz1 chaperone presents the variant dimer to the remodeling complex, Swr1, which then incorporates the dimer into chromatin (Kobor et al., 2004; Krogan et al., 2004; Luk et al., 2007; Mizuguchi et al., 2004). Recently, another remodeler has been indicated in H2A.Z regulation. Ino80 has been suggested to perform the opposite reaction to Swr1, by catalyzing the exchange of H2A.Z/H2B dimers for the canonical dimer H2A/H2B (Papamichos-Chronakis et al., 2011).

H2A.Z is found at distinct regions of the chromosomes. In budding yeast, two H2A.Z-containging nucleosomes flank the nucleosome-depleted region of the transcriptional start site (Guillemette and Gaudreau, 2006). A similar organization is found in human cells (Barski et al., 2007). In fission yeast and Drosophila, H2A.Z is found only at the first transcribed nucleosome (the +1 nucleosome) (Buchanan et al., 2009; Mavrich et al., 2008). In yeast, the presence of H2A.Z at gene promoters is generally inversely correlated with transcription, whereas it is positively correlated in human cells and fly (Billon and Cote, 2012). It has been suggested that this difference can be explained by downstream effects of H2A.Z. The variant has been proposed to direct or regulate the position of downstream nucleosomes, and depending on where the nucleosomes are repositioned, positive or negative effects on gene transcription can be observed (Marques et al., 2010).

In addition to its role in transcription, H2A.Z also appears to be involved in defining chromatin boundaries and domains. In budding yeast, H2A.Z is found at heterochromatin boundaries where it blocks the spread of heterochromatin (Meneghini et al., 2003). H2A.Z has also been implicated in centromere function and chromosome segregation in mammals and in budding and fission yeast (Carr et al., 1994). It is localized at centromeres in mammals but not in yeast (Buchanan et al., 2009; Greaves et al., 2007; Zhang et al., 2005).

H2A.X is found in all eukaryotes studied except for nematodes (Malik and Henikoff, 2003). It is characterized by a C-terminal SQE motif. Upon DNA damage, the serine residue of this motif becomes phosphorylated. This phosphorylation is thought to function as a signal that recruits DNA damage response proteins and cell cycle checkpoint factors (Ismail and Hendzel, 2008).

In mammalian cells, H2A.X is evenly distributed throughout the genome and represents 2-25% of the histone H2A pool. In budding and fission yeast, the canonical H2A has the SQE motif and functions as the mammalian H2A.X variant (Ismail and Hendzel, 2008).

2.1.2 H3 variants

Two variants of histone H3 are found in all eukaryotes: H3.3 and CENP-A (Table 1). Additional H3 variants vary among species. The core H3 in yeast is a hybrid between H3.1 (the canonical histone) and H3.3 (Ahmad and Henikoff, 2002).

H3.3 differs from core H3 at only 3 to 4 amino acids in plants and animals. Despite this small sequence difference, it preforms functions separated from H3 and is enriched at specific genomic locations. Three of the differentiated amino acids (located in the α2 helix of the histone fold domain) make it possible for H3.3 to be incorporated into chromatin throughout the cell cycle, whereas core H3 is deposited strictly during S phase (Ahmad and Henikoff, 2002). Moreover, changing these amino acids to the canonical H3 sequence alters its genome-wide enrichment pattern (Goldberg et al., 2010). Thus, the amino acids specific for H3.3 are essential for determining its genomic localization, possibly through specific interactions with distinct assembly machineries.

H3.3 was originally found within actively transcribed regions and therefore it has been thought to have a role linked with gene activity (Szenker et al., 2011). It has been suggested that this role could be to compensate for the eviction of nucleosomes during the progression of the RNA polymerase complex in the gene body, or to function as an epigenetic memory of an activated state. However, H3.3 is not exclusively a marker of transcriptionally active genes and has been found at transcriptionally silent regions such as telomeres and pericentric chromatin (Goldberg et al., 2010). The role of H3.3 at these regions is unknown.

CENP-A is the most divergent of the H3 variants with only 60% identity to H3.1 at its histone fold domain, and even less similarity in its N-terminal tail (Hamiche and Shuaib, 2012). It is found at active centromeres where it is indispensible for centromere function and chromosome segregation (Takahashi et al., 2000). The variant appears to function as both an epigenetic mark for centromere identity and as a structural foundation for the assembly of the kinetochore -the multi-protein complex that forms the attachment site for spindle microtubule during cell division (Figure 2).

The overall protein structure of CENP-A is very similar to histone H3, although there are some features that differ between the two histones. For example, in alpha helix 2 of the histone fold domain, CENP-A has a unique domain called CENP-A targeting domain (CATD). This domain is sufficient for centromere localization and may confer a unique rigidity to the CENP-A nucleosome (Black et al., 2004; Black et al., 2007).

The composition and structure of the CENP-A nucleosome is heavily debated (reviewed in (Black and Cleveland, 2011). Different models that have been proposed and they include: 1) a classical octameric CENP-A nucleosome with two copies of each histone, 2) a tetrasome lacking H2A:H2B dimers, 3) a hemisome with one copy of each histone, and 4) a hexasome or trisome where Scm3 replaces H2A/H2B. Which one of these structures that is the correct one or if they all represent versions of the CENP-A nucleosome in different phases of cell cycle remains to be determined.

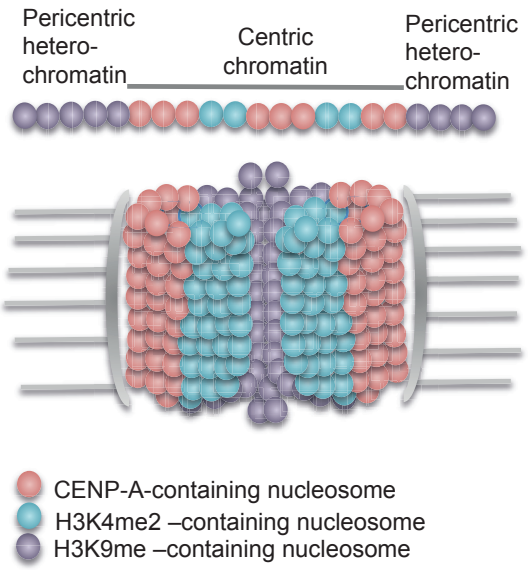


Figure 2. Schematic representation of a human centromere. Top: On the linear two-dimensional chromatin fiber, centric chromatin contains domains of CENP-A-containing nucleosomes (pink) that are interspersed with H3 nucleosomes methylated on lysine 4 (H3K4me2) (blue). Pericentric heterochromatin with H3K9me (purple) flanks the centric chromatin.

Bottom: Model of 3D structure of mitotic centromeres. CENP-A nucleosomes are presented on the chromosome surface, allowing for kinetochore assembly and association with spindle microtubules (grey lines).

As CENP-A is a fundamental determinant of centromere identity, many studies have focused on the mechanisms that specifically recruit and load CENP-A onto centromeric chromatin. The DNA sequence itself is neither necessary nor sufficient for CENP-A recruitment, instead it appears to rely on chromatin features. Principally, the CENP-A deposition pathway can be broken down into three steps that involve distinct protein complexes: priming, deposition, and stabilization/maintenance (Boyarchuk et al., 2011). Different factors involved in each step are described in Table 2.

Priming. During priming, specific factors, including the Mis18 complex and the RbAp46/48 chaperones, are recruited to the centromeres where they provide a "competent" chromatin state that prepares the centromeres for the loading of new CENP-A (Fujita et al., 2007; Hayashi et al., 2004). This priming appears to involve changing the histone acetylation status.

Deposition. CENP-A deposition to centromeric chromatin is governed by the histone chaperone HJURP (*sp*Scm3) (Dunleavy et al., 2009; Foltz et al., 2009). HJURP associates directly with CENP-A and centromeric CENP-A levels are reduced after depleting HJURP by siRNA. However, HJURP requires the priming activity of Mis18 to incorporate CENP-A.

Another factor that is important for the deposition of CENP-A is the constitutive centromere-associated network (CCAN). It is composed of 16 proteins that colocalize with CENP-A throughout the cell cycle (Cheeseman and Desai, 2008).

Table 2. Factors required for CENP-A incorporation

Function during CENP-A incorporation	Factor	Name in *S. pombe*	Properties	Ref
Priming/ licensing	RbAp46/48	Mis16	Histone chaperone. Part of a HAT complex	(Fujita et al., 2007; Hayashi et al., 2004)
	Mis18α/β	Mis18	Interacts with Mis16	(Fujita et al., 2007) (Hayashi et al., 2004)
Deposition	HJURP	Scm3	Histone chaperone	(Camahort et al., 2007; Dunleavy et al., 2009; Foltz et al., 2009; Mizuguchi et al., 2007; Stoler et al., 2007)
	CCAN (CENP-C, CENP-H/I/K, CENP-L/M/N, CENP-O/P/Q/R/U, CENP-T/W, CENP-S/X)	Mis6 $^{CENP-I}$ Mis15^{CENP-N} Mis17^{CENP-M} Sim4^{CENP-H}	Multi-protein complex required for kinetochore assembly.	(Hayashi et al., 2004; Pidoux et al., 2003; Takahashi et al., 2000)
	FACT complex (SSRP1 & SPT16)	Pob3 & Spt16	Histone chaperone	(Okada et al., 2009)
	CHD1	Hrp1	ATP-dependent chromatin remodelling	(Choi et al., 2011; Okada et al., 2009; Walfridsson et al., 2005)
		Ams2	GATA-like transcription factor	(Chen et al., 2003)
Stabilization/ Maintenance	RSF complex (Rsf-1, SNF2h)		Chromatin remodelling	(Perpelescu et al., 2009)
	MgcRacGAP		Rho family GTPase activating protein	(Lagana et al., 2010)

Other factors required for CENP-A deposition, e.g. the FACT complex (Okada et al., 2009), the CHD1 remodeling complex (Walfridsson et al., 2005), and the GATA-like transcription factor (Chen et al., 2003), are tightly linked to transcription and, interestingly, transcripts have recently been identified from CENP-A chromatin (Choi et al., 2011; Topp et al., 2004). These findings have led to the hypothesis that transcription through centromeric chromatin evicts histone H3, which then facilitates CENP-A incorporation (Choi et al., 2011). The link between transcription and CENP-A incorporation has been studied in paper II of this thesis.

Stabilization/maintenance. A growing amount of evidence supports the idea that CENP-A nucleosomes are not fully stable after their initial deposition. The ATP-dependent nucleosome remodeling and spacing factor (RSF) complex is recruited to centromeres after deposition of new CENP-A and seems to assemble the predeposited CENP-A into a mature, stable chromatin state (Perpelescu et al., 2009). The CENP-A chromatin is then further stabilized by a Rho family small GTPase molecular switch, activated by MgcRacGAP (Lagana et al., 2010).

In addition to centromeric regions, a low level of CENP-A associates with gene promoters (Choi et al., 2011; Lefrancois et al., 2009). In *S. pombe,* the recruitment of CENP-A to these promoters appears to be dependent on the remodeler Hrp1 (Choi et al., 2011). However it is not known if these promoters share any structural or mechanistic feature with the centromere. This was studied in paper II of this thesis.

2.1.3 H4 and H2B histone variants

Unlike the H3 and H2A histones, no ubiquitously expressed H4 or H2B variant has been reported so far. However, a few tissue-specific H2B variants have been described. These include for example a sperm specific variant (spH2B) and testis specific variants (TH2B and H2BFWT) (Yuan and Zhu, 2012).

The reason for the nonexistence of H4 variants and the very small number of H2B variants can probably be explained by the arrangement of the histones in the nucleosome core particle. The interaction between the H3-H4 tetramer and the H2A-H2B dimer is established through contacts made by H2B with H4. Therefore H2B may not be preferred for variation (Pusarla and Bhargava, 2005). Histone H4 makes extensive contacts with all of the other histones and therefore sequence variations are constrained; only some positions in the $\alpha 2$ helix appear to tolerate any amino acid substitutions (Malik and Henikoff, 2003).

2.2 HISTONE MODIFICATIONS

Histones are extensively modified post-translationally and over one hundred distinct modifications are described in the literature (Rando, 2012). These modifications include small chemical groups (e.g. acetylation, phosphorylation and methylation) and larger peptides (e.g. ubiquitylation and sumoylation). Most of them occur in the N-terminal tails of the histones, which protrude from the nucleosomes, but also within the globular core region. Together, the modifications provide an important regulatory platform for processes such as gene expression, DNA replication and repair, chromosome condensation and segregation as well as apoptosis (Fullgrabe et al., 2011).

The large quantity of possible modifications have led to the hypothesis that specific combinations of histone marks would specify unique biological outcomes, the so called histone code hypothesis (Strahl and Allis, 2000). Genome-wide analysis from different organisms show that many histone modifications co-occur. For example, actively transcribed promoters in all studied organisms are associated with a range of histone acetylation marks. However, today there is little evidence to support the hypotheses that these combinations of marks lead to unique outcomes (Rando, 2012).

For each post-translational histone modification, enzymes exist that can either add or remove the mark. Major factors in this include histone acetyltransferases (HATs) which acetylate histones and histone deacetylases (HDACs) which remove acetyl groups. Histone methyltransferases (HMTs) and histone demethylases (HDMs) add and remove methyl groups respectively, whereas kinases and phosphatases regulate the phosphorylation of histones (Bannister and Kouzarides, 2011).

It is thought that the covalent modifications exert their effects via two main mechanisms. The modification could either directly influence the chromatin structure or form a dynamic binding platform that promotes, or hinders, recruitment of chromatin-modifying enzymes (Bannister and Kouzarides, 2011).

Histones are highly basic proteins with numerous positively charged residues (lysines and arginines). This gives the proteins a high affinity for the negatively charged DNA. Histone acetylation and phosphorylation reduce this positive charge and this probably weakens the histone-DNA contact, creating a more open chromatin with increased access to DNA (Bannister and Kouzarides, 2011). In agreement with this, histone acetylation is often found at chromatin regions that are undergoing active processing, such as gene transcription and DNA replication. Ubiquitylation adds a very large molecule to the histone protein and therefore it is likely that this modification induces a change in the nucleosome conformation and the chromatin structure. In contrast, small neutral modifications, e.g. phosphorylation, are not thought to change the chromatin structure but appear to act by regulating the binding of chromatin-associated proteins.

Many chromatin-regulating proteins carry domains that bind specifically to a type of covalent modification. For example, bromodomains bind to acetylated lysines, while PHD fingers and chromodomains bind to methylated residues (Bannister and Kouzarides, 2011). The interaction between the modification and the protein domain

has been suggested to direct the recruitment of the protein to certain chromatin locations (e.g. HP1 is recruited to heterochromatic regions via H3K9me3).
While this is true in many cases, an emerging number of studies show that the domains might play a role in regulating the complex's behavior rather than its recruitment (Rando, 2012). One example of this is the Rpd3S complex (an HDAC complex) in *S. cerevisiae*. This complex carries a chromodomain protein that recognizes H3K36me3, which is present over coding regions of genes. This led to the speculation that the methylation mark recruits the complex to the body of transcribed genes (Carrozza et al., 2005). However, deletion of the chromodomain does not change the location of the Rpd3S complex (Drouin et al., 2010). Instead the function of the complex is lost as acetylation levels increase over genes (Li et al., 2007). Thus, it appears as if the H3K36me3 serves to activate the Rpd3S complex, rather than recruit it. Another example is the Drosophila chromatin remodeler Chd1, in which tandem chromodomain regulates its function but not its genomic location (Morettini et al., 2011).

Histone modifications can also function to inhibit the binding of chromatin-associated factors to chromatin. For instance, H3K4me3 prevents the NuRD complex from binding histones (Zegerman et al., 2002).

2.3 NUCLEOSOME POSITIONING

It is not only the composition or modifications of the nucleosomes that affect nuclear processes, but also the exact position of nucleosome on the DNA. Depending on whether a particular DNA sequence is wrapped around a histone octamer or if it is located in a linker region between two nucleosomes, it will be differently accessible for DNA binding proteins. Nucleosomes can have both activating and inhibitory roles. Nucleosomes positioned in a silent promoter can inhibit transcription by blocking the binding of basal transcription factors to the DNA. In contrast, nucleosomes can also bring distant DNA sequences into close proximity and thereby promote transcription. Thus, information about nucleosome positions and about the mechanisms determining the positioning is necessary to understand all DNA-related processes (reviewed in (Iyer, 2012)).

Genome-wide maps of nucleosome positions have been generated for several organisms and they all revealed a high degree of well-defined nucleosome positions (reviewed in (Iyer, 2012)). Particularly at gene starts there is often a conserved stereotypic organization with a broad (100-200 bp) nucleosome depleted region (NDR) just upstream of the transcription start site (Figure 3). The first nucleosome downstream of the TSS (the +1 nucleosome) usually occupies a highly distinct position. In yeast, the +1 nucleosome is typically followed by an array of regularly spaced nucleosomes extending into the gene body. Metazoans and plants appear to lack this array of positioned nucleosomes, but they still have a highly positioned +1 nucleosome downstream the NDR. In paper I of this thesis, we generated the first genome-wide nucleosome positioning map for *S. pombe*.

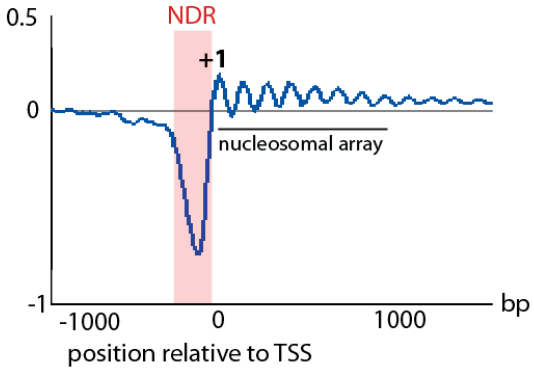


Figure 3. Nucleosome positions at the beginning of genes. Average enrichment of nucleosomes over all *S. pombe* genes. Genes were aligned at transcriptional start site (TSS) using Podbat (Sadeghi et al., 2011). Data is from (Lantermann et al., 2010).

Genes display a nucleosome depleted region (NDR) before TSS, a well-positioned +1 nucleosome, and a array of regularly spaced nucleosomes extending into the gene body.

The mechanisms that determine the positioning of nucleosomes are still poorly understood. Most likely a combination of different mechanisms –including intrinsic sequence biases; stacking against a fixed barrier; DNA-binding proteins and chromatin remodelers– function together to position nucleosomes.

2.3.1 The role of the DNA sequence

Nucleosomes can form on virtually any DNA sequence, but it is clear that some DNA sequences are more readily bound to the histone octamer than others. Sequences that bend more easily have a high affinity for histones, whereas stiff DNA sequences are poorly incorporated into nucleosomes. The existence of sequence preferences led to the idea that the *in vivo* nucleosome positions are intrinsically encoded in the DNA sequence. In agreement with this, a computer model trained on yeast DNA could predict the *in vivo* occupancy of nucleosomes fairly accurately in *S. cerevisiae* (75%) and to a lesser degree in *C. elegans* (60%), indicating that DNA sequence plays an important, conserved, role in determine the nucleosome positions (Kaplan et al., 2009). However, it is important to note that this model predicts the occupancy of nucleosomes (i.e. the probability of a given base pair to reside in any nucleosome) and not the precise position of the nucleosomes. When nucleosome position was considered, only 15-20% of the positions could be correctly predicted (Iyer, 2012). Thus, DNA sequences preference can explain quite accurately the nucleosome occupancy, but only few proper nucleosome positions and hardly any regular arrays. In paper I of this thesis we investigate the role the DNA sequence plays in nucleosome positioning.

2.3.2 Formation of the NDR

All organisms studied so far display a nucleosome-depleted region upstream of the TSS of highly expressed genes. It appears as if this NDR, at least partly, is encoded in the DNA sequence (Kaplan et al., 2009). In *S. cerevisiae* NDRs are enriched in poly(dA-dT) sequences (Field et al., 2008). This sequence strongly disfavors bending of DNA and thus prevents nucleosome formation. However, the NDRs are smaller in *in vitro* maps, in which nucleosomes are allowed to form on naked DNA and the positioning is therefore governed only by the intrinsic sequence preferences, than in *in vivo* maps.

This strongly indicates that other mechanisms than the DNA sequence also contribute to the NDR formation (Kaplan et al., 2009). Moreover, not all organisms have poly(dA-dT) sequences enriched in their NDRs (Lantermann et al., 2010).

One suggested mechanism is DNA-binding proteins (e.g. transcription factors), which, when binding to DNA, may inhibit the histone octamer, preventing nucleosome formation. In agreement with this, there is evidence that the budding yeast Reb1 and Abf1 and mammalian CTCF contribute to NDR formation (Fu et al., 2008; Jansen and Verstrepen, 2011; Kaplan et al., 2009). Moreover, the chromatin remodeling complex RSC has a role in NDR generation (Hartley and Madhani, 2009; Parnell et al., 2008). These factors are also thought to position nucleosomes surrounding their binding sites.

2.3.3 Formation of nucleosomal arrays

A striking difference between *in vitro* and *in vivo* nucleosomal maps is the absence of the regularly spaced array of nucleosomes following the +1 nucleosome in the *in vitro* maps. The position of the nucleosomes in the array seems therefore not to be encoded in the DNA sequence. One explanation for the array formation has instead been the so-called barrier model or statistical positioning. This model is based on the fact that nucleosomes are located at fixed distances from each other, and therefore if one nucleosome (e.g. the +1 nucleosome) is highly positioned, it would also determine the positions of the adjacent nucleosomes. The one positioned nucleosome functions as a barrier against which the rest of the nucleosomes are passively stacked (Iyer, 2012). However, this model has been disputed and correct positioning of the array seems to depend on ATP (Korber 2012). Therefore an active packing mechanism, where ATP-dependent chromatin remodelers align the nucleosomes against the barrier has been proposed. In agreement with this, it has been shown in *S. cerevisiae* that the remodelers ISWI, ISW2 and CHD1 can position nucleosomes. Deletion of the individual remodeler genes does not drastically affect the overall nucleosome positioning, but deletion of all three together resulted in a significant reduction in the regular array of nucleosomes downstream of the TSS (Gkikopoulos et al., 2011). Interestingly, the remodelers did not affect the NDR or the +1 nucleosome. This function seems to be conserved since deletion of the *S. pombe* CHD-remodelers, Hrp1 and Hrp3, give similar results (Pointner et al., 2012).

3 TRANSCRIPTION

Transcription is a process that copies the genetic information from DNA into RNA. The process is mediated by RNA polymerases that read the DNA sequence and synthesize a complementary RNA strand. Three types of RNA polymerases exist in all eukaryotes: Pol I, Pol II and Pol III. The three polymerases transcribe different substrates. Pol I transcribes rRNA genes, which encode the RNA components of the ribosomes. Pol II transcribes all protein coding genes as well as several small nuclear RNAs. Pol III transcribes 5S rRNA, tRNA, and other small RNAs.

Transcription by Pol II and Pol III will be described in the sections that follow.

3.1 POL II TRANSCRIPTION

The transcription cycle of a Pol II gene starts with Pol II binding to the promoter – a DNA sequence located upstream of the coding region. However, Pol II cannot bind to the promoter and initiate transcription without the aid of general transcription factors (GTFs). The GTFs help to position Pol II correctly at the promoter, aid in pulling apart the DNA strands to allow the transcription to begin, and release Pol II from the promoter into elongation. Once elongating, Pol II requires a series of elongation factors which help the polymerase to move through the gene and decrease the likelihood of the polymerase dissociating before it reaches the end. During the elongation, the new RNA molecule is modified by the addition of a cap (a methylated guanine nucleotide) at its 5' end, and introns are removed by splicing. When the polymerase reaches the end of the gene, the RNA is cleaved and 3' polyadenylated.

Transcription needs to be highly regulated in order for the cell to respond to developmental and environmental signals. The most common point of gene regulation is to control transcriptional initiation. This is achieved by a wide range of gene- and tissue-specific transcription factors which bind specific regulatory elements within the core promoter or at more distant regions (reviewed in (Thomas and Chiang, 2006)). If these distant elements have a positive effect on gene expression, they are commonly referred to as enhancers (in humans) or upstream activating sequences (in yeast). Cofactors are often required to transmit the signal between the gene-specific transcription factors and the general transcription machinery.

3.2 POL III TRANSCRIPTION

Genes transcribed by Pol III can be divided into three types (type 1, 2, and 3) depending on their promoter type and the transcription factors that are required for their transcription (reviewed in (Huang and Maraia, 2001)). Type 1 genes include the 5S rRNA genes. These genes contain a C-box to which the transcription factor TFIIIA binds. TFIIIA binding allows the recruitment of another transcription factor TFIIIC, which then directs a third transcription factor TFIIIB to bind upstream of the transcription start site (TTS). Once this complex is assembled, Pol III is recruited and transcription is initiated. Type 2 genes –including tRNA genes– do not have C-box sequences and do not require TFIIIA. Instead, tRNA genes have internal promoters

consisting of two highly conserved sequence elements called A- and B-box. These sequences recruit TFIIIC, which then recruit TFIIIB and Pol III. Type 3 genes are present only in higher eukaryotes and they utilize an upstream TATA element that recruits a specific TFIIIB variant.

3.3 CHROMATIN AND TRANSCRIPTION

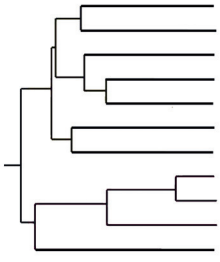
Nucleosomes can conceal regulatory elements on the DNA and prevent binding of regulatory proteins. For example, the general transcription factors seem unable to assemble onto a promoter that is packaged in a conventional nucleosome. There exist two main mechanisms that can regulate the repressive effect of chromatin structure. The first mechanism involves ATP-dependent chromatin remodeling factors that use the energy from hydrolysis of ATP to alter the contacts between DNA and histones. They can reveal DNA regulatory elements by removing or sliding nucleosomes. They can also exchange the canonical histones for histone variants that might aid or hinder transcription. Chapter 4 describes the ATP-dependent remodeling complexes in more detail.

The second mechanism involves posttranslational modifications of histone proteins (see section 2.2). Many modifications, and in particular histone acetylation, is thought to loosen the interaction between histones and DNA, creating a more open chromatin structure that increases the efficiency of transcriptional initiation and elongation. Histone modifications can also affect the recruitment of regulatory proteins that interact with the transcription machinery. The enzymes that regulate histone modifications (e.g. HATs and HDACs) are together with ATP-dependent remodeling factors considered as transcriptional cofactors.

4 ATP-DEPENDENT CHROMATIN REMODELING FACTORS

To enable dynamic access to packaged DNA, cells have evolved a set a specialized protein complexes, called chromatin remodeling complexes. These enzymes use the energy of ATP hydrolysis to move, destabilize, eject or restructure nucleosomes. The remodelers are involved in a wide variety of genomic processes. For example, they have important roles in transcriptional regulation, where they by remodeling nucleosomes can expose or cover DNA sequences for binding of transcriptional activators or repressors. Moreover, several remodelers are recruited to sites of DNA damage, where they assist the many steps of DNA repair and recombination. Remodelers are also important for many steps in DNA replication (Clapier and Cairns, 2009).

Since chromatin remodelers are involved in most chromosomal processes, it is not surprising that a very large number of different chromatin remodeling complexes exist. The catalytic subunits (the remodeler) all contain a similar helicase-like ATPase domain, but they differ in unique domains within or adjacent to the ATPase domain and in their associated subunits. Based on these features, the remodelers can be divided into four families: SWI/SNF, ISWI, CHD, and INO80 (Figure 4) (Clapier and Cairns, 2009). The families are conserved from yeast to human, although there are some variations between species.



family	subfamily	name in *S.pombe*	name in *S.cerevisiae*	name in human
ISWI	ALC1	-	-	CHD1L
	Iswi	-	isw1, isw2	SMARCA1/hSNF2L SMARCA5/hSNF2H
Mi2-CHD	CHD7	-	-	CHD6, CHD,7 CHD8, CHD9
	Chd1	hrp1, hrp3	Chd1	CHD1 CHD2
	Mi-2	Mit1	-	CHD5, CHD4/mi-2β, CHD3/mi-2α
SWI/SNF	Lsh	-	IRC5	HELLS
	Snf2	snf21, snf22	snf2, sth1	SMARCA2/BRM, SMARCA4/BRG1
INO80	EP400	-	-	EP400
	SWR1	Swr1	Swr1	SRCAP
	INO80	Ino80	Ino80	INO80
	Etl1	Fft1, Fft2. Fft3	Fun30	SMARCAD1

Figure 4. Families of ATP-dependent chromatin remodelers. Right: A tree showing the sequence homology between different families of remodelers. Data is from (Flaus et al., 2006). Left: A table showing the different families and subfamilies of chromatin remodelers.

4.1 MI2-CHD FAMILY

The Mi2-CHD family of remodelers is characterized by two tandem chromodomains in the N terminus of the catalytic subunit. Chromodomains are known for recognizing and binding methylated lysine residues, and accordingly human CHD1 binds H3K4me2 and me3 (Flanagan et al., 2005; Sims et al., 2005). The presence of additional domains has been used to divide the group into three subfamilies (Hargreaves and Crabtree, 2011). The first subfamily, Chd1, contains remodelers with a C-terminal DNA-binding domain that binds to AT-rich DNA. Members of the second subfamily, Mi-2, lacks the DNA-binding domain, but have instead two PHD domains. The enzymes of the third subfamily, CHD7, possess additional domains that differ between members. The different Chd remodelers do not only differ in their functional motif but also in their biochemical properties. Some of the remodelers exist as monomers whereas others function in large multiprotein complexes. Moreover, one remodeler can exist in several different protein complexes (Murawska and Brehm, 2011).

4.1.1 Recruitment of CHD remodelers

Many CHD remodelers lack a DNA binding domain and therefore need to be recruited to chromatin via chromatin-associated proteins, e.g. histones and transcription factors (reviewed in (Murawska and Brehm, 2011)). The recruitment is regulated by modifications (methylation, SUMOylation or PARylation) of these factors.

Human CHD1 interacts with methylated histones, in particular H3K4me. This histone modification is abundant at the 5' region of actively transcribed genes and concordantly CHD1 is enriched at promoters of highly expressed genes (Flanagan et al., 2005; Sims et al., 2005).

The NuRD complex, which contains CHD3/4 (mi2-α/mi2-β) and HDAC1/2, is involved in transcriptional repression and is recruited to the promoters of its target genes via interaction with a myriad of transcription factors and co-regulators (Murawska and Brehm, 2011). However, recent evidence suggests that Mi-2 remodelers bind SUMOylated residues of proteins. It is therefore possible that Mi-2 containing complexes are recruited by SUMOylated transcription factors. This would explain how Mi-2 can interact with so many unrelated transcription factors (Murawska and Brehm, 2011).

Moreover, CHD remodeling complexes can be recruited via methylation of DNA. NuRD contains a methyl-CpG-binding subunit, MBD2, which can interact with methylated DNA and tethers NuRD to methylated promoters (Feng and Zhang, 2001; Zhang et al., 1999).

4.1.2 CHD remodelers and their roles in transcription

Most of the CHD remodelers have been connected to transcriptional regulation. However, different remodelers act at separate phases of the transcription cycle. Some have a role in the initiation of transcription, whereas others in the elongation or

termination. Some also participate in RNA processing events, such as pre-mRNA splicing and 3' end formation (Murawska and Brehm, 2011).

Roles in repression of transcription:
One CHD remodeling complex, the NuRD complex, has a role in transcriptional repression. This complex contains both a remodeler (CHD3/4) and an HDAC (HDAC1/2) and it is thought that the nucleosome remodeling combined with histone deacetylation generate a compact, hypoacetylated chromatin that is unfavorable for transcription (Denslow and Wade, 2007). The *S. pombe* equivalent of the NuRD complex, SHREC, is also involved in transcriptional repression. It is localized to heterochromatic regions where it is critical for transcriptional gene silencing (Sugiyama et al., 2007).

Roles in transcriptional initiation:
The *S. pombe* CHD1 homologs, Hrp1 and Hrp3, localize to a subset of gene promoters where they remove nucleosomes near the transcriptional start site (Walfridsson et al., 2007). Other CHD remodelers function at enhancer elements. For example, CHD7 binds to enhancers and this appears to activate the transcription of the genes (Schnetz et al., 2009). CHD8 and CHD4 also play a role at enhancers (Murawska and Brehm, 2011). Thus, CHD remodelers seem to create nucleosome-depleted regions at both promoter and enhancer regions. This nucleosome removal is thought to facilitate the binding of transcription factors, co-activators and the RNA pol II machinery and thereby stimulates transcriptional initiation.

Roles in transcriptional elongation:
The chromatin structure is actively modulated during transcriptional elongation. Nucleosomes ahead of elongating Pol II are temporarily disassembled and then reassemble when Pol II has passed through. Two CHD remodelers have been implicated in this process: KisL and CHD1 (Murawska and Brehm, 2011).
Drosophila and yeast CHD1 associate with actively transcribed genes where they are thought to deposit new nucleosomes after the elongating Pol II (Murawska and Brehm, 2011). Budding yeast Chd1 interacts with elongation factors but does not appear to directly affect the elongating Pol II (Murawska and Brehm, 2011). Instead it is thought to prevent transcription initiation from cryptic promoters inside the gene bodies by maintaining an appropriate nucleosome positioning over the transcribed region (Gkikopoulos et al., 2011; Quan and Hartzog, 2010). In agreement with this, three recent studies from *S. pombe* showed that Hrp1 and Hrp3 are involved in preventing cryptic antisense transcription by regulating nucleosome positioning over coding regions (Hennig et al., 2012; Pointner et al., 2012; Shim et al., 2012).

4.1.3 CHD remodelers and histone variant deposition

Chd1 is providing proper chromatin structure at several loci by promoting deposition of histone H3 variants. In Drosophila, Chd1 puts in the H3.3 variant instead of core H3 after the elongating RNA pol II machinery (Konev et al., 2007). Chicken and fission yeast Chd1 is involved in the deposition of the centromeric H3 variant, CENP-A, both at centromeres and at certain gene promoters (Choi et al., 2011; Okada et al., 2009;

Walfridsson et al., 2005). The incorporation of both variants, H3.3 and CENP-A, appears to be coupled to transcription and it is thought that eviction of H3 during the transcription facilitates the incorporation of the variants (Choi et al., 2011). This process was studied in paper II of this thesis.

4.1.4 CHD remodelers and nucleosome positioning

CHD remodelers also have important roles in positioning nucleosomes. In yeast, most genes have stereotypic organization with a nucleosome free region just upstream of the transcription start site and a well-positioned +1 nucleosome followed by an array of regularly spaced nucleosomes extending into the gene body (see Figure 3 and section 2.3). It has been shown in *S. cerevisiae* that if you delete Chd1 together with the remodelers Isw1 and Isw2, the array of positioned nucleosomes after the +1 nucleosome is lost (Gkikopoulos et al., 2011). Similar results were obtained in *S. pombe* when Hrp1 and Hrp3 were deleted (Pointner et al., 2012). In paper I of this thesis we investigated the role of another CHD-remodeler Mit1 in nucleosome positioning.

4.2 INO80 FAMILY

The INO80 family of remodelers is the evolutionary most conserved family of remodeling complexes and appear to have crucial roles in nearly all DNA metabolic process, including gene transcription, DNA repair, and DNA replication. The family consists of three classes of proteins: Ino80, Swr1 (SRCAP and EP400 in human), and Etl1/Fun30.

The defining characteristic of the Ino80 family is the presence of a split ATPase domain, which contains a large insertion between motifs III and IV (Watanabe and Peterson, 2010). The length of this insertion varies between species, being small in yeast (247-282 amino acids) and large in mammals (>1000 amino acids). This unique domain retains ATPase activity and functions as a scaffold for the association of two RuvB-like proteins, Rvb1 and Rvb2.

The split ATPase domain seems to give the Ino80 group of remodeler a unique ability to catalyze ATP-dependent histone dimer exchange reactions, which gives them the ability incorporate of histone variants. Swr1 specifically removes one or both H2A/H2B dimers from the nucleosome and replaces them with H2A.Z/H2B dimer(s) in ATP-dependent reaction (see section 2.1.1). Ino80 has been suggested to perform the opposite reaction, i.e. exchanging a H2A.Z/H2B dimer for a H2A/H2B (Papamichos-Chronakis et al., 2011). Together these enzymes regulate the chromosomal location of the H2A.Z variant.

4.2.1 ETL1/Fun30 remodelers

Etl1 is one of the first remodeling enzymes identified in mammals (Soininen et al., 1992), yet it has received little attention and is poorly characterized. Only in very recently years have we started to understand its important roles in chromatin regulation.

The family is conserved from yeast to human, and includes Fun30 in *S. cerevisiae*; Fft1, Fft2 and Fft3 in *S. pombe*; Etl1 in mouse; and SMARCAD1 in human (see Figure 4 and Table 3) (Adra et al., 2000; Barton and Kaback, 1994; Clark et al., 1992; Neves-Costa et al., 2009; Soininen et al., 1992). It is expressed throughout development and in virtually all adult tissues (Schoor et al., 1999; Soininen et al., 1992). The protein is nonessential in all organisms examined but its deletion or overexpression causes severe defects in chromosome stability, integrity and segregation (Ouspenski et al., 1999; Rowbotham et al., 2011; Stralfors et al., 2011). Furthermore removal of Etl1 causes grave developmental defects in mouse (Schoor et al., 1999), and the human homolog, SMARCAD1, has been mapped to a chromosomal region (4q22) that is involved in several human diseases, such as head and neck cancer, liver cancer and immigration delay disease (absence of fingerprints) (Bluteau et al., 2002; Cetin et al., 2008; Nousbeck et al., 2011). SMARCAD1 has also been identified as a regulator of pluripotency and self-renewal and cells depleted of the remodeler lose the ability to maintain their stemness state (Hong et al., 2009; Lim et al., 2006; Seki et al., 2010).

Table 3. Identity between Fun30-remodelers sequences. Percent identity was calculated using NCBI BLASTp.

	*sp*Fft3	*sp*Fft2	*sp*Fft1	*sc*Fun30	*hs*SMARCAD1
***sp*Fft3**	100%	53%	47%	52%	40%
***sp*Fft2**	53%	100%	53%	60%	42%
***sp*Fft1**	47%	53%	100%	52%	60%
***sc*Fun30**	52%	60%	52%	100%	53%
***hs*SMARCAD1**	40%	42%	60%	53%	100%

Several recent reports have highlighted the importance of the Etl1/Fun30 family in chromatin regulation and the maintenance of functional chromatin domains. Depletion of the remodeler generates an open chromatin structure at regions that normally are transcriptionally silent. This function appears to be conserved between human, *S. cerevisiae* and *S. pombe* (Durand-Dubief et al., 2012; Neves-Costa et al., 2009; Rowbotham et al., 2011; Stralfors et al., 2011; Yu et al., 2011). The protein is enriched at boundaries between chromatin domains, and in its absence normally silent regions display increased levels of histone acetylation, an altered nucleosome positioning and decreased levels of histone methylation and heterochromatic proteins (e.g. HP-1). Importantly, the silent regions also become transcriptionally active and genes in these regions start to be transcribed (Durand-Dubief et al., 2012; Stralfors et al., 2011). SMARCAD1 directly interacts with the histone deacetylases HDAC1/2 and it has been suggested that SMARCAD1, through this interaction, controls removal of histone acetylation marks, the first step in formation of repressive chromatin (Rowbotham et al., 2011). Together, these results strongly suggest that the Etl1/Fun30 remodeler functions as key factors for the re-establishment and maintenance of repressive chromatin (Rowbotham et al., 2011; Yu et al., 2011).

Three recent studies show that Fun30 and SMARCAD1 have important roles in double-strand break repair by promoting DNA end-resection (Chen et al., 2012;

Costelloe et al., 2012; Eapen et al., 2012). Whether this function is conserved in *S. pombe* needs to be determined.

In vitro, biochemical experiments demonstrated that budding yeast Fun30, like other chromatin remodelers, is capable of binding nucleosomes and DNA, hydrolyzing ATP and disrupting nucleosomes in an ATP-dependent manner (Awad et al., 2010). Moreover, Fun30 was shown to be better in catalyzing histone dimer exchange in comparison to nucleosome sliding. This is similar to the two other proteins in the Ino80 family, Ino80 and Swr1, which both have been implicated in histone dimer exchange. Together, these results suggest that Fun30/Etl1 functions as a bona fine chromatin remodeling factor and in agreement with this, in vivo experiments where the ATPase domain of the protein has been destroyed by a point-mutation show the same phenotype as when the full protein has been depleted (Neves-Costa et al., 2009; Rowbotham et al., 2011).

5 CHROMATIN TYPES

A functional genome is carefully organized into different chromosomal domains of gene activity and noncoding structural domains. All the different chromosomal domains have a distinct chromatin structure that make the DNA more or less accessible. Defects in the maintenance of specific chromatin domains lead to aberrant gene expression and chromosome instability.

The first indication of that chromatin exists in different configurations came as early as 1928 when Heitz, using a cytological analysis, was able to distinguish between two general types of chromatin. Heitz noted that some fractions of the chromosomes were stained very intensely with nuclear dyes, whereas other areas were only weakly stained (Heitz, 1928). Based on these findings, the weakly stained domains were designated as euchromatin and the strongly stained domains as heterochromatin. Since then, molecular biologists have been able to provide a deeper understanding of the properties and functions of these two chromatin domains. Euchromatin is typically a gene-rich open chromatin, whereas heterochromatin is maintained in a condensed state that replicates late and contains primary repetitive sequences and relatively few genes.

Recent studies have shown that the division of chromatin into two different types is a simplistic view and multiple forms of both classes of chromatin exist. In the fruit fly, at least two different types of transcriptionally active euchromatin and three types of repressed heterochromatin has been identified (Filion et al., 2010). More detailed analysis will probably identify even more classes of chromatin.

5.1 HETEROCHROMATIN

Heterochromatin is highly condensed chromatin that is generally inaccessible to DNA binding factors and refractory to gene expression. In multicellular organisms two types of heterochromatin have been described, namely constitutive and facultative. Constitutive heterochromatin is silenced in all cell types and is found mainly at chromosome regions that contain high density of repetitive elements, such as the centromeres and telomeres (Grewal and Elgin, 2002). Facultative heterochromatin is found at regions that are differently expressed during development and differentiation, such at the inactive X-chromosome, imprinted loci, and at genes that are permanently silenced in a given cell type (Bannister and Kouzarides, 2011). Emerging evidence suggests that there exist more types of repressive chromatin. For example, 48% of the *Drosophila* genome is folded into a third type of repressed chromatin (so called black chromatin) (Filion et al., 2010).

At the molecular level heterochromatin is characterized by histone modifications that are recognized by repressor/silencing complexes. Both facultative and constitutive heterochromatin contain nucleosomes that are underacetylated but they can be distinguished by the presence of differential methylation marks. For example, facultative heterochromatin is enriched for H3K29me3 that recruits the polycomb silencing complex, while histone H3 in constitutive heterochromatin is methylated at lysine 9 (H3K9me2/3), to which heterochromatin protein 1, HP1, binds (Bannister and

Kouzarides, 2011). The third type of heterochromatin described in *Drosophila* (the black chromatin) has low levels of polycomb and HP1 but is instead highly enriched for lamin, indicating that these domains are localized near the nuclear membrane (Filion et al., 2010). In agreement with this, the nuclear periphery is thought to be a repressive environment refractory to gene expression (see chapter 6).

5.2 EUCHROMATIN

Transcriptionally active genes are located in euchromatin regions. This type of chromatin is more accessible to enzymatic probes and DNA nucleases than heterochromatin, indicating that it has a more "open" structure. The open structure is thought to be generated by a combination of histone modifications, including high levels of acetylation and methylation of H3K4 and H3K79 as well as depletion of the linker histone H1 (Valenzuela and Kamakaka, 2006). The open structure makes DNA elements, such as promoters and enhancers, accessible to transcription factors and other regulatory proteins, thus facilitating transcription by RNA polymerases.

As with heterochromatin there exist several types of euchromatin that differs in their histone modification and DNA-binding proteins. Based on these differences it is possible to divide the euchromatin in *Drosophila* into two different types (yellow and red) (Filion et al., 2010). Interestingly, these two types of euchromatin seem to regulate different types of genes. Yellow chromatin is enriched for H3K36me3 and contains genes with a broad expression pattern that is constant in many developmental stages and tissues (e.g. housekeeping genes), whereas red chromatin contains genes linked to more specific processes and lacks H3K36me3 (Filion et al., 2010).

5.3 SUBTELOMERIC CHROMATIN

Subtelomeres are DNA sequences placed between chromosome-specific regions and chromosome ends (i.e. the telomeres). The length of the subtelomeres are around 100 kb in fission yeast and between 10 to 300 kb in human cells. The borders of the subtelomeres in fission are yeast marked by LTR elements and the binding of the chromatin remodeling factor Fft3 (Buchanan et al., 2009; Stralfors et al., 2011). The subtelomeres contain genes but they are lowly expressed (Buchanan et al., 2009; Robyr et al., 2002) and if you place a gene inside the subtelomeres they become transcriptionally silenced (Baur et al., 2001; Gottschling et al., 1990). In budding and fission yeast, the subtelomeric genes are repressed by HDACs and they became upregulated during nutritional stress (Hansen et al., 2005; Robyr et al., 2002). Studies in *S. pombe* have shown that subtelomeres do not display "normal" heterochromatin structure but have a unique type of chromatin (Buchanan et al., 2009). This subtelomeric chromatin (named ST-chromatin) is characterized by low levels of both the repressive heterochromatic mark, H3K9me2, and the active euchromatic mark, H3K4me2. It is also depleted in several histone acetylation marks (H4K5Ac, H4K12Ac, H4K16Ac and H3K14Ac) and in the histone variant H2A.Z (Buchanan et al., 2009).

5.4 CENTROMERIC CHROMATIN

The centromere is a specialized chromosomal region that serves as the assembly site of the kinetochore - the multi-protein complex that regulates chromosome segregation. In most species the centromeres assemble at repetitive sequences. However, these sequences are neither necessary nor sufficient for centromere function. Instead centromere identity relies largely on chromatin features.

In most eukaryotes, the centromeres contain two types of chromatin: the centric chromatin, which serves as the site of kinetochore formation, and the surrounding pericentric heterochromatin (Figure 2 and Figure 5). The pericentric heterochromatin is constitutive heterochromatin, which is highly methylated on DNA and enriched in hypoacetylated histones, H3K9me2/3, and HP1 protein. The formation of the pericentric heterochromatin is dependent on the RNAi interference (RNAi) machinery in *S. pombe* (Volpe et al., 2002). The heterochromatin is important, but not essential for centromere function (Folco et al., 2008). The centric chromatin has a unique chromatin structure that is characterized by the incorporation if the centromere-specific histone H3 variant CENP-A. This histone variant forms the basis for kinetochore assembly and is essential for centromere function in all organisms. In metazoans, blocks of CENP-A-containing nucleosome are interspersed with nucleosome containing canonical histone H3 as well as CENP-T/W/S/X "nucleosomes" (Nishino et al., 2012).

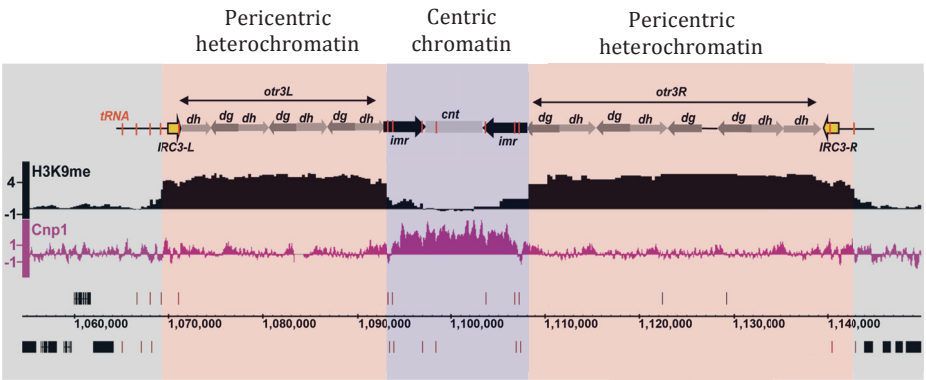


Figure 5. Schematic presentation of a *S. pombe* centromere. ChIP-chip data for $Cnp1^{CENP-A}$ (pink) and H3K9me2 (black). Centric chromatin (consisting of *imr* repeats and *cnt*) is occupied by the histone H3 variant $Cnp1^{CENP-A}$ and surrounded be pericentric heterochromatin with H3 methylated on lysine 9. Modified with permission from (Stralfors et al., 2011)

6 NUCLEAR ORGANIZATION AND INSULATORS

The cell nucleus is a structurally and functionally complex organelle with a non-random positioning of chromosomal loci and other nuclear components. This non-random positioning is believed to place genomic loci into functionally distinct nuclear compartments. Some compartments seem to favor transcription whereas others favor silencing. One important aspect of this organization is the interactions between chromatin and the nuclear periphery. Several studies from different organisms have shown that nuclear periphery is a repressive environment and that chromatin near the nuclear membrane contains repressive chromatin marks and silent or lowly expressed genes (Guelen et al., 2008; Pickersgill et al., 2006; Steglich et al., 2012). Remarkably, the borders of these peripheral-associated domains are highly enriched for insulators (Guelen et al., 2008; van Bemmel et al., 2010; Zullo et al., 2012) and this has led to a model in which insulators orchestrate genome organization.

6.1 INSULATOR ELEMENTS

Chromatin insulators are regulatory elements that protect genes from their surrounding environment (reviewed in (Strålfors and Ekwall, 2012)). They recruit proteins to either establish boundaries between adjacent chromatin domains (e.g. stopping the spread of heterochromatin into euchromatic domains), or to block the communication between enhancer elements and nearby promoters. Insulator elements are abundant throughout the eukaryotic genomes. For example, the insulator protein CTCF is bound to thousands of independent sites on the *Drosophila* chromosomes and tens of thousands of sites in human cell lines (Van Bortle and Corces, 2012).

6.1.1 TFIIIC-bound elements

One important class of insulators are TFIIIC-bound elements, which are the only described insulators conserved from yeast to human. TFIIIC is a transcription factor for the RNA polymerase III (Pol III) complex, which transcribes e.g. tRNA genes. Its insulating activity was first shown in *S. cerevisiae* at the silent mating type locus, *HMR*. This locus is surrounded by euchromatic genes that need to be insulated from the repressive chromatin. Molecular studies of the border between active and silent domains led to a demonstration that the insulating activity was due to a tRNA gene that blocked the spreading of heterochromatin into adjacent euchromatin and thereby protected nearby genes (Donze and Kamakaka, 2001). Furthermore, it was shown that the insulating activity of the tRNA gene required TFIIIC binding (Donze and Kamakaka, 2001). Since then, TFIIIC-bound tRNA genes have been shown to have insulating activities also in *S. pombe* and mammalian cells (Raab et al., 2012; Scott et al., 2006). Interestingly, TFIIIC is not only found at tRNA genes but also at regions depleted of Pol III. These sites are called *ETC* (Extra-TFIIIC) or *COC* (chromosome-organizing clamps) and also show insulating activities (Moqtaderi and Struhl, 2004; Moqtaderi et al., 2010; Noma et al., 2006). Thus, TFIIIC has an evolutionary conserved function in insulating chromatin domains.

6.1.2 CTCF

CTCF is another well-described insulator protein present in metazoans. It is a DNA-binding protein that was originally identified as a transcription factor. Further characterization showed that CTCF is located at known insulator elements and has an enhancer-blocking activity (Valenzuela and Kamakaka, 2006). Its enhancer-blocking activity is thought to be mediated by chromatin loops - two CTCF molecules interact with each other and thereby generate chromatin loops that position the enhancer and promoters in separate loops, preventing them from communicating. Recent studies have shown that CTCF also can promote communication between regulatory elements (Handoko et al., 2011). Hence, CTCF appear to have major roles in controlling gene expression. Furthermore, CTCF binding sites appear to change during development, indicating that CTCF generates cell-specific chromatin interactions that guide gene expression programs (Handoko et al., 2011).

Recent studies have reported significant enrichment of CTCF at boundaries between topological domains. For example, CTCF is found between domain of different transcription activities and epigenetic marks (Handoko et al., 2011). Specifically, CTCF demarcates chromatin-nuclear membrane attachment (Guelen et al., 2008) and boundaries between euchromatin and heterochromatin (Cuddapah et al., 2009). Thus, CTCF appears to be one of the main organizers of the genome.

6.2 ORGANIZING THE 3D STRUCTURE OF CHROMATIN

Most, if not all, known insulator elements can mediate long-range intra- and interchromosomal interactions across the genome, through insulator-insulator contacts (reviewed in (Van Bortle and Corces, 2012)). For example, high-throughput 3C-based techniques showed that tRNA genes in *S. cerevisiae* were significantly enriched for interactions with other tRNA genes (Duan et al., 2010). Furthermore, nuclear staining of insulator proteins show a clear concentration of insulators at distinct nuclear foci, often near the nuclear periphery, indicating that they interact with and localize to structural elements within the nucleus (Van Bortle and Corces, 2012). By doing this, the insulator elements tether associated chromatin to defined nuclear compartments (Figure 6). Different nuclear compartments would contain high concentrations of different enzymes and other factors and thereby, depending on which factors present in the particular compartment, provide a transcriptionally repressive or permissive environment. Recent evidence suggests that different insulators collaborate with each other. For example, CTCF and TFIIIC may cluster together to efficiently recruit essential cofactors important for robust insulation and stable long-range interactions (Van Bortle and Corces, 2012). Moreover the insulator-chromatin contacts seem to change during development, indicating that developmentally regulated genes are dynamically targeted by insulators to specific nuclear subcompartments favoring either transcriptional repression or activation (Van Bortle and Corces, 2012). These findings suggest that insulator elements play major roles in the three dimensional organization of chromatin.

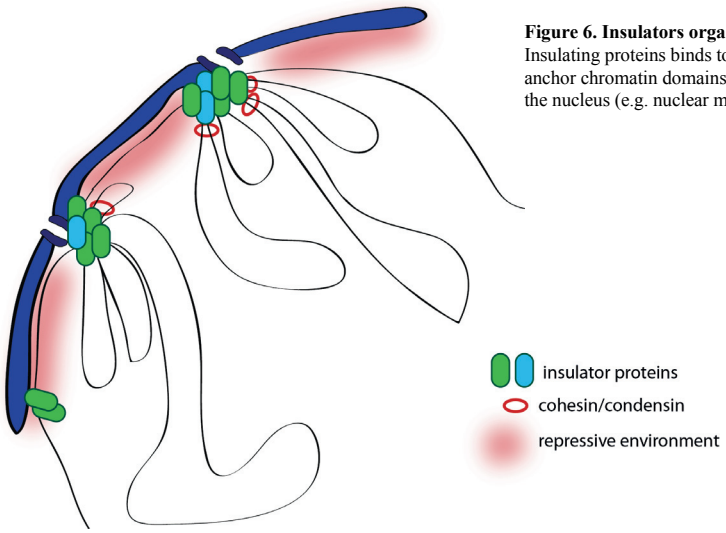


Figure 6. Insulators organize the chromatin. Insulating proteins binds to insulator elements and anchor chromatin domains to structural elements within the nucleus (e.g. nuclear membrane).

6.3 CONDENSIN AND COHESIN

Cohesin and condensin are two multiprotein complexes that form ring-like structures that hold DNA helices together. Cohesin holds sister chromatids together from S phase until mitosis while condensin compacts chromosomes during mitosis (Wood et al., 2010). However, both complexes also have roles during interphase. It was recently found that the interphase binding sites of cohesin largely coincide with those of CTCF in human cells (Wendt et al., 2008). Giving its ability to hold DNA helices together, it has been suggested that cohesin stabilizes chromatin loops arranged by CTCF. Similarly, TFIIIC-binding sites are associated with cohesin in mouse embryonic stem cells and human cells (Carriere et al., 2012; Moqtaderi et al., 2010). In fission and budding yeast, TFIIIC sites function as loading sites for condensin (D'Ambrosio et al., 2008; Iwasaki et al., 2010). Thus, condensin and cohesin appear to play fundamental roles at insulators and help regulating interphase genome organization.

6.4 CHROMATIN REMODELERS AND INSULATORS

The role of ATP-dependent chromatin remodeling complexes at insulators has so far not been extensively studied. However, there are some reports indicating that they have important functions in remodeling chromatin at insulator elements. For example the human CHD-remodeler CHD8 interacts *in vitro* and *in vivo* with CTCF and is found at many CTCF-binding sites (Ishihara et al., 2006). Further analyses showed that CHD8 is recruited to these sites via CTCF, but depletion of CHD8 abolishes CTCF insulator activity. These results suggest that remodeling of insulator elements by CHD8 is essential for proper insulation.

TFIIIC-bound insulators have also been associated with chromatin remodeling complexes. In *S. cerevisiae* the SWI/SNF remodeling complex Rsc is recruited to tRNA genes and removes nucleosomes from them, forming a histone-depleted region (Dhillon et al., 2009). This nucleosome-depleted region is required for proper barrier insulation. Furthermore, direct recruitment of remodeling factors to a synthetic insulator is sufficient to block the spread of silent chromatin in *S. cerevisiae* (Oki et al., 2004). The function of the Fun30-remodeler Fft3 at TFIIIC bound insulators has been studied in paper III and IV in this thesis.

7 METHODS

7.1 SCHIZOSACCAROMYCES POMBE

In this thesis, all experiments have been performed in *Schizosaccharomyces pombe. S. pombe*, also known as fission yeast, is a rod-shaped unicellular yeast that grows by tip elongation and divides by medial fission (hence its name). It has a relatively small genome, approximately 14 million bp, containing close to 5000 protein coding genes, divided between three chromosomes. The relatively large chromosomes share numerous features with the human chromosomes. They have large and complex centromeres containing repetitive sequences; "typical" heterochromatin and epigenetic silencing mechanisms; large replication origins; and conserved telomere proteins. These features have made fission yeast the model of choice for studying eukaryotic chromosome structure.

7.2 CHROMATIN IMMUNOPRECIPITATION

Chromatin immunoprecipitation (ChIP) is one of the most commonly used methods for analyzing DNA-protein interactions. In summary, protein-DNA interactions are fixed by e.g. formaldehyde. The cross-linked DNA is then fragmented by sonication to approximately 300-500 bp. Other methods for fragmentation, e.g. MNase digestion, can be used. After this, the chromatin extracts are incubated with antibodies specific for the protein you want to study, and the antibody-protein-DNA complex is pulled down by protein A beads. After several washes of the antibody-protein-DNA complex, the cross-linking is reversed and the immunoprecipitated DNA is precipitated.

The immunoprecipitated DNA can be identified by PCR or by tiling microarrays. ChIP combined with microarray (ChIP-chip) gives the genome-wide localization of the protein studied and is a powerful tool for understanding the potential roles and mechanisms of DNA binding proteins. In recent years, tiling microarrays are starting to be replaced by high-throughput sequencing methods.

7.3 DAM-ID

DamID is a method analogous to chromatin immunoprecipitation (Vogel et al., 2007). The technique is based on the creation of a fusion protein consisting of *Escherichia coli* DNA adenine methyltransferase (Dam) and the protein you want to study. Dam methylates adenine in the sequence GATC, a modification that is not present in most eukaryotes. When the fusion protein is expressed Dam will be targeted to the native binding sites of the protein of interest. This will result in methylation of nearby GATC sequences. In paper IV of this thesis, we fused Dam to the inner nuclear membrane protein Man1, which results in methylation of DNA in close proximity to the nuclear membrane. The methylated sequences are subsequently amplified by methylation specific PCR and identified by hybridization to microarrays (Figure 7).

In the methylation specific PCR, methylated DNA fragments are cut between GA^{me} and TC nucleotides with the methylation-sensitive restriction enzyme *DpnI*. This results in blunt-ended DNA fragments with 5' TC and 3'GA^{me} . In the next step, a double-stranded adaptor is ligated to the blunt ends. The adaptors have a 32 bp 5'overhang to ensure directional ligation. All unmethylated GATC is then cleaved with *DpnII*, which only recognizes and cut unmethylated DNA. Finally, a PCR reaction, with primers identical to the 3' end of the adaptor plus the first two nucleotides (TC) of the DNA fragments, amplifies the methylated DNA sequences. Only DNA fragments that have been methylated and cut by *DpnI* on both sides, and thus have the adaptor ligated to both sides, will be amplified. The amplified DNA can then be fragmented, labeled and hybridized to microarrays.

The Dam-fusion protein is never perfectly targeted to the native binding sites and this could cause background methylation of non-target sites. Moreover, depending on their genomic location, some GATCs are more accessible to Dam then others. These background problems are corrected for by including a parallel control experiment with unfused "free" Dam.

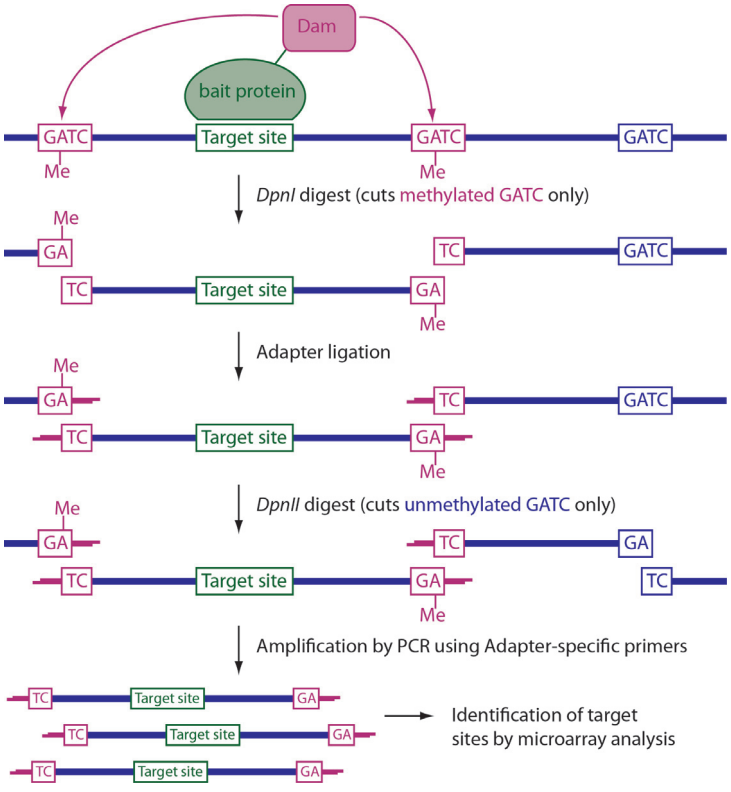


Figure 7. Principle of amplification of methylated fragments with the DamID protocol. Methylated GATC sequences (Me) are cut with *DpnI*. An adaptor (pink) is ligated to ends cut by *DpnI* and unmethylated GATC sequences are cut with *DpnII*. Fragments with adaptor ligated on both sides are amplified with PCR.

7.3.1 Comparison between DamID and ChIP

DamID has some advantages over ChIP. First it is not dependent on the availability of high-quality antibodies, which in theory makes it applicable to any protein. Furthermore, it does not require cross-linking of the DNA and this eliminates potential cross-linking artifacts. However, the DamID requires a fusion protein, which might not behave the same as the endogenous protein. Also, as the DamID experiment represents the average binding of the protein over a time period of ~24h or more, it is not suitable for e.g. time-course experiments. Moreover, it cannot be used for mapping post-translation modifications of e.g. histones. The resolution of the DamID experiment depends on the frequency of GATC sequences in the genome (on average every 0.2-2.5 kb), whereas the resolution of ChIP depends on the fragmentation method used (e.g. sonication).

7.4 GENOME-WIDE MAPPING OF NUCLEOSOME POSITIONS

DNA is relatively protected in the nucleosome but is more accessible in linker regions. Consequently, there exist various enzymes and reagents that cleave linker DNA but leave nucleosomal DNA intact. These can be used to assay nucleosome positioning in chromatin. Most commonly used is micrococcal nuclease (MNase), but other enzymes (such as DNaseI) or chemical reagents (e.g. methidiumpropyl-EDTA) can be used (Thoma, 1996). Until recently, nucleosome positions were analyzed by small-scale locus specific techniques, but now it has become possible to determine nucleosome positions genome-wide by the use of microarrays or high-throughput sequencing.

In paper I of this thesis the method described in (Lantermann et al., 2009) has been used to map genome-wide positions of nucleosomes in *S. pombe*. Briefly, chromatin is digested with MNase and mononucleosomal DNA is purified and hybridized to high-resolution tiling microarrays. Before hybridization, the DNA fragments need to be further fragmented from ~150 bp to an average length of 50 bp. This step is necessary to avoid a shift of nucleosome position due to bias of stronger hybridization at borders of the nucleosome core (Lantermann et al., 2009). As a control for hybridization efficiency, whole-genome DNA is analyzed and used for normalization.

A well-positioned nucleosome will give a high signal over a continuous series of probes covering around 120-140 bp of DNA. If the position of the nucleosome varies within the cell population, the peak will be broader and cover more probes. Conversely, a nucleosome free region will show low hybridization signal and a negative peak.

7.5 S. POMBE TILING 1.0FR ARRAY

DNA microarray technologies have been developed to interrogate thousands of nucleic acid probes in parallel. Throughout this thesis, GeneChip® *S. pombe* 1.0FR Arrays from Affymetrix has been used. This array is a tiling microarray, meaning that its probes cover the entire *S. pombe* genome, including both coding and non-coding

regions and centromeres. The probes are 25 bp long and have a 5 bp overlap, giving a resolution of 20bp (www.affymetrix.com).

RNA and DNA samples were fragmented, labeled, hybridized and scanned by our core facility, BEA (www.bea.ki.se). For data analysis TAS (Transcription Analysis Software), IGB (Integrated Genome Browser) and R/Bioconductor were used.

8 RESULTS AND DISCUSSIONS

8.1 PAPER I: SCHIZOSACCHAROMYCES POMBE GENOME-WIDE NUCLEOSOME MAPPING REVEALS POSITIONING MECHANISMS DISTINCT FROM THOSE OF SACCHAROMYCES CEREVISIAE.

Genome-wide maps of nucleosome positions have been generated for several organisms and they all show striking similarities in nucleosome positions at gene promoters (reviewed in (Piatti et al., 2011)). Just upstream of the transcription start site (TSS) you often find a broad (100-200 bp) nucleosome depleted region (NDR) that is flanked up- and downstream by well-positioning nucleosomes (denoted the -1 and +1 nucleosome). In yeast, the +1 nucleosome is typically followed by an array of regularly spaced nucleosomes extending into the gene body. In this paper we provide the first genome-wide nucleosome positioning map for *S. pombe* and compare it to published maps from *S. cerevisiae*.

To generate the genome-wide map of nucleosome positions we prepared mononucleosomal DNA by digesting chromatin with MNase and hybridized it to tiling microarrays. When analyzing the data we observed that the nucleosome position pattern at promoter regions was very similar to the pattern described in *S. cerevisiae*. We observed a NDR upstream transcriptional start site and a regular nucleosomal array downs stream of the TSS with a well-positioned +1 nucleosome. However, there were also several differences between the two yeast species. The nucleosome repeat length was considerably shorter in *S. pombe* (154 bp in *S. pombe* and 167 bp in *S. cerevisiae*) and most genes in *S. pombe* lacked the upstream nucleosomal array found in *S. cerevisiae*. To further analyze promoter regions we clustered promoters on the basis of their nucleosome occupancy pattern. We found six clusters and the most striking difference between them was the size of the NDR region. Interestingly, clusters with a deeper NDR had significantly higher expression levels. However, this correlation was not as strong when performing gene-by-gene correlation of promoter nucleosome occupancy and gene expression level.

We could also show that the regular nucleosome array downstream TSS is linked to transcription. First, arrays are only observed after promoter NDRs and not after other types of NDRs. Second, the arrays are formed in the transcriptional orientation and Pol II enrichment underlies the arrays. Third, the length of the transcripts correlates with the lengths of the arrays. Short open reading frames have short arrays and long genes have long arrays. Finally, silent genes do not display any nucleosomal array.

Nucleosomes can form on virtually any DNA sequence, but it is clear that some sequences are more readily bound to the histone octamer than others. These sequence preferences have been used to design computer models that predict nucleosome occupancy fairly accurate from DNA sequence alone (Kaplan et al., 2009). To test if this is true also for *S. pombe* we trained the N-score algorithm (Yuan and Liu, 2008) on hybridization data from *S. pombe* and *S. cerevisiae* and applied both model versions to the genome sequences of both yeasts. The computer algorithm could predict the NDR and overall nucleosome occupancy well for the species it was trained for, but

performed considerably worse when applied cross species. Moreover, several studies have shown that poly(dA-dT) sequences are strong nucleosome exclusion signal in *S. cerevisiae* (Yuan et al., 2005). We did not observe this in *S. pombe*. Instead we found that NDR are enriched for the sequence CGTTA. Hence, the DNA sequence seems to guide nucleosome positioning differently in the two yeasts. This argues for species-specific nucleosome positioning factors that override purely biophysical DNA sequence properties.

In *S. cerevisiae,* ATP-dependent chromatin remodelers have been shown to position nucleosomes onto energetically unfavorable DNA sequences. For example, deletion of *isw1, isw2* and *chd1* results in impaired nucleosomal arrays (Gkikopoulos et al., 2011). *S. pombe* lacks ISWI remodelers, but has three CHD remodelers, Mit1, Hrp1 and Hrp3 (see Figure 4). Here, we investigated the role of Mit1 in nucleosome positioning and found that cells lacking Mit1 display less pronounced nucleosomal arrays downstream of the TSS compared to wild type. However, with improved methodology, we could, in a later study, not detect any substantial differences between wild type and *mit1Δ* cells (Pointner et al., 2012). So, we now conclude that Mit1 does not play a major role in nucleosome positioning in *S. pombe*. However, the other two CHD remodelers, Hrp1 and Hrp3, appear to be essential for linking regular arrays to most TSSs (Pointner et al., 2012). However, the remodelers did not affect the NDR or the +1 nucleosome, so the factors determining them still need to be identified.

In summary, comparing genome-wide maps of nucleosome positions between the two yeasts *S. cerevisiae* and *S. pombe* revealed different mechanisms of nucleosome positioning. The two yeasts showed differences in nucleosome spacing, the roles of DNA sequence features and in the regular nucleosome arrays. This argues for an evolutionary plasticity of nucleosome positioning mechanisms and against the existence of a universal nucleosome positioning code.

8.2 PAPER II: IDENTIFICATION OF NONCODING TRANSCRIPTS FROM WITHIN CENP-A CHROMATIN AT FISSION YEAST CENTROMERES.

The histone variant CENP-A replaces histone H3 in centromeric chromatin and is essential for kinetochore formation and accurate chromosome segregation. It is not clear how CENP-A is specifically delivered to and assembled into centromeric chromatin, although our lab have previously shown that the CHD remodeling factor, Hrp1, has a role in this (Walfridsson et al., 2005). Hrp1 also has a role in transcription where it removes histone H3 at a subset of gene promoters (Walfridsson et al., 2007). In this study we investigate the function of Hrp1 and transcription at centromeric chromatin.

Fission yeast centromeres consist of a central core domain (over which CENP-A^{Cnp1} and the kinetochore assembles) and surrounding pericentric heterochromatin. Placement of an *arg3+* gene within the central domain results in its transcriptional silencing. We found that this silencing was partially impaired in *hrp1Δ* cells, and fully abolished when *hrp1Δ* was combined with mutations of either of the kinetochore proteins Mis6 and CENP-A^{Cnp1}. Consistent with this, we show that the level of CENP-

A^{Cnp1} was reduced and H3 increased at central domain in *hrp1Δ* cells. These results are consistent with earlier observations that Hrp1 is required for correct CENP-A^{Cnp1} deposition.

Our genome-wide ChIP-chip data revealed that CENP-A^{Cnp} is not only present at centromeric chromatin but also at a subset of gene promoters. Interestingly, a significant amount of these promoters are promoters at which Hrp1 acts to disassemble H3-containing nucleosomes. Moreover, CENP-A^{Cnp1} enrichment was reduced at some, but not all, of these promoters in *hrp1Δ* cells. These results indicate that Hrp1 participates in a remodeling process that evicts H3 from promoters, which then facilitates CENP-A^{Cnp1} incorporation. These results led us to the hypothesis that centromeric chromatin might also have promoter element where Hrp1 removes H3. To explore this we first wanted to investigate if the central cores of the *S. pombe* centromeres are transcribed. Using RT-PCR, we could not detect any transcript in wild type cells. However, several studies have observed that cryptic transcripts are degraded by exoribonucleases and that their stability depends on 3'end processing (Houseley and Tollervey, 2009). We therefore looked for centromeric transcripts in mutants of Psf2 (polyadenylation factor I subunit 2), Dhp1 (a 5'-3'-exoribonuclease) and Dis3 (a 3'-5' exoribonuclese in the exosome). Indeed, using these mutant strains, we could detect RNA homologous to the central domain. Hence, the central domain is transcribed but the transcripts are normally undetectable due to rapid turnover. We named these transcripts TUKs (Transcripts from Under Kinetochore).

Interestingly, Northern blot analysis revealed that TUKs were also detected in cells carrying mutations in CENP-A^{Cnp1} and other kinetochore proteins (Mis6, Mis16, and Mis18), suggesting that an intact CENP-A^{Cnp1} chromatin inhibits transcription of TUKs. Moreover, we could, using 5'RACE-PCR, demonstrate that the TUKs were produced from the central domain and made by read through from outer repeat transcripts. We also showed that TUKs are poly-adenylated and 5'-capped and therefore probably produced by RNA polymerase II. Together, these analyses indicate that cryptic transcription is prevalent in the central kinetochore domain of *S. pombe* and revealed only in cells defective in RNA turnover or formation of subkinetochore chromatin.

To conclude, this paper demonstrates an analogy between the subkinetochore CENP-A^{Cnp1}-contaning chromatin of centromeres and genes whose promoters are associated with Hrp1. Since Hrp1 promotes eviction of H3, which facilitates CENP-A^{Cnp1} incorporation, at these promoters, we suggest that a similar process occurs at promoters within the centromeres. We suggest that transcription through centromeric chromatin evicts histone H3, which then facilitates CENP-A incorporation.

It is known that CENP-A^{Cnp1} competes with histone H3 for incorporation into centromeric chromatin (Castillo et al., 2007) and in a recent paper we showed that factors such as FACT and Clr6-CII, which actively promote the integrity of H3 chromatin during Pol II transcription, prevent the incorporation of excess CENP-A^{Cnp1} (Choi et al., 2012). We therefore propose that CENP-A^{Cnp1} is opportunistic in nature and its assembly into chromatin is strongly affected by its availability relative to histone H3. Thus, factors that promote transcription-coupled recycling of H3 nucleosomes (e.g.

FACT) prevent incorporation of CENP-A^{Cnp1}, while factors that disassemble H3 nucleosomes (e.g. Hrp1) promote CENP-A^{Cnp1} assembly.

8.3 PAPER III: THE FUN30 CHROMATIN REMODELER, FFT3, PROTECTS CENTROMERIC AND SUBTELOMERIC DOMAINS FROM EUCHROMATIN FORMATION.

The Fun30 chromatin remodeler was first identified in a screen for genes that affect chromosome stability in *S. cerevisiae* (Ouspenski et al., 1999). This prompted us to investigate if the *S. pombe* homolog, Fft3, also has a role in chromosome stability. We observed that cells where *fft3* is deleted display a high rate of unequal chromosome segregation and reduced central core silencing, indicating malfunctioning centromeres. Furthermore, we found that the remodeler was highly enriched over the central domain of the three centromeres, suggesting that Fft3 has a direct role in maintaining centromere integrity. Fft3 was depleted from the pericentric heterochromatin, which surrounds the central core region. The most prominent peaks of Fft3 enrichment were found at centromeric insulator elements, both at the transition between the central core and the pericentric heterochromatin and at the border between pericentric heterochromatin and the surrounding euchromatin.

The localization of Fft3 suggests that the remodeler could have a function at insulator elements, which block the spreading of chromatin domains beyond their natural borders. To test this, we analyzed the distribution of histone H3, Cnp1 and H2A.Z in *fft3Δ* cells. These data revealed that in the absence of Fft3, H3 spreads beyond its normal boundary into the central core domain. Concurrently, the Cnp1 domain shrinks, and H2A.Z becomes incorporated. Surprisingly, the H3 that was incorporated into the central core region was, unlike the surrounding heterochromatin, not methylated at lysine 9 (H3K9me), but was instead acetylated at this residue. In agreement with this we also found that acetylation of K12 of histone H4 (H4K12Ac) increased in the central domain in *fft3Δ* cells. Thus, our data shows that the properties of the central domain are altered and show a more active chromatin structure in the *fft3Δ* mutant.

Expression profiling of *fft3Δ* cells revealed that 61 genes were upregulated and 15 genes were downregulated in *fft3Δ* versus wild type (using a 2-fold cutoff). Interestingly more than 60% of the upregulated genes lay within 100 kb of the chromosome ends, i.e. within subtelomeric regions. Thus, Fft3 appears to affect the silencing of subtelomeric genes.

Interestingly, the most prominent noncentromeric peaks of Fft3 enrichment were seen at the border of the subtelomeres, at the transition between euchromatin and silent subtelomeric chromatin, strongly suggesting that Fft3 has a function at subtelomeric insulator elements. To explore this, we performed ChIP-chip experiments for two euchromatic marks, H2A.Z and H4K12Ac, which are normally depleted from subtelomeric chromatin. Remarkably, the ChIP-chip data showed that when Fft3 is removed, both H2A.Z and H4K12Ac expand beyond the euchromatin domain into the subtelomeric chromatin. From these results, we conclude that Fft3 marks the boundary

between euchromatin and subtelomeres and that the remodeler has are role in insulating subtelomeres from surrounding euchromatin domains.

At the left subtelomere on chromosome II, the transition from euchromatin to subtelomeric chromatin coincides exactly with the presence of four long terminal repeats (LTRs) located just upstream of the promoter of four copies of one gene encoding a membrane transporter. Fft3 is present at the four LTR elements but not at the membrane transporter genes. We observed that the H3 density over these elements changes in *fft3Δ* cells, indicating that Fft3 remodels the chromatin over this putative insulator element. This remodeling appears to be essential for maintaining an accurate silent chromatin structure at subtelomeres.

In summary, we have found that the Fun30 remodeler, Fft3, regulates the chromatin structure over insulator elements and thereby ensures correct chromatin structure and gene expression of silent domains. When Fft3 is absent, euchromatin invades the centromeres and subtelomeres, causing a change in histone modification, incorrect incorporation of histone variants, mis-regulation of gene expression, and severe chromosome segregation effects. Similar observations have later been seen in *S. cerevisiae* (Durand-Dubief et al., 2012). The mechanism that Fft3 uses to insulate chromatin domains is further explored in paper IV.

8.4 PAPER IV: THE FUN30 CHROMATIN REMODELER FFT3 CONTROLS THE SPATIAL ORGANIZATION OF POL III GENES AND SUBTELOMERES IN THE FISSION YEAST NUCLEUS.

In paper III we showed that the Fun30 chromatin remodeler Fft3 has an important role in insulating chromatin domains from euchromatic surroundings. For example, we showed that subtelomeric genes on chromosome I and II became upregulated in an *fft3Δ* mutant (Stralfors et al., 2011). To investigate this further, we performed a ChIP-chip analysis of RNA polymerase II (Pol II), and in agreement with the expression analysis we saw a clear increase of Pol II enrichment at all subtelomeres. We also observed an increase in two active chromatin marks typically not present at subtelomeres (H4K12Ac and H2A.Z). Thus, Fft3 affects the silent chromatin structure of subtelomeres.

A recent study from our lab showed that subtelomeres are in close proximity to the nuclear envelope (Steglich et al., 2012). The same study also suggested that the nuclear periphery is a repressive environment refractory to gene expression. This led us to hypothesize that the change in chromatin modifications and gene expression of subtelomeres in *fft3Δ* cells might coincide with a change in their peripheral association. To test this, we performed a DamID experiment in which we mapped DNA in close proximity to the inner nuclear membrane protein Man1. As expected, we found that subtelomeres in wild type cells were enriched for Man1, indicating that they are in close proximity to the nuclear membrane. However, this association was lost in *fft3Δ* cells, which instead showed a depletion of Man1 at subtelomeres. This result strongly suggests that the subtelomeres lose their membrane association in the absence of the Fft3 remodeler.

The transitions between subtelomeric chromatin and the euchromatic chromosome arms are marked by LTR elements. Interestingly, Fft3 is enriched over these elements. In fact, Fft3 is highly enriched over most LTR elements in the genome. Interestingly, LTR elements are also associated with Man1, indicating that they are located at the nuclear periphery. Many LTR elements reduce this membrane association in *fft3Δ* cells. Hence, Fft3 is not only present at LTR elements but also plays a functional role in their peripheral localization.

To explore if the effects of Fft3 depend on its ATP-dependent remodeling activity we created a mutant strain carrying a point mutation (K418R) in the ATPase domain that results in a catalytically inactive enzyme. The ATPase mutant was expressed at similar levels to the wild type protein and was recruited to the same locations. Nevertheless, the ATPase mutant displayed the same phenotypes as the full deletion of the gene, e.g. subtelomeric genes were upregulated. This result strongly suggests that ATP-dependent chromatin remodeling by Fft3 is required to maintain a silent chromatin structure at subtelomeres.

When searching for other genomic loci that could be affected by Fft3 we found that the remodeler is enriched over tRNA genes. In addition, Fft3 co-localizes with the RNA Polymerase III and its transcription factor, TFIIIC, which are components of the machinery that transcribes tRNA genes. Moreover, we discovered, using a yeast-two hybrid screen and CoIP, that Fft3 physically interacts with a subunit of TFIIIC, Sfc4. Interestingly, we found that tRNA genes have significantly higher levels of Man1-DamID signals compared to the rest of the genome, indicating that they are localized close to the nuclear membrane. Remarkably, this association was lost in the *fft3Δ* mutant. Thus, Fft3 appears to affect the nuclear organization of several genomic elements, e.g. subtelomeres, LTR elements and tRNA genes.

DNA loci that are in close proximity to nuclear pores were recently mapped in *S. pombe* (Woolcock et al., 2012). When analyzing this data set we found that the DNA regions enriched in Nup85-DamID overlap with Fft3 targets. For example, both tRNA genes and LTR elements are strong Nup85-DamID targets. To test if nuclear pores, like Fft3, insulate subtelomeric chromatin we measured gene expression of two subtelomeric genes in a *nup61Δ* mutant. Interestingly, we found that the subtelomeric genes were upregulated in *nup61Δ* cells, though the increased expression was not as pronounced as in *fft3Δ* mutant cells. Thus, these results show that targets of Fft3 are localized to and depend on components of the nuclear pores.

Fft3 is located at borders between chromatin domains (Stralfors et al., 2011) and two of the main targets of Fft3, tRNA genes and LTR elements, seem to function as insulators (Carabana et al., 2011; Kirkland et al., 2012). Most, if not all, known insulator elements can mediate long-range intra- and interchromosomal interactions across the genome, through insulator-insulator contacts (reviewed in (Van Bortle and Corces, 2012)). Furthermore, clusters of insulators often interact with structural elements within the nucleus, e.g. nuclear pores. By doing this, the insulator elements tether associated chromatin to defined nuclear compartments. Here, we show that tRNA and LTR elements are localized to nuclear pores and since Fft3 is required for tethering tRNAs,

and to a certain extent also LTR elements, to the nuclear periphery it is possible that remodeling by Fft3 has a role in anchoring these regions to the pores. This would organize the chromatin into different domains and protect them from neighboring chromatin. We therefore propose that Fft3 is important for both spatial and functional organization of chromatin in *S. pombe*.

9 CONCLUSIONS

In this thesis we have shown that:

- Intrinsic DNA sequence properties do not provide an evolutionarily conserved genomic code for nucleosome positioning. Instead, species-specific nucleosome positioning factors (e.g. chromatin remodeling factors) appear to override the biophysical properties of the DNA sequence.

- Transcription from promoters, both in euchromatin and within centromeric chromatin, promotes eviction of histone H3 through the associated remodeling factor $Chd1^{Hrp1}$. This facilities the incorporation of the H3 variant $CENP\text{-}A^{Cnp1}$.

- The Fun30 remodeler, Fft3, regulates the chromatin structure over insulator elements and thereby ensures correct chromatin structure and gene expression of silent domains (e.g. subtelomeres and centromeres).

- Fft3 has important roles in the spatial and functional organization of chromatin. It anchors insulator elements to the inner nuclear membrane close to nuclear pores. This organizes the chromatin into different domains and protects them from neighboring chromatin.

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11 REFERENCES

Adra, C.N., Donato, J.L., Badovinac, R., Syed, F., Kheraj, R., Cai, H., Moran, C., Kolker, M.T., Turner, H., Weremowicz, S., *et al.* (2000). SMARCAD1, a novel human helicase family-defining member associated with genetic instability: cloning, expression, and mapping to 4q22-q23, a band rich in breakpoints and deletion mutants involved in several human diseases. Genomics *69*, 162-173.

Ahmad, K., and Henikoff, S. (2002). The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. Mol Cell *9*, 1191-1200.

Alen, C., Kent, N.A., Jones, H.S., O'Sullivan, J., Aranda, A., and Proudfoot, N.J. (2002). A role for chromatin remodeling in transcriptional termination by RNA polymerase II. Mol Cell *10*, 1441-1452.

Awad, S., Ryan, D., Prochasson, P., Owen-Hughes, T., and Hassan, A.H. (2010). The Snf2 homolog Fun30 acts as a homodimeric ATP-dependent chromatin-remodeling enzyme. J Biol Chem *285*, 9477-9484.

Bannister, A.J., and Kouzarides, T. (2011). Regulation of chromatin by histone modifications. Cell Res *21*, 381-395.

Barski, A., Cuddapah, S., Cui, K., Roh, T.Y., Schones, D.E., Wang, Z., Wei, G., Chepelev, I., and Zhao, K. (2007). High-resolution profiling of histone methylations in the human genome. Cell *129*, 823-837.

Barton, A.B., and Kaback, D.B. (1994). Molecular cloning of chromosome I DNA from Saccharomyces cerevisiae: analysis of the genes in the FUN38-MAK16-SPO7 region. J Bacteriol *176*, 1872-1880.

Baur, J.A., Zou, Y., Shay, J.W., and Wright, W.E. (2001). Telomere position effect in human cells. Science *292*, 2075-2077.

Billon, P., and Cote, J. (2012). Precise deposition of histone H2A.Z in chromatin for genome expression and maintenance. Biochim Biophys Acta *1819*, 290-302.

Black, B.E., and Cleveland, D.W. (2011). Epigenetic centromere propagation and the nature of CENP-a nucleosomes. Cell *144*, 471-479.

Black, B.E., Foltz, D.R., Chakravarthy, S., Luger, K., Woods, V.L., Jr., and Cleveland, D.W. (2004). Structural determinants for generating centromeric chromatin. Nature *430*, 578-582.

Black, B.E., Jansen, L.E., Maddox, P.S., Foltz, D.R., Desai, A.B., Shah, J.V., and Cleveland, D.W. (2007). Centromere identity maintained by nucleosomes assembled with histone H3 containing the CENP-A targeting domain. Mol Cell *25*, 309-322.

Bluteau, O., Beaudoin, J.C., Pasturaud, P., Belghiti, J., Franco, D., Bioulac-Sage, P., Laurent-Puig, P., and Zucman-Rossi, J. (2002). Specific association between alcohol intake, high grade of differentiation and 4q34-q35 deletions in hepatocellular carcinomas identified by high resolution allelotyping. Oncogene *21*, 1225-1232.

Boyarchuk, E., Montes de Oca, R., and Almouzni, G. (2011). Cell cycle dynamics of histone variants at the centromere, a model for chromosomal landmarks. Curr Opin Cell Biol *23*, 266-276.

Buchanan, L., Durand-Dubief, M., Roguev, A., Sakalar, C., Wilhelm, B., Stralfors, A., Shevchenko, A., Aasland, R., Ekwall, K., and Francis Stewart, A. (2009). The Schizosaccharomyces pombe JmjC-protein, Msc1, prevents H2A.Z localization in centromeric and subtelomeric chromatin domains. PLoS Genet *5*, e1000726.

Camahort, R., Li, B., Florens, L., Swanson, S.K., Washburn, M.P., and Gerton, J.L. (2007). Scm3 is essential to recruit the histone h3 variant cse4 to centromeres and to maintain a functional kinetochore. Mol Cell *26*, 853-865.

Carabana, J., Watanabe, A., Hao, B., and Krangel, M.S. (2011). A barrier-type insulator forms a boundary between active and inactive chromatin at the murine TCRbeta locus. J Immunol *186*, 3556-3562.
Carr, A.M., Dorrington, S.M., Hindley, J., Phear, G.A., Aves, S.J., and Nurse, P. (1994). Analysis of a histone H2A variant from fission yeast: evidence for a role in chromosome stability. Mol Gen Genet *245*, 628-635.
Carriere, L., Graziani, S., Alibert, O., Ghavi-Helm, Y., Boussouar, F., Humbertclaude, H., Jounier, S., Aude, J.C., Keime, C., Murvai, J.*, et al.* (2012). Genomic binding of Pol III transcription machinery and relationship with TFIIS transcription factor distribution in mouse embryonic stem cells. Nucleic Acids Res *40*, 270-283.
Carrozza, M.J., Li, B., Florens, L., Suganuma, T., Swanson, S.K., Lee, K.K., Shia, W.J., Anderson, S., Yates, J., Washburn, M.P.*, et al.* (2005). Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription. Cell *123*, 581-592.
Castillo, A.G., Mellone, B.G., Partridge, J.F., Richardson, W., Hamilton, G.L., Allshire, R.C., and Pidoux, A.L. (2007). Plasticity of fission yeast CENP-A chromatin driven by relative levels of histone H3 and H4. PLoS Genet *3*, e121.
Cetin, E., Cengiz, B., Gunduz, E., Gunduz, M., Nagatsuka, H., Bekir-Beder, L., Fukushima, K., Pehlivan, D., N, M.O., Nishizaki, K.*, et al.* (2008). Deletion mapping of chromosome 4q22-35 and identification of four frequently deleted regions in head and neck cancers. Neoplasma *55*, 299-304.
Cheeseman, I.M., and Desai, A. (2008). Molecular architecture of the kinetochore-microtubule interface. Nat Rev Mol Cell Biol *9*, 33-46.
Chen, E.S., Saitoh, S., Yanagida, M., and Takahashi, K. (2003). A cell cycle-regulated GATA factor promotes centromeric localization of CENP-A in fission yeast. Mol Cell *11*, 175-187.
Chen, X., Cui, D., Papusha, A., Zhang, X., Chu, C.D., Tang, J., Chen, K., Pan, X., and Ira, G. (2012). The Fun30 nucleosome remodeller promotes resection of DNA double-strand break ends. Nature *489*, 576-580.
Choi, E.S., Stralfors, A., Castillo, A.G., Durand-Dubief, M., Ekwall, K., and Allshire, R.C. (2011). Identification of noncoding transcripts from within CENP-A chromatin at fission yeast centromeres. J Biol Chem *286*, 23600-23607.
Choi, E.S., Stralfors, A., Catania, S., Castillo, A.G., Svensson, J.P., Pidoux, A.L., Ekwall, K., and Allshire, R.C. (2012). Factors That Promote H3 Chromatin Integrity during Transcription Prevent Promiscuous Deposition of CENP-A(Cnp1) in Fission Yeast. PLoS Genet *8*, e1002985.
Clapier, C.R., and Cairns, B.R. (2009). The biology of chromatin remodeling complexes. Annu Rev Biochem *78*, 273-304.
Clark, M.W., Zhong, W.W., Keng, T., Storms, R.K., Barton, A., Kaback, D.B., and Bussey, H. (1992). Identification of a Saccharomyces cerevisiae homolog of the SNF2 transcriptional regulator in the DNA sequence of an 8.6 kb region in the LTE1-CYS1 interval on the left arm of chromosome I. Yeast *8*, 133-145.
Costelloe, T., Louge, R., Tomimatsu, N., Mukherjee, B., Martini, E., Khadaroo, B., Dubois, K., Wiegant, W.W., Thierry, A., Burma, S.*, et al.* (2012). The yeast Fun30 and human SMARCAD1 chromatin remodellers promote DNA end resection. Nature *489*, 581-584.
Cuddapah, S., Jothi, R., Schones, D.E., Roh, T.Y., Cui, K., and Zhao, K. (2009). Global analysis of the insulator binding protein CTCF in chromatin barrier regions reveals demarcation of active and repressive domains. Genome Res *19*, 24-32.
D'Ambrosio, C., Schmidt, C.K., Katou, Y., Kelly, G., Itoh, T., Shirahige, K., and Uhlmann, F. (2008). Identification of cis-acting sites for condensin loading onto budding yeast chromosomes. Genes Dev *22*, 2215-2227.

Denslow, S.A., and Wade, P.A. (2007). The human Mi-2/NuRD complex and gene regulation. Oncogene *26*, 5433-5438.
Dhillon, N., Raab, J., Guzzo, J., Szyjka, S.J., Gangadharan, S., Aparicio, O.M., Andrews, B., and Kamakaka, R.T. (2009). DNA polymerase epsilon, acetylases and remodellers cooperate to form a specialized chromatin structure at a tRNA insulator. Embo J *28*, 2583-2600.
Donze, D., and Kamakaka, R.T. (2001). RNA polymerase III and RNA polymerase II promoter complexes are heterochromatin barriers in Saccharomyces cerevisiae. Embo J *20*, 520-531.
Drouin, S., Laramee, L., Jacques, P.E., Forest, A., Bergeron, M., and Robert, F. (2010). DSIF and RNA polymerase II CTD phosphorylation coordinate the recruitment of Rpd3S to actively transcribed genes. PLoS Genet *6*, e1001173.
Duan, Z., Andronescu, M., Schutz, K., McIlwain, S., Kim, Y.J., Lee, C., Shendure, J., Fields, S., Blau, C.A., and Noble, W.S. (2010). A three-dimensional model of the yeast genome. Nature *465*, 363-367.
Dunleavy, E.M., Roche, D., Tagami, H., Lacoste, N., Ray-Gallet, D., Nakamura, Y., Daigo, Y., Nakatani, Y., and Almouzni-Pettinotti, G. (2009). HJURP is a cell-cycle-dependent maintenance and deposition factor of CENP-A at centromeres. Cell *137*, 485-497.
Durand-Dubief, M., Will, W.R., Petrini, E., Theodorou, D., Harris, R.R., Crawford, M.R., Paszkiewicz, K., Krueger, F., Correra, R.M., Vetter, A.T., *et al.* (2012). SWI/SNF-Like Chromatin Remodeling Factor Fun30 Supports Point Centromere Function in S. cerevisiae. PLoS Genet *8*, e1002974.
Eapen, V.V., Sugawara, N., Tsabar, M., Wu, W.H., and Haber, J.E. (2012). The Saccharomyces cerevisiae chromatin remodeler Fun30 regulates DNA end-resection and checkpoint deactivation. Mol Cell Biol.
Feng, Q., and Zhang, Y. (2001). The MeCP1 complex represses transcription through preferential binding, remodeling, and deacetylating methylated nucleosomes. Genes Dev *15*, 827-832.
Field, Y., Kaplan, N., Fondufe-Mittendorf, Y., Moore, I.K., Sharon, E., Lubling, Y., Widom, J., and Segal, E. (2008). Distinct modes of regulation by chromatin encoded through nucleosome positioning signals. PLoS Comput Biol *4*, e1000216.
Filion, G.J., van Bemmel, J.G., Braunschweig, U., Talhout, W., Kind, J., Ward, L.D., Brugman, W., de Castro, I.J., Kerkhoven, R.M., Bussemaker, H.J., *et al.* (2010). Systematic protein location mapping reveals five principal chromatin types in Drosophila cells. Cell *143*, 212-224.
Flanagan, J.F., Mi, L.Z., Chruszcz, M., Cymborowski, M., Clines, K.L., Kim, Y., Minor, W., Rastinejad, F., and Khorasanizadeh, S. (2005). Double chromodomains cooperate to recognize the methylated histone H3 tail. Nature *438*, 1181-1185.
Flaus, A., Martin, D.M., Barton, G.J., and Owen-Hughes, T. (2006). Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. Nucleic Acids Res *34*, 2887-2905.
Folco, H.D., Pidoux, A.L., Urano, T., and Allshire, R.C. (2008). Heterochromatin and RNAi are required to establish CENP-A chromatin at centromeres. Science *319*, 94-97.
Foltz, D.R., Jansen, L.E., Bailey, A.O., Yates, J.R., 3rd, Bassett, E.A., Wood, S., Black, B.E., and Cleveland, D.W. (2009). Centromere-specific assembly of CENP-a nucleosomes is mediated by HJURP. Cell *137*, 472-484.
Fu, Y., Sinha, M., Peterson, C.L., and Weng, Z. (2008). The insulator binding protein CTCF positions 20 nucleosomes around its binding sites across the human genome. PLoS Genet *4*, e1000138.

Fujita, Y., Hayashi, T., Kiyomitsu, T., Toyoda, Y., Kokubu, A., Obuse, C., and Yanagida, M. (2007). Priming of centromere for CENP-A recruitment by human hMis18alpha, hMis18beta, and M18BP1. Dev Cell *12*, 17-30.
Fullgrabe, J., Kavanagh, E., and Joseph, B. (2011). Histone onco-modifications. Oncogene *30*, 3391-3403.
Gamble, M.J., and Kraus, W.L. (2010). Multiple facets of the unique histone variant macroH2A: from genomics to cell biology. Cell Cycle *9*, 2568-2574.
Gkikopoulos, T., Schofield, P., Singh, V., Pinskaya, M., Mellor, J., Smolle, M., Workman, J.L., Barton, G.J., and Owen-Hughes, T. (2011). A role for Snf2-related nucleosome-spacing enzymes in genome-wide nucleosome organization. Science *333*, 1758-1760.
Goldberg, A.D., Banaszynski, L.A., Noh, K.M., Lewis, P.W., Elsaesser, S.J., Stadler, S., Dewell, S., Law, M., Guo, X., Li, X.*, et al.* (2010). Distinct factors control histone variant H3.3 localization at specific genomic regions. Cell *140*, 678-691.
Gonzalez-Romero, R., Mendez, J., Ausio, J., and Eirin-Lopez, J.M. (2008). Quickly evolving histones, nucleosome stability and chromatin folding: all about histone H2A.Bbd. Gene *413*, 1-7.
Gottschling, D.E., Aparicio, O.M., Billington, B.L., and Zakian, V.A. (1990). Position effect at S. cerevisiae telomeres: reversible repression of Pol II transcription. Cell *63*, 751-762.
Greaves, I.K., Rangasamy, D., Ridgway, P., and Tremethick, D.J. (2007). H2A.Z contributes to the unique 3D structure of the centromere. Proc Natl Acad Sci U S A *104*, 525-530.
Grewal, S.I., and Elgin, S.C. (2002). Heterochromatin: new possibilities for the inheritance of structure. Curr Opin Genet Dev *12*, 178-187.
Guelen, L., Pagie, L., Brasset, E., Meuleman, W., Faza, M.B., Talhout, W., Eussen, B.H., de Klein, A., Wessels, L., de Laat, W.*, et al.* (2008). Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. Nature *453*, 948-951.
Guillemette, B., and Gaudreau, L. (2006). Reuniting the contrasting functions of H2A.Z. Biochem Cell Biol *84*, 528-535.
Hamiche, A., and Shuaib, M. (2012). Chaperoning the histone H3 family. Biochim Biophys Acta *1819*, 230-237.
Handoko, L., Xu, H., Li, G., Ngan, C.Y., Chew, E., Schnapp, M., Lee, C.W., Ye, C., Ping, J.L., Mulawadi, F.*, et al.* (2011). CTCF-mediated functional chromatin interactome in pluripotent cells. Nat Genet *43*, 630-638.
Hansen, K.R., Burns, G., Mata, J., Volpe, T.A., Martienssen, R.A., Bahler, J., and Thon, G. (2005). Global effects on gene expression in fission yeast by silencing and RNA interference machineries. Mol Cell Biol *25*, 590-601.
Hargreaves, D.C., and Crabtree, G.R. (2011). ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. Cell Res *21*, 396-420.
Hartley, P.D., and Madhani, H.D. (2009). Mechanisms that specify promoter nucleosome location and identity. Cell *137*, 445-458.
Hayashi, T., Fujita, Y., Iwasaki, O., Adachi, Y., Takahashi, K., and Yanagida, M. (2004). Mis16 and Mis18 are required for CENP-A loading and histone deacetylation at centromeres. Cell *118*, 715-729.
Heitz, E. (1928). Das heterochromatin der Moose. Jahrb Wiss Bot *69*, 728-818.
Hennig, B.P., Bendrin, K., Zhou, Y., and Fischer, T. (2012). Chd1 chromatin remodelers maintain nucleosome organization and repress cryptic transcription. EMBO Rep.

Hong, F., Fang, F., He, X., Cao, X., Chipperfield, H., Xie, D., Wong, W.H., Ng, H.H., and Zhong, S. (2009). Dissecting early differentially expressed genes in a mixture of differentiating embryonic stem cells. PLoS Comput Biol *5*, e1000607.
Houseley, J., and Tollervey, D. (2009). The many pathways of RNA degradation. Cell *136*, 763-776.
Huang, Y., and Maraia, R.J. (2001). Comparison of the RNA polymerase III transcription machinery in Schizosaccharomyces pombe, Saccharomyces cerevisiae and human. Nucleic Acids Res *29*, 2675-2690.
Ishihara, K., Oshimura, M., and Nakao, M. (2006). CTCF-dependent chromatin insulator is linked to epigenetic remodeling. Mol Cell *23*, 733-742.
Ismail, I.H., and Hendzel, M.J. (2008). The gamma-H2A.X: is it just a surrogate marker of double-strand breaks or much more? Environ Mol Mutagen *49*, 73-82.
Iwasaki, O., Tanaka, A., Tanizawa, H., Grewal, S.I., and Noma, K. (2010). Centromeric localization of dispersed Pol III genes in fission yeast. Mol Biol Cell *21*, 254-265.
Iyer, V.R. (2012). Nucleosome positioning: bringing order to the eukaryotic genome. Trends Cell Biol *22*, 250-256.
Jansen, A., and Verstrepen, K.J. (2011). Nucleosome positioning in Saccharomyces cerevisiae. Microbiol Mol Biol Rev *75*, 301-320.
Kaplan, N., Moore, I.K., Fondufe-Mittendorf, Y., Gossett, A.J., Tillo, D., Field, Y., LeProust, E.M., Hughes, T.R., Lieb, J.D., Widom, J.*, et al.* (2009). The DNA-encoded nucleosome organization of a eukaryotic genome. Nature *458*, 362-366.
Kirkland, J.G., Raab, J.R., and Kamakaka, R.T. (2012). TFIIIC bound DNA elements in nuclear organization and insulation. Biochim Biophys Acta.
Kobor, M.S., Venkatasubrahmanyam, S., Meneghini, M.D., Gin, J.W., Jennings, J.L., Link, A.J., Madhani, H.D., and Rine, J. (2004). A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. PLoS Biol *2*, E131.
Konev, A.Y., Tribus, M., Park, S.Y., Podhraski, V., Lim, C.Y., Emelyanov, A.V., Vershilova, E., Pirrotta, V., Kadonaga, J.T., Lusser, A.*, et al.* (2007). CHD1 motor protein is required for deposition of histone variant H3.3 into chromatin in vivo. Science *317*, 1087-1090.
Krogan, N.J., Baetz, K., Keogh, M.C., Datta, N., Sawa, C., Kwok, T.C., Thompson, N.J., Davey, M.G., Pootoolal, J., Hughes, T.R.*, et al.* (2004). Regulation of chromosome stability by the histone H2A variant Htz1, the Swr1 chromatin remodeling complex, and the histone acetyltransferase NuA4. Proc Natl Acad Sci U S A *101*, 13513-13518.
Lagana, A., Dorn, J.F., De Rop, V., Ladouceur, A.M., Maddox, A.S., and Maddox, P.S. (2010). A small GTPase molecular switch regulates epigenetic centromere maintenance by stabilizing newly incorporated CENP-A. Nat Cell Biol *12*, 1186-1193.
Lantermann, A., Stralfors, A., Fagerstrom-Billai, F., Korber, P., and Ekwall, K. (2009). Genome-wide mapping of nucleosome positions in Schizosaccharomyces pombe. Methods *48*, 218-225.
Lantermann, A.B., Straub, T., Stralfors, A., Yuan, G.C., Ekwall, K., and Korber, P. (2010). Schizosaccharomyces pombe genome-wide nucleosome mapping reveals positioning mechanisms distinct from those of Saccharomyces cerevisiae. Nat Struct Mol Biol *17*, 251-257.
Lefrancois, P., Euskirchen, G.M., Auerbach, R.K., Rozowsky, J., Gibson, T., Yellman, C.M., Gerstein, M., and Snyder, M. (2009). Efficient yeast ChIP-Seq using multiplex short-read DNA sequencing. BMC Genomics *10*, 37.

Li, B., Gogol, M., Carey, M., Pattenden, S.G., Seidel, C., and Workman, J.L. (2007). Infrequently transcribed long genes depend on the Set2/Rpd3S pathway for accurate transcription. Genes Dev *21*, 1422-1430.
Lim, D.A., Suarez-Farinas, M., Naef, F., Hacker, C.R., Menn, B., Takebayashi, H., Magnasco, M., Patil, N., and Alvarez-Buylla, A. (2006). In vivo transcriptional profile analysis reveals RNA splicing and chromatin remodeling as prominent processes for adult neurogenesis. Mol Cell Neurosci *31*, 131-148.
Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. (1997). Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature *389*, 251-260.
Luk, E., Vu, N.D., Patteson, K., Mizuguchi, G., Wu, W.H., Ranjan, A., Backus, J., Sen, S., Lewis, M., Bai, Y*., et al.* (2007). Chz1, a nuclear chaperone for histone H2AZ. Mol Cell *25*, 357-368.
Malik, H.S., and Henikoff, S. (2003). Phylogenomics of the nucleosome. Nat Struct Biol *10*, 882-891.
Marques, M., Laflamme, L., Gervais, A.L., and Gaudreau, L. (2010). Reconciling the positive and negative roles of histone H2A.Z in gene transcription. Epigenetics *5*, 267-272.
Mavrich, T.N., Jiang, C., Ioshikhes, I.P., Li, X., Venters, B.J., Zanton, S.J., Tomsho, L.P., Qi, J., Glaser, R.L., Schuster, S.C*., et al.* (2008). Nucleosome organization in the Drosophila genome. Nature *453*, 358-362.
Meneghini, M.D., Wu, M., and Madhani, H.D. (2003). Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. Cell *112*, 725-736.
Mizuguchi, G., Shen, X., Landry, J., Wu, W.H., Sen, S., and Wu, C. (2004). ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science *303*, 343-348.
Mizuguchi, G., Xiao, H., Wisniewski, J., Smith, M.M., and Wu, C. (2007). Nonhistone Scm3 and histones CenH3-H4 assemble the core of centromere-specific nucleosomes. Cell *129*, 1153-1164.
Moqtaderi, Z., and Struhl, K. (2004). Genome-wide occupancy profile of the RNA polymerase III machinery in Saccharomyces cerevisiae reveals loci with incomplete transcription complexes. Mol Cell Biol *24*, 4118-4127.
Moqtaderi, Z., Wang, J., Raha, D., White, R.J., Snyder, M., Weng, Z., and Struhl, K. (2010). Genomic binding profiles of functionally distinct RNA polymerase III transcription complexes in human cells. Nat Struct Mol Biol *17*, 635-640.
Morettini, S., Tribus, M., Zeilner, A., Sebald, J., Campo-Fernandez, B., Scheran, G., Worle, H., Podhraski, V., Fyodorov, D.V., and Lusser, A. (2011). The chromodomains of CHD1 are critical for enzymatic activity but less important for chromatin localization. Nucleic Acids Res *39*, 3103-3115.
Murawska, M., and Brehm, A. (2011). CHD chromatin remodelers and the transcription cycle. Transcription *2*, 244-253.
Neves-Costa, A., Will, W.R., Vetter, A.T., Miller, J.R., and Varga-Weisz, P. (2009). The SNF2-family member Fun30 promotes gene silencing in heterochromatic loci. PLoS One *4*, e8111.
Nishino, T., Takeuchi, K., Gascoigne, K.E., Suzuki, A., Hori, T., Oyama, T., Morikawa, K., Cheeseman, I.M., and Fukagawa, T. (2012). CENP-T-W-S-X forms a unique centromeric chromatin structure with a histone-like fold. Cell *148*, 487-501.
Noma, K., Cam, H.P., Maraia, R.J., and Grewal, S.I. (2006). A role for TFIIIC transcription factor complex in genome organization. Cell *125*, 859-872.

Nousbeck, J., Burger, B., Fuchs-Telem, D., Pavlovsky, M., Fenig, S., Sarig, O., Itin, P., and Sprecher, E. (2011). A mutation in a skin-specific isoform of SMARCAD1 causes autosomal-dominant adermatoglyphia. Am J Hum Genet *89*, 302-307.
Okada, M., Okawa, K., Isobe, T., and Fukagawa, T. (2009). CENP-H-containing complex facilitates centromere deposition of CENP-A in cooperation with FACT and CHD1. Mol Biol Cell *20*, 3986-3995.
Oki, M., Valenzuela, L., Chiba, T., Ito, T., and Kamakaka, R.T. (2004). Barrier proteins remodel and modify chromatin to restrict silenced domains. Mol Cell Biol *24*, 1956-1967.
Ouspenski, II, Elledge, S.J., and Brinkley, B.R. (1999). New yeast genes important for chromosome integrity and segregation identified by dosage effects on genome stability. Nucleic Acids Res *27*, 3001-3008.
Papamichos-Chronakis, M., Watanabe, S., Rando, O.J., and Peterson, C.L. (2011). Global regulation of H2A.Z localization by the INO80 chromatin-remodeling enzyme is essential for genome integrity. Cell *144*, 200-213.
Parnell, T.J., Huff, J.T., and Cairns, B.R. (2008). RSC regulates nucleosome positioning at Pol II genes and density at Pol III genes. Embo J *27*, 100-110.
Perpelescu, M., Nozaki, N., Obuse, C., Yang, H., and Yoda, K. (2009). Active establishment of centromeric CENP-A chromatin by RSF complex. J Cell Biol *185*, 397-407.
Piatti, P., Zeilner, A., and Lusser, A. (2011). ATP-Dependent Chromatin Remodeling Factors and Their Roles in Affecting Nucleosome Fiber Composition. Int J Mol Sci *12*, 6544-6565.
Pickersgill, H., Kalverda, B., de Wit, E., Talhout, W., Fornerod, M., and van Steensel, B. (2006). Characterization of the Drosophila melanogaster genome at the nuclear lamina. Nat Genet *38*, 1005-1014.
Pidoux, A.L., Richardson, W., and Allshire, R.C. (2003). Sim4: a novel fission yeast kinetochore protein required for centromeric silencing and chromosome segregation. J Cell Biol *161*, 295-307.
Pointner, J., Persson, J., Prasad, P., Norman-Axelsson, U., Stralfors, A., Khorosjutina, O., Krietenstein, N., Peter Svensson, J., Ekwall, K., and Korber, P. (2012). CHD1 remodelers regulate nucleosome spacing in vitro and align nucleosomal arrays over gene coding regions in S. pombe. The EMBO Journal.
Pusarla, R.H., and Bhargava, P. (2005). Histones in functional diversification. Core histone variants. Febs J *272*, 5149-5168.
Quan, T.K., and Hartzog, G.A. (2010). Histone H3K4 and K36 methylation, Chd1 and Rpd3S oppose the functions of Saccharomyces cerevisiae Spt4-Spt5 in transcription. Genetics *184*, 321-334.
Quenet, D., and Dalal, Y. (2012). The CENP-A nucleosome: a dynamic structure and role at the centromere. Chromosome Res *20*, 465-479.
Raab, J.R., Chiu, J., Zhu, J., Katzman, S., Kurukuti, S., Wade, P.A., Haussler, D., and Kamakaka, R.T. (2012). Human tRNA genes function as chromatin insulators. Embo J *31*, 330-350.
Rando, O.J. (2012). Combinatorial complexity in chromatin structure and function: revisiting the histone code. Curr Opin Genet Dev *22*, 148-155.
Robyr, D., Suka, Y., Xenarios, I., Kurdistani, S.K., Wang, A., Suka, N., and Grunstein, M. (2002). Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases. Cell *109*, 437-446.
Rowbotham, S.P., Barki, L., Neves-Costa, A., Santos, F., Dean, W., Hawkes, N., Choudhary, P., Will, W.R., Webster, J., Oxley, D*., et al.* (2011). Maintenance of silent chromatin through replication requires SWI/SNF-like chromatin remodeler SMARCAD1. Mol Cell *42*, 285-296.

Sadeghi, L., Bonilla, C., Stralfors, A., Ekwall, K., and Svensson, J.P. (2011). Podbat: a novel genomic tool reveals Swr1-independent H2A.Z incorporation at gene coding sequences through epigenetic meta-analysis. PLoS Comput Biol *7*, e1002163.
Schnetz, M.P., Bartels, C.F., Shastri, K., Balasubramanian, D., Zentner, G.E., Balaji, R., Zhang, X., Song, L., Wang, Z., Laframboise, T., *et al.* (2009). Genomic distribution of CHD7 on chromatin tracks H3K4 methylation patterns. Genome Res *19*, 590-601.
Schoor, M., Schuster-Gossler, K., Roopenian, D., and Gossler, A. (1999). Skeletal dysplasias, growth retardation, reduced postnatal survival, and impaired fertility in mice lacking the SNF2/SWI2 family member ETL1. Mech Dev *85*, 73-83.
Scott, K.C., Merrett, S.L., and Willard, H.F. (2006). A heterochromatin barrier partitions the fission yeast centromere into discrete chromatin domains. Curr Biol *16*, 119-129.
Seki, Y., Kurisaki, A., Watanabe-Susaki, K., Nakajima, Y., Nakanishi, M., Arai, Y., Shiota, K., Sugino, H., and Asashima, M. (2010). TIF1beta regulates the pluripotency of embryonic stem cells in a phosphorylation-dependent manner. Proc Natl Acad Sci U S A *107*, 10926-10931.
Shim, Y.S., Choi, Y., Kang, K., Cho, K., Oh, S., Lee, J., Grewal, S.I., and Lee, D. (2012). Hrp3 controls nucleosome positioning to suppress non-coding transcription in eu- and heterochromatin. The EMBO Journal.
Sims, R.J., 3rd, Chen, C.F., Santos-Rosa, H., Kouzarides, T., Patel, S.S., and Reinberg, D. (2005). Human but not yeast CHD1 binds directly and selectively to histone H3 methylated at lysine 4 via its tandem chromodomains. J Biol Chem *280*, 41789-41792.
Soininen, R., Schoor, M., Henseling, U., Tepe, C., Kisters-Woike, B., Rossant, J., and Gossler, A. (1992). The mouse Enhancer trap locus 1 (Etl-1): a novel mammalian gene related to Drosophila and yeast transcriptional regulator genes. Mech Dev *39*, 111-123.
Steglich, B., Fillion, G., van Steensel, B., and Ekwall, K. (2012). The inner nuclear membrane proteins Man1 and Ima1 link to two different types of chromatin at the nuclear periphery in *S. pombe*. Nucleus *3*, 78-87.
Stoler, S., Rogers, K., Weitze, S., Morey, L., Fitzgerald-Hayes, M., and Baker, R.E. (2007). Scm3, an essential Saccharomyces cerevisiae centromere protein required for G2/M progression and Cse4 localization. Proc Natl Acad Sci U S A *104*, 10571-10576.
Strahl, B.D., and Allis, C.D. (2000). The language of covalent histone modifications. Nature *403*, 41-45.
Strålfors, A., and Ekwall, K. (2012). Heterochromatin and Euchromatin -Organization, Boundaries, and Gene Regulation. In Epigenetic Regulation and Epigenomics: Advances in Molecular Biology and Medicin, R. Meyers, ed. (Wiley-VCH Verlag GmbH & Co. KGaA), pp. 171-187.
Stralfors, A., Walfridsson, J., Bhuiyan, H., and Ekwall, K. (2011). The FUN30 chromatin remodeler, Fft3, protects centromeric and subtelomeric domains from euchromatin formation. PLoS Genet *7*, e1001334.
Straube, K., Blackwell, J.S., Jr., and Pemberton, L.F. (2010). Nap1 and Chz1 have separate Htz1 nuclear import and assembly functions. Traffic *11*, 185-197.
Sugiyama, T., Cam, H.P., Sugiyama, R., Noma, K., Zofall, M., Kobayashi, R., and Grewal, S.I. (2007). SHREC, an effector complex for heterochromatic transcriptional silencing. Cell *128*, 491-504.
Szenker, E., Ray-Gallet, D., and Almouzni, G. (2011). The double face of the histone variant H3.3. Cell Res *21*, 421-434.
Takahashi, K., Chen, E.S., and Yanagida, M. (2000). Requirement of Mis6 centromere connector for localizing a CENP-A-like protein in fission yeast. Science *288*, 2215-2219.
Thoma, F. (1996). Mapping of nucleosome positions. Methods Enzymol *274*, 197-214.

Thomas, M.C., and Chiang, C.M. (2006). The general transcription machinery and general cofactors. Crit Rev Biochem Mol Biol *41*, 105-178.
Topp, C.N., Zhong, C.X., and Dawe, R.K. (2004). Centromere-encoded RNAs are integral components of the maize kinetochore. Proc Natl Acad Sci U S A *101*, 15986-15991.
Valenzuela, L., and Kamakaka, R.T. (2006). Chromatin insulators. Annu Rev Genet *40*, 107-138.
van Bemmel, J.G., Pagie, L., Braunschweig, U., Brugman, W., Meuleman, W., Kerkhoven, R.M., and van Steensel, B. (2010). The insulator protein SU(HW) fine-tunes nuclear lamina interactions of the Drosophila genome. PLoS One *5*, e15013.
Van Bortle, K., and Corces, V.G. (2012). Nuclear Organization and Genome Function. Annu Rev Cell Dev Biol.
Vogel, M.J., Peric-Hupkes, D., and van Steensel, B. (2007). Detection of in vivo protein-DNA interactions using DamID in mammalian cells. Nat Protoc *2*, 1467-1478.
Volpe, T.A., Kidner, C., Hall, I.M., Teng, G., Grewal, S.I., and Martienssen, R.A. (2002). Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi. Science *297*, 1833-1837.
Walfridsson, J., Bjerling, P., Thalen, M., Yoo, E.J., Park, S.D., and Ekwall, K. (2005). The CHD remodeling factor Hrp1 stimulates CENP-A loading to centromeres. Nucleic Acids Res *33*, 2868-2879.
Walfridsson, J., Khorosjutina, O., Matikainen, P., Gustafsson, C.M., and Ekwall, K. (2007). A genome-wide role for CHD remodelling factors and Nap1 in nucleosome disassembly. Embo J *26*, 2868-2879.
Watanabe, S., and Peterson, C.L. (2010). The INO80 family of chromatin-remodeling enzymes: regulators of histone variant dynamics. Cold Spring Harb Symp Quant Biol *75*, 35-42.
Wendt, K.S., Yoshida, K., Itoh, T., Bando, M., Koch, B., Schirghuber, E., Tsutsumi, S., Nagae, G., Ishihara, K., Mishiro, T., *et al.* (2008). Cohesin mediates transcriptional insulation by CCCTC-binding factor. Nature *451*, 796-801.
Wood, A.J., Severson, A.F., and Meyer, B.J. (2010). Condensin and cohesin complexity: the expanding repertoire of functions. Nat Rev Genet *11*, 391-404.
Woolcock, K.J., Stunnenberg, R., Gaidatzis, D., Hotz, H.R., Emmerth, S., Barraud, P., and Buhler, M. (2012). RNAi keeps Atf1-bound stress response genes in check at nuclear pores. Genes Dev *26*, 683-692.
Yu, Q., Zhang, X., and Bi, X. (2011). Roles of chromatin remodeling factors in the formation and maintenance of heterochromatin structure. J Biol Chem *286*, 14659-14669.
Yuan, G., and Zhu, B. (2012). Histone variants and epigenetic inheritance. Biochim Biophys Acta *1819*, 222-229.
Yuan, G.C., and Liu, J.S. (2008). Genomic sequence is highly predictive of local nucleosome depletion. PLoS Comput Biol *4*, e13.
Yuan, G.C., Liu, Y.J., Dion, M.F., Slack, M.D., Wu, L.F., Altschuler, S.J., and Rando, O.J. (2005). Genome-scale identification of nucleosome positions in S. cerevisiae. Science *309*, 626-630.
Zegerman, P., Canas, B., Pappin, D., and Kouzarides, T. (2002). Histone H3 lysine 4 methylation disrupts binding of nucleosome remodeling and deacetylase (NuRD) repressor complex. J Biol Chem *277*, 11621-11624.
Zhang, H., Roberts, D.N., and Cairns, B.R. (2005). Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. Cell *123*, 219-231.

Zhang, Y., Ng, H.H., Erdjument-Bromage, H., Tempst, P., Bird, A., and Reinberg, D. (1999). Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. Genes Dev *13*, 1924-1935.
Zullo, J.M., Demarco, I.A., Pique-Regi, R., Gaffney, D.J., Epstein, C.B., Spooner, C.J., Luperchio, T.R., Bernstein, B.E., Pritchard, J.K., Reddy, K.L., *et al.* (2012). DNA sequence-dependent compartmentalization and silencing of chromatin at the nuclear lamina. Cell *149*, 1474-1487.