

From DEPARTMENT OF BIOSCIENCES AND NUTRITION
Karolinska Institutet, Stockholm, Sweden

**EXPLORING CHROMATIN
ORGANIZATION AND TRANSCRIPTION
IN S.POMBE AND HEMATOPOIETIC
DEVELOPMENT**

Wenbo Dong



**Karolinska
Institutet**

Stockholm 2020

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet.

Printed by US-AB Tryckeriet

© Wenbo Dong, 2020

ISBN 978-91-7831-859-9

Exploring chromatin organization and transcription in *S.pombe* and hematopoietic development

THESIS FOR DOCTORAL DEGREE (Ph.D.)

The thesis will be defended on Thursday 18th of June 2020, at 10.00

Location: Gene 5108, Neo, Blickagången 16, Flemingsberg

By

Wenbo Dong

Principal Supervisor:

Associate Professor Andreas Lennartsson
Karolinska Institutet
Department of Biosciences and Nutrition

Co-supervisor(s):

Professor Karl Ekwall
Karolinska Institutet
Department of Biosciences and Nutrition

Mickaël Durand-Dubief
Karolinska Institutet
Department of Biosciences and Nutrition

Punit Prasad
Institute of Life Sciences (ILS)

Opponent:

Professor Stefan Åström
Stockholm University
Department of Molecular Biosciences,
The Wenner-Gren Institute

Examination Board:

Professor Anthony Wrigh
Karolinska Institutet
Department of Laboratory Medicine

Vicente Pelechano
Karolinska Institutet
Department of Microbiology, Tumor and Cell
Biology

Professor Mattias Mannervik
Stockholm University
Department of Molecular Biosciences,
The Wenner-Gren Institute

To my beloved family

特别送给姥姥

ABSTRACT

DNA in the eukaryotic nucleus is organized into histone-DNA complex, so-called chromatin, through forming an array of nucleosomes. Each nucleosome consists of a 147bp DNA wrapped around a histone octamer harboring two of each H2A-H2B and H3-H4. Chromatin is orderly packed several times forming a chromosome structure. Active euchromatin and repressive heterochromatin are defined according to the degree of DNA compaction, of which euchromatin is open, and heterochromatin is condensed. Chromatin organization and its regulation always affect downstream gene transcriptions through different mechanisms, which consequently play crucial roles in many cellular and biological processes.

In this thesis, we explored mechanisms of chromatin organization and its associated regulatory factors by using *Schizosaccharomyces pombe*. We identified an uncovered role of Abo1 in different heterochromatin locus. We demonstrated that Abo1 is involved in Clr4 mediated heterochromatin assembly through regulating H3K9me2 to H3K9me3 transition, related to distinct silencing machinery.

We also performed multiple in vitro experiments to investigate the functional role of the chromatin remodeler Hrp3, which is the orthologue of human CHD1. We generated several mutant strains where the non-catalytic domains were individually deleted. Our result suggested non-catalytic domains could further affect ATP hydrolyzing activity, and may further affect the chromatin remodeling function.

In this thesis, we also investigated the outcomes of epigenetic and transcriptional regulation in hematopoietic development. We performed analysis on CAGE libraries in various primary cell types from the Fantom 5 project to study the usage of alternative transcriptional start site (TSS). Through mapping the TSS to Refseq, we identified alternative TSS that can lead protein domain loss. The alternative TSSs were shown to be expressed at different levels in different cell types or developmental stages, particularly in blood cells. We further investigated the functional consequence of alternative TSSs usage for KDM2B in Jurkat T-cells.

To identify critical novel epigenetic regulators for myeloid differentiation, we performed a CRISPR-cas9 screen. We identified the chromatin remodeler CHD2 as a crucial regulator for megakaryocyte differentiation in the PMA inducible K-562 cell model.

LIST OF SCIENTIFIC PAPERS

- I. **Abo1 is required for the H3K9me2 to H3K9me3 transition in heterochromatin**
Dong, W., E. Oya, Y. Zahedi, P. Prasad, J. P. Svensson, A. Lennartsson, K. Ekwall and M. Durand-Dubief. Scientific reports 2020 Apr 8;10(1): 1-13.
- II. **The role of non-catalytic domains of Hrp3 in chromatin remodeling**
Wenbo Dong, Punit Prasad, Andreas Lennartsson¹, Karl Ekwall
- III. **Investigation of protein coding sequence exclusion by alternative transcription start site usage across the human body**
Wenbo Dong*, Berit Lilje*, Farzaneh Shahin Varnoosfaderani, Erik Arner, The FANTOM consortium, Andreas Lennartsson, Albin Sandelin
- IV. **A regulatory role for CHD2 in myelopoiesis**
Shahin Varnoosfaderani, F., A. Palau, W. Dong, J. Persson, M. Durand-Dubief, J. P. Svensson and A. Lennartsson Epigenetics 2020 Jan 10:1-13

*shared first authors

CONTENTS

1. Introduction.....	1
1.1 Chromatin organization.....	1
1.1.1 Chromatin type	2
1.1.1.1 Euchromatin	2
1.1.1.2 Heterochromatin.....	2
1.1.1.3 Chromatin dynamic.....	7
1.1.2 Histone modifications	9
1.1.2.1 H3K9me2/3	13
1.1.2.2 PRC complex	14
1.1.3 ATP dependent chromatin remodeler.....	16
1.1.3.1 CHD chromatin remodeling family.....	17
1.1.3.1.1 CHD1 and CHD2.....	18
1.1.4 Mechanisms of nucleosome assembly and remodeling.....	19
1.2 Fission yeast <i>Schizosaccharomyces pombe</i>	20
1.3 Hematopoietic development	21
1.3.1 Hematopoiesis	21
1.3.2 Epigenetics of hematopoietic development.....	22
1.3.3 Disorders of hematopoietic development.....	24
1.3.3.1 Epigenetic changes in AML	24
2. Aims of The Thesis	26
3. Methods	27
3.1 Cell culture	27
3.2 Chromatin immunoprecipitation.....	27
3.3 Overall gene expression analysis	28
3.4 Biochemical assays <i>in vitro</i>	31
3.5 siRNA knocking down.....	32
4. Results and Discussion	33
4.1 Study I.....	33
4.2 Study II.....	35
4.3 Study III	36
4.4 Study IV	39
5. Acknowledgements	41
6. References.....	44

LIST OF ABBREVIATIONS

5hmC	5-hydroxymethyl-cytosine
5mC	5-methyl-cytosine
ADP	Adenosine diphosphate
ALL	Acute lymphocytic leukemia
AML	Acute myeloid leukemia
ASXL1	Additional sex combs like 1
BACH 1/2	BTB domain and CNC homolog 1/2
BER	Base excision repair
CAGE	Cap analysis of gene expression
CBX	Chromobox
CD	Cluster of differentiation
CENP-A	Histone H3-like centromeric protein A
CHD	Chromodomain heliase DNA-binding
ChIP	Chromatin immunoprecipitation
CLL	Chronic lymphoid leukemia
CLP	Common lymphoid progenitors
CML	Chronic myeloid leukemia
CMP	Common myeloid progenitors
CRLC	Calcitonin receptor-like receptor complex
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
DSR	Determinant of selective removal
EMCs	Embryonic stem cells
EPOP	Elongin BC and PRC2 associated protein
EZH1/2	Enhancer of zeste homolog1/2
FACT	Facilitates chromatin transcription
FLT3	Fms related receptor tyrosine kinase 3
GATA-1	GATA binding protein-1
GFP	Green fluorescent protein
HAS	Helicase-SANT
HBD	Histone-binding domain
HDAC	Histone deacetylase
HMTs	Histone methyltransferases
HOR	Higher ordered tandem repeats
HP1	Heterochromatin protein 1
HSCs	Hematopoietic stem cells
IDH	Isocitrate dehydrogenase
INO80	INOsitol-requiring 80
ISWI	Imitation switch

JMJD1A	Jumonji domain-containing 1A
K	Lysine
KDM2B	Lysine demethylase 2B
KLF4	Kruppel like factor 4
lncRNA	long non-coding RNA
MET2	Methyltransferase 2
MLL	Myeloid/lymphoid or mixed-lineage leukemia 1
NCP	Nucleosome core particle
ncRNA	non-coding RNA
NPM1	Nucleophosmin
PCGF	Polycomb group ring finger
PCL	Polycomb like
PHD	Plant homeodomain
PIWI	P-element induced wimpy
PMA	Phorbol 12-myristate 13-acetate
Pol	Polymerase
PRC	Polycomb repressive complex
PRDM1	PR domain zinc finger protein 1
PTM	Post-translational modifications
PU.1	PU-box.1
rDNA	Ribosomal DNA
RDRC	RNA-directed RNA polymerase complex
RITS	RNA-induced transcriptional silencing
RNA	Ribonucleic acid
RNAi	RNA interference
RUNX1	Runt-related transcription factor 1
S.cerevisiae	Saccharomyces cerevisiae/budding yeast
S.pombe	Shizosaccharomyces pombe/fission yeast
SANT	Swi3, Ada2, N-cor and TF IIIB
SET-25	SET domain containing-25
sgRNA	Single guid RNAi
SHL2	Superhelical location 2
SHREC	Snf2/Hdac repressive complex
SLIDE	SANT-like ISWI
SnAC	Snf2 ATP coupling
snRNP	Small nuclear ribonucleoproteins
SUV39H1/2	Suppressor of variegation 3-9 homolog1/2
SWI/SNF	Switch/sucrose non-fermentable
TAS	Telomeric associated sequence
TC	Tag cluster
TDG	Thymine DNA glycosylase
TGS	Transcriptional gene silencing

TIN2	Interacting nuclear factor 2
TPE	Telomere position effect
TPM	Tags per million
TPP1	Tripeptidyl peptidase 1
TRF1/2	Telomeric repeat binding factor 1/2
tRNA	transfer RNA
TSS	Transcriptional start site

1. Introduction

1.1 Chromatin organization

Eukaryotic genomes consist of chromosomes, each of which is packaged from a single linear DNA. Although the genome sizes are diverse, the basic chromosome structure is the same in all eukaryotic species (Alberts, Johnson et al. 2002). A double-stranded helical DNA, tightly wrapped around a histone octamer composed of H2A-H2B dimers and H3-H4 tetramer constitute the nucleosome. Each nucleosome consists of a 147bp DNA formed nucleosome core particle (NCP), with additional a linker DNA and a histone protein H1 (Cooper 2000). This DNA-histone proteins interacted complex is called chromatin. To be compressed into a nucleus, chromatin is hierarchically packed into 30-nm fiber and 300-nm fiber, which is finally folded into a chromosome (Figure 1) (Alberts, Johnson et al. 2002, Annunziato 2008). The packaging of chromatin is regulated mainly through biochemically modified histones and remodeling enzymes, which cause remodeled chromatin structures and consequently affect downstream transcription of genes (Hubner, Eckersley-Maslin et al. 2013).

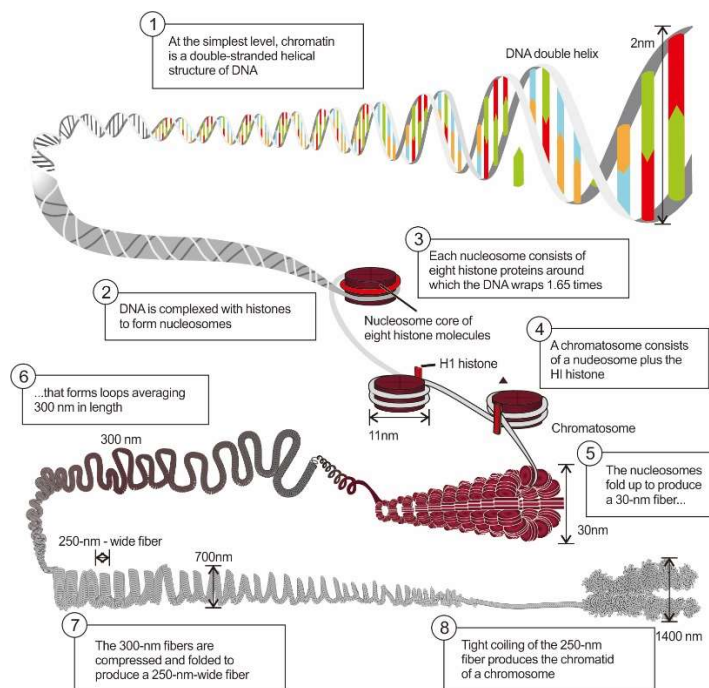


Figure 1. View of chromatin organization and orderly compaction. Linear DNA is compacted into a chromosome hierarchically form string containing 11-nm nucleosomes. The nucleosome string then coils into 30-nm fiber that forms loops around 300-nm. The 300-nm fiber subsequently is folded to produce 250nm-fiber, which finalize to coil into a chromosome. © 2013 Nature Education. All rights reserved. Figure reprinted with the permission from publisher.

1.1.1 Chromatin types

In 1928, Heitz distinguished two types of chromatin according to the cytological difference detected through staining the nucleus of moss with nuclei dye. The chromatin regions with light staining were defined as euchromatin, while the regions with intense staining were defined as heterochromatin (Heitz 1928). Later, studies identified that the different intensity of staining in the two chromatin regions comes from the distinct compacted DNA density (Babu and Verma 1987). Nowadays, we know that euchromatin is featured by its less packed open structure and enrichment of genes. Transcription in euchromatin is usually active. On the contrary, heterochromatin is featured by its tightly packed structure with condensed DNA. Transcription in heterochromatin is usually repressed, but not always. Within the eukaryotic nucleus, cytogenetic studies also revealed that euchromatin is found in the inner body, while heterochromatin is more located in the inner face of the periphery (Oberdoerffer and Sinclair 2007, Kalverda, Röling et al. 2008).

Besides these two main classified categories, there are also multiple chromatin types reported. For instance, a study in *Drosophila melanogaster* revealed five principal chromatin types, which provided a global view and potential possibility of chromatin diversity in metazoan cells (Filion, van Bommel et al. 2010).

1.1.1.1 Euchromatin

Most actively transcribed genes are located in the euchromatin, such as the housekeeping genes. The unfolded structure of euchromatin relies on the high level of histone acetylation and the absence of linker histone H1. Methylation on H3K4 and H3K79 are also present in euchromatin. Keeping the chromatin structure open allows for the recruitment of transcriptional regulatory proteins and RNA polymerase, and consequently initiate the transcription of genes (Hubner, Eckersley-Maslin et al. 2013).

1.1.1.2 Heterochromatin

Heterochromatin is a key chromatin feature of the eukaryotic genome. One crucial role of heterochromatin is to maintain the stability of the chromosome. It virtually participates in many cellular processes, from gene regulation to chromosome replication. The coverage of the genome for heterochromatin is quite diverse through eukaryotic organisms. Typical heterochromatin domains situate in particular positions on a chromosome, such as in the middle (centromere), at the ends (subtelomere/telomere) (Allshire and Madhani 2018, Janssen, Colmenares et al. 2018). In the 1960s, researchers performed a kinetic analysis of DNA renaturation and

characterized that heterochromatin contains more highly repeated DNA sequences compared to euchromatin (Britten and Kohne 1968, Vanrobays, Thomas et al. 2018). These highly repetitive sequences are required for the genome integrity during replication, such as repeats in centromere and telomere (discussed later). Besides the repetitive sequences, heterochromatin regions also comprise repressive genes that can only be transcribed in certain conditions/processes, such as cell cycling, environmental stress response, and development. Therefore, depending on the formation and strength of silencing, heterochromatin can be classically subdivided into constitutive and facultative heterochromatin (Brown 1966, Vanrobays, Thomas et al. 2018). Additionally, researchers reported a distinct repressive chromatin type (so-called black chromatin in the article) that is prevalent in *Drosophila*'s genome (~50%) (Filion, van Bommel et al. 2010).

Constitutive heterochromatin is always associated with permanent silent regions throughout the cell cycle. It is mostly composed of tandem repeats (so-called satellite repeats), but poor of genes. In most eukaryotic organisms, bulk constitutive heterochromatin situates at the pericentromeric region of centromere and at telomere on a chromosome (Saksouk, Simboeck et al. 2015). In humans, it is significantly more found on chromosome 1, 9, 16 19, and chromosome Y (Strachan and Read 2003).

The centromere is a conserved structure on a eukaryotic chromosome where the kinetochore is constituted during mitotic segregation. It is essential for equal distribution of genetic information from mother to daughter cell during mitosis. Typical centromere chromatin contains two sub-domains: a centromere core flanked by pericentromeric regions. The nucleosomes in centromere core are specialized by the composition of H3-variant CENP-A, which is essential for kinetochore assembly. The length of centromere core varies in species, from around 120bp occupying single nucleosome in budding yeast *Saccharomyces cerevisiae* to megabases-long satellite repeats array occupying hundreds of nucleosomes in humans (Black, Jansen et al. 2007, Aldrup-Macdonald and Sullivan 2014). Pericentromeric region also comprises non-conserved repetitive sequences, from *dg-dh* in *Schizosaccharomyces pombe* to higher ordered tandem repeats (HOR) in human. Heterochromatin formation in the pericentromeric region depends on hypoacetylation and methylation of H3 lysine 9 and associated heterochromatin protein 1 (HP1). This heterochromatin structure is also essential for pericentromeric recruitment and stabilization of cohesin to construct sister chromatid cohesion (Nakayama, Rice et al. 2001, Nonaka, Kitajima et al. 2002, Volpe, Kidner et al. 2002, Smurova and De Wulf 2018). The silencing of the pericentromeric region, which is well established in *S.pombe*, provides a typical model of RNA interference (RNAi) silencing machinery in constitutive heterochromatin regions

(Volpe, Kidner et al. 2002). Repetitive sequences, *dg-dh* repeats (Outer repeats/otr) in *S.pombe*, is transcribed as long non-coding RNA and turned into dsRNA molecules by RNA-directed RNA polymerase complex (RDRC). This RDRC contains an RNA-mediated RNA polymerase Rdp1, a helicase Hrr1, and a poly (A) RNA polymerase Cid12 (Motamedi, Verdel et al. 2004). The Dicer enzyme then cleaves the dsRNA into siRNA, which is captured by Ago1, the orthologue of the human PIWI family, and consequently constitutes transcriptional gene silencing (RITS) complex together with chromodomain containing protein 1 (Chp1) and Tas3 (Verdel, Jia et al. 2004). siRNA induced RITS complex helps to recruit and promote the RDRC complex to further establish a siRNA circular enhance system (Motamedi, Verdel et al. 2004, Sugiyama, Cam et al. 2005). Meanwhile, siRNA induced RITS is also associated with gathering and activating cryptic loci regulator complex (CLRC). Clr4 in CLRC, the orthologue of human methyltransferase SUV39H, can methylate lysine 9 of H3 and consequently engage the binding of the HP1 orthologue Swi6 and the Snf2/Hdac repressive complex (SHREC), which finally result in the establishment and spreading of repressive heterochromatin (Volpe, Kidner et al. 2002, Locke and Martienssen 2009). The SHREC complex, which consists of histone deacetylases Clr1, Clr2, Clr3, and chromatin remodeler Mit1, can accumulate to most types of heterochromatin region via Swi6 to generate transcriptional gene silencing (TGS) (Figure2) (Job, Brugger et al. 2016).

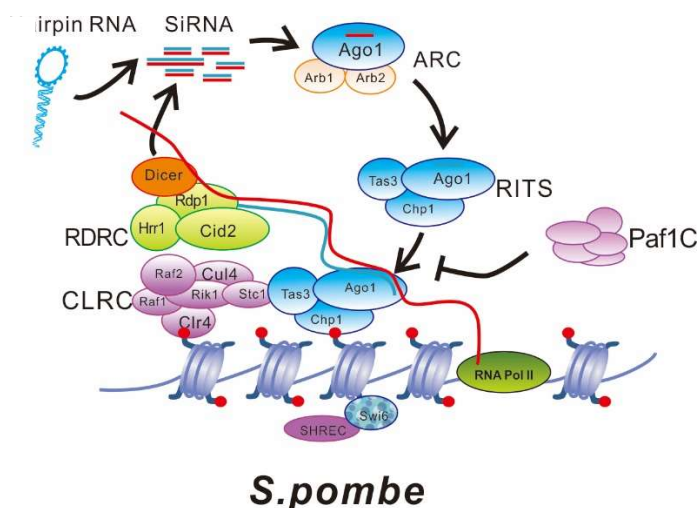


Figure 2. RNAi silencing machinery in *S.pombe*. Long non-coding RNA is transcribed to produce siRNA via the function of the RDRC complex with components, as illustrated. siRNA interacted Ago1 induces the recruitment of the RITS complex, which goes back to help to produce the second strand of lncRNA. Meanwhile, the activated RITS complex recruit CLRC to methylated H3K9. The binding of associated factors, such as Swi6, SHREC finalizes the chromatin compaction and transcriptional silencing (Biscotti, Canapa et al. 2015). Reprinted by permission from Springer Nature Customer Service Centre GmbH.

In mammalian cells, the silencing of the pericentromeric region relies on ncRNA (eg. HOR sequences) induced enrichment of H3K9me3 and the recruitment of HP1. Additional histone mark H4K20me3 also present via SUV4-20H. However, in *Suv39h* knockout mice, the H3K9me3 mark is abolished in the pericentromeric region. Instead, the Polycomb Repressive Complex 2 (PRC2) for H3K27me3 and the BEND3-NuRD complex-mediated pathway facilitate forming the heterochromatin (Saksouk, Simboeck et al. 2015, Nishibuchi and Déjardin 2017).

The telomere is a heterochromatin region located at the end of the eukaryotic chromosome. It is composed of G-rich DNA repetitive sequences and binding of specific proteins. In mammalian cells, this tandem array is formed by numbers of TTAGGG repeats (O'Sullivan and Karlseder 2010). In eukaryotic cells, the telomere is shortened by the “end replication problem” in each cell division process, which leads to the 3' end single-strand overhang and triggers the DNA damage response (Wynford-Thomas and Kipling 1997). Therefore, the main function of the telomere complex is to prevent the loss of genetic information and chromosome fusion during replication. The enzyme telomerase helps to elongate the telomeric region to heal the shortening. It is not usually active in somatic cells, but active in germs cells, stem cells, and many cancer cells (Collins and Mitchell 2002). The telomere binding proteins (also called “shelterin”) in human is similar to *S.pombe*. Among them, telomere binding protein Rap1 is recruited to the telomeric region through double-stranded telomeric repeats binding protein Taz1 in *S.pombe* or TRF1/2 in human to inhibit the double-stranded DNA repairing pathway. Pot1 binds the single-strand end and links to the double-stranded region through the bridge of Ccq1- Tpz1-Poz1 in *S.pombe* and TPP1-TIN2 in human (Kanoh and Ishikawa 2001, Diotti and Loayza 2011, Audry and Runge 2019). In *S.pombe*, the establishment of heterochromatin proteins and H3K9 methylation enrichment in telomeric ends is dependent on Taz1 (Kanoh, Sadaie et al. 2005, Deng, Norseen et al. 2009, Bandaria, Qin et al. 2016).

Taz1, coupled with Ccq1, helps to recruit Clr3 of SHREC complex in the telomeric region. However, Clr3 enrichment is still retained in deletions of Taz1 and Ccq1. It is eliminated in the deletions of Chp1 and Taz1, suggesting an indirect role of RNAi machinery in the silencing of the telomere (Sugiyama, Cam et al. 2007). Epigenetic histone marks are diverse in human telomeric region because of the various subtypes in different cell types. In many human cell lines, the staining of H3K9me3 and HP1 is lowly co-localized with the staining of TRF1 or TRF2, which is further confirmed by ChIP studies, revealing the less enriched heterochromatin mark H3K9me3 in the telomeric region (Cubiles, Barroso et al. 2018).

Another example of constitutive heterochromatin is located in subtelomeric heterochromatin, where *dg-dh* repeats are also present. The silencing at this cen-like loci is also associated with pericentromeric-like RNAi silencing machinery (Chikashige, Kinoshita et al. 1989, Kanoh, Sadaie et al. 2005).

Facultative heterochromatin, as discussed above, is transcriptional silent regions containing genes that can still potentially be converted into euchromatin. In mammalian cells, facultative heterochromatin includes regions such as inactive X chromosome, autosomal imprinted genomic loci, long-range silencing, local gene silencing (Trojer and Reinberg 2007). The molecular features of these silent regions are modifications like DNA methylation, H3K9 methylation, H3K27 methylation, H2AK119 ubiquitination, and related chromatin factors of SUV39H1/2, HP1, Polycomb Repressive Complex 1 and 2 (PRC1 and PRC2) that we will discuss in a later section. Here we want to introduce the silencing machinery in the subtelomeric region and heterochromatin islands as examples, especially in *S.pombe*.

Subtelomere situates on a chromosome adjacent to the telomere ends. The length of the subtelomere region varies in different organisms, which is around ~50kb in *S.pombe* and 100~300kb in human. The composition is also highly variable. In budding yeast, subtelomere encompasses of X and Y elements. In human, subtelomere is even more polymorphic for the mixing of multiple types of segments containing different ORFs. In *S.pombe*, subtelomeric heterochromatin features by the enrichment of H3K9 methylation. It is located between constitutive telomere ends and highly condensed “knob”. In “knob” region, both repressive and active histone modification levels are shallow (compare to bulk heterochromatin and euchromatin) (Buchanan, Durand-Dubief et al. 2009, Matsuda, Chikashige et al. 2015). Deletion of subtelomeric heterochromatin sequences causes silence inbreak from telomere end to knob region, but does not affect the process of mitosis or meiosis. It is suggested that subtelomeric heterochromatin functions as a “buffer area” to prevent heterochromatin spreading from the telomere (Tashiro, Nishihara et al. 2017). Both repressive genes and *dg-dh* repeats present in the subtelomeric heterochromatin region. Telomeric associated sequence (TAS) is also found in subtelomere within around 6kb to the telomere with a low nucleosome occupancy. The silencing here is controlled by telomeric Ccq1 (van Emden, Forn et al. 2019). Therefore the silencing in subtelomeric heterochromatin is based on several types of machinery. The silencing for the repressive genes in subtelomeric regions requires hypoacetylation via deacetylase, such as Clr3, Sir2. It is also essential for the recruitment of CLRC (especially Clr4) and related methylation on H3K9 in order to recruit Swi6. The assembly of Swi6 and methylated H3K9 steadies the gathering of Clr4, which builds the platform of the heterochromatin structure through interacting

with other heterochromatin components to develop transcriptional gene silencing (TGS) (Buscaino 2019).

Facultative heterochromatin islands in fission yeast are pinpointed through mapping the enrichment of H3K9 methylation in chromosomal euchromatin bodies (Zofall, Yamanaka et al. 2012). A subset of facultative heterochromatin islands, known as “determinant of selective removal” (DSR) islands, contains meiotic genes that can only express during meiosis. DSR islands are silenced through RNA degradation machinery by RNA elimination complex, together with heterochromatin Clr4 associated transcriptional silencing (Zofall, Yamanaka et al. 2012). A recent 3D architecture analysis in *Drosophila* showed that membrane-less pericentromeric heterochromatin (PCH) domains interact with heterochromatin islands interspersed in euchromatin. It revealed the potential crosstalk between heterochromatin domains in space (Lee, Ogiyama et al. 2020).

1.1.1.3 Chromatin dynamics

Euchromatin and heterochromatin enable mutual conversion under the regulation of many factors, such as histone modification enzymes, ATP dependent chromatin remodelers, transcriptional factors (Figure 3) (Trojer and Reinberg 2007). These regulations can also be influenced by each other. According to our interests, we focus more on the heterochromatin side. Abolishment on RNAi dependent/ independent silencing machinery (detailed as discussed above) and correlated factors, can disrupt the assembly and maintaining of heterochromatin structure and turn it into active regions. Another way of the transition between heterochromatin to euchromatin is to disturb the heterochromatin spreading.

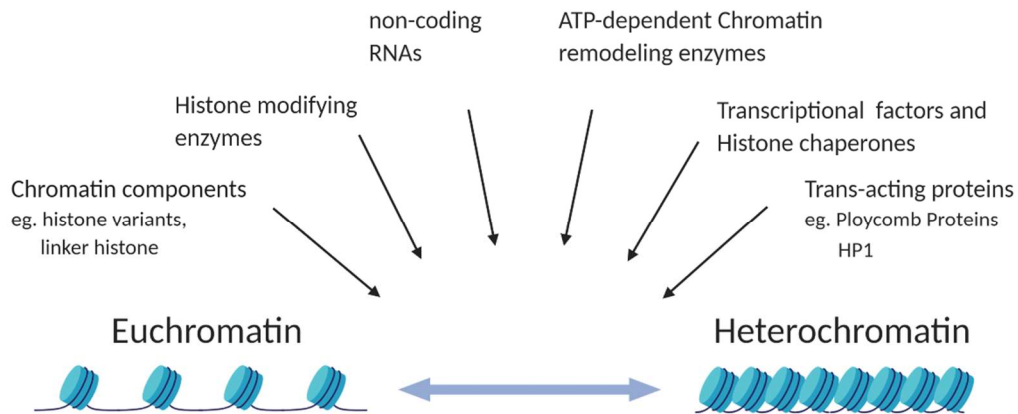


Figure 3. Euchromatin and Heterochromatin can mutually convert. The transition of chromatin compaction and transcription between these two types of chromatin is regulated by several types of factors, as mentioned in the illustration. It is adapted to the figure from Trojer and Reinberg 2007.

In the 1930s, Muller described the position effect variegation (PEV) on eye color controlled gene in *Drosophila melanogaster*: the expression of the white gene located in euchromatin leads to the red eyes. Translocation of the gene near pericentromeric heterochromatin caused by chromosome rearrangement results in the transcriptional silence and the phenotype of mottled eyes (Muller 1930, Elgin and Reuter 2013). Another similar phenomenon was later observed in budding yeast *Saccharomyces cerevisiae*, where reporter genes became repressed by being placed in the region near telomeres, so-called telomere position effect (TPE) (Gottschling, Aparicio et al. 1990, Mondoux and Zakian 2006). Both PEV and TPE have been found in many other organisms, from plant to mammal, indicating that constitutive heterochromatin can spread its repressive state to the neighbor's active genes (Dillon and Festenstein 2002, Elgin and Reuter 2013). This silencing expansion plays a crucial role in altering the transcription of regulatory genes during the cellular developmental process (Lippman, Gendrel et al. 2004). The abnormal heterochromatin spreading can also suppress the transcription of genes improperly and consequently induce disease (Kleinjan and Lettice 2008).

Consequently, cells have developed the structure of chromatin boundary in order to prohibit the expansion of heterochromatin to euchromatin. Generally, the boundary consists of particular DNA elements “insulator” flanking at heterochromatin domain and relevant proteins (Wei, Liu et al. 2005, Valenzuela and Kamakaka 2006, Wang,

Lawry et al. 2014). In *S.pombe*, transfer RNA (tRNA) gene and IRC element function as insulators in centromere (Matsuda, Asakawa et al. 2017). Transcriptional factor TFIIC and Histone demethylase LSD 1 act virtually for the boundary of tRNA genes (Lan, Zaratiegui et al. 2007). Negative regulator of subtelomere Epe1 that has the histone demethylation JmjC domain and bromodomain protein Bdf2 for histone acylation are required to IRC element boundary (Buchanan, Durand-Dubief et al. 2009, Wang, Tadeo et al. 2013).

Inversely, wild type heterochromatin domains should also be protected to maintain its natural function. A chromatin remodeler Fun 30 protein Fft3 in *S.pombe* as an example, sits at tRNA and IRC elements boundaries of centromere and LTR element boundary of subtelomere to shield the expansion from euchromatin (Steglich, Strålfors et al. 2015).

A recent study in *S.pombe* analyzed the heterochromatin proteins through Swi6-associated proteome and euchromatin proteins through acetylated histone binding Bdf1/2 associated proteome. They revealed that euchromatin and heterochromatin proteomes have over-lapped proteins besides the fundamental chromatin component like histone proteins, but also unique proteins for each of them. If these shared proteins between heterochromatin and euchromatin help the inter-conversion is still unclear (Iglesias, Paulo et al. 2020).

1.1.2 Histone modifications

Post-translational modifications (PTM) usually occur at the N terminal tails of histone proteins, which play crucial roles in modulating chromatin structure and regulating gene expression. PTM on histones was firstly correlated to functional outcome in 1964 when Allfrey described his observation that high level of histone acetylation was relevant to the less inhibition of RNA synthesis (Allfrey, Faulkner et al. 1964). Till now, many histone modifications, such as acetylation, methylation, ubiquitination, phosphorylation, ADP ribosylation, sumoylation, and even lactylation reported last year, have been found at various residues of different histone proteins. They are correlated with transcription switch on/off and consequently involved in diverse cellular processes (Kouzarides 2007, Zhang, Tang et al. 2019). Most of the histone modifications are dynamically regulated by “writer”-enzymes adding the modifying groups and “eraser”-enzymes removing the modifying groups (Nicholson, Veland et al. 2015). Here I list acetylation and methylation on residues of canonical H3 and H4, together with their writers, erasers, and proposed function (Table 1).

Histone modifications carry out functions with two mechanisms mutually dependent or independent. 1) Some histone PTMs influence the chromatin structure directly through disturbing the electronegativity of the nucleosome. For instance, acetylation and phosphorylation reduce the positive charge through neutralizing the histone tail and introducing a negative charge respectively, which may disrupt the interaction between DNA and histones. Ubiquitination affects the architecture of nucleosome because of the large size of the multi-ubiquitins attachment. 2) Histone PTMs influence the chromatin structure indirectly through recruiting other factors (Bannister and Kouzarides 2011, Lawrence, Daujat et al. 2016). Some protein domains can specifically recognize and bind their target modified histones. For instance, PHD domain, chromodomain, tudor domain, and MBT domain bind to methylated lysine, while bromodomain binds to acetylated lysine. The proteins embracing these recognizing domains play diverse roles in modulating chromatin structure. For instance, it can be ATP-dependent chromatin remodelers, such as CHD1 with chromodomain targeting H3K4me_{2/3}. It can be interactive chromatin factors, such as heterochromatin protein HP1 with chromodomain targeting H3K9me_{2/3}. It can also be histone modifying enzyme itself, such as GCN5 of histone acetyltransferase HATs family targeting acetylated lysine on H4 (Sanchez and Zhou 2009, Bannister and Kouzarides 2011, Yun, Wu et al. 2011).

Histone modifications also affect each other (Figure 4). As an example, we have mentioned in the previous section that hypoacetylation from HDACs is essential for installing H3K9me₃ in heterochromatin regions. Another example is H3K27me₃ and H2AK119ub from the Polycomb Repressive Complex (PRC), which we will discuss in detail later.

Histone	Modified Residuals	Modification	Writer	Eraser	Proposed Function
H3	Lys4 (<i>S. cerevisiae</i>)	Acetylation	Esa1, Hpa2		transcriptional activation
	Lys9	Acetylation	Gcn5, SRC-1		transcriptional activation, histone deposition
	Lys14	Acetylation	Gcn5, PCAF, Esa1, Tip60, SRC-1, Elp3, Hpa2, hTFIIIC90, TAF1, Sas2, Sas3,	Class I :HDAC1,2,3,8 /Rpd3 <i>S. cerevisiae</i> /Clr6 <i>S.pombe</i> Class IIa :HDAC4,5,7,9 /Had1 <i>S. cerevisiae</i> /Clr3 <i>S.pombe</i>	histone deposition, transcriptional activation, DNA repair , RNA polymerase II & III transcription
	Lys18	Acetylation	Gcn5, p300/CBP	Class IIb :HDAC6,10	transcriptional activation, DNA repair and replication
	Lys23	Acetylation	unknown, Gcn5, SAs3, p300/CBP	Class III : SIRT1,6,7/Sir2 yeast	histone deposition , transcriptional activation, DNA repair
	Lys27	Acetylation	Gcn5		transcriptional activation
	Lys56 (<i>S. cerevisiae</i>)	Acetylation	Spt10		transcriptional activation, DNA repair
	Lys4	Methylation	Set1 (<i>S. cerevisiae</i>), MLL, ALL-1, SMYD,SET7/9, PRDM9	Jhd2(<i>S. cerevisiae</i>), LSD, NO66, JARID1	permissive euchromatin (di-Me), transcriptional activation
	Arg8	Methylation	PRMT5	JMJD6	transcriptional repression
	Lys9	Methylation	Suv39h,Clr4(<i>S.pombe</i>), G9a,GLP, SETDB1, PRDM family	Rph1(<i>S. cerevisiae</i>), JHDM2, JHDM3, PHF8	transcriptional silencing (tri-Me), transcriptional repression, genomic imprinting, transcriptional activation
	Arg17	Methylation	CARM1	PAD4	transcriptional activation
	Lys27	Methylation	Ezh2, G9a	unkown, UTX, JMJD3, KIAA1718, PHF8	transcriptional silencing, X inactivation (tri-Me)
	Lys36	Methylation	Set2(<i>S. cerevisiae</i>), SET2D, NSD, SMYD2, ASH1L, SETD3	JHDM1 and JHDM2 family	transcriptional activation (elongation)
	Lys79	Methylation	Dot1	unknown	transcriptional activation (elongation), checkpoint response

Histone	Modified Residuals	Modification	Writer	Eraser	Proposed Function
H4	Lys5	Acetylation	Hat1, Esal, Tip60, ATF2, Hpa2, p300	HDAC3	histone deposition, transcriptional activation, DNA repair
	Lys8	Acetylation	Gcn5, PCAF, Esal, Tip60, ATF2, Elp3, p300	Class I :HDAC1,2,3,8 /Rpd3 <i>S. cerevisiae</i> /Clr6 <i>S.pombe</i>	transcriptional activation, DNA repair
	Lys12	Acetylation	Hat1, Esal, Tip60, Hpa2, p300	Class IIa :HDAC4,5,7,9 /Had1 <i>S. cerevisiae</i> /Clr3 <i>S.pombe</i>	histone deposition, telomeric silencing, transcriptional activation, DNA repair
	Lys91 (<i>S. cerevisiae</i>)	Acetylation	Hat1/Hat2	Class IIb :HDAC6,10 Class III : SIRT1,6,7/Sir2 yeast	chromatin assembly
	Arg3	Methylation	PRMT1	PAD4	transcriptional activation
	Lys20	Methylation	PR-Set7, Suv4-20h, Set9 (<i>S. pombe</i>)	PHF8,PHF2	transcriptional silencing (mono-Me), heterochromatin (tri-Me),transcriptional activation, checkpoint response
	Lys59	Methylation	unknown, CK2	unkown	mitosis, chromatin assembly, DNA repair

Table 1. Acetylation and Methylations on residues of canonical H3 and H4 was conclude in the table, together with the “writer” ,“eraser” and their proposed function. Most presented histone modification are conserved from human to yeast. Specie specific modification was labelled as in brackets(Nicholson, Veland et al. 2015, Hyun, Jeon et al. 2017).

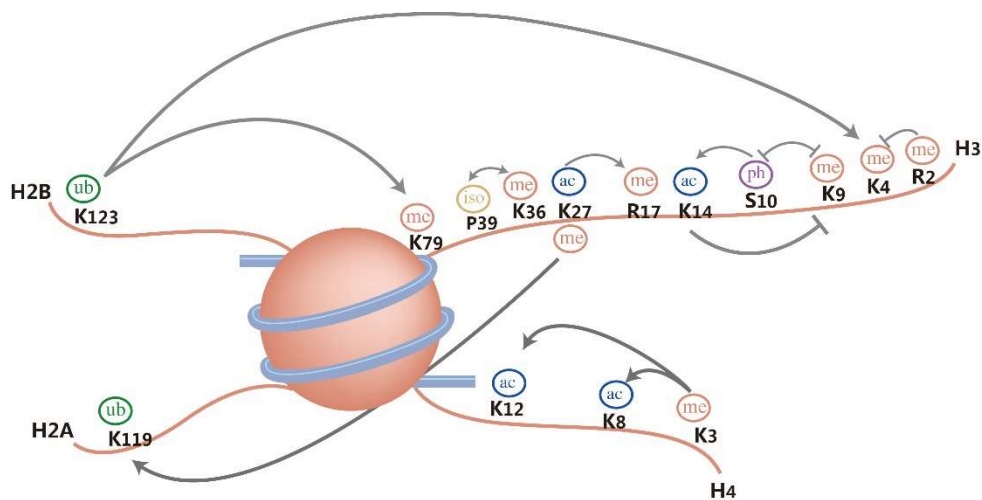


Figure 4 Crosstalk between histone modifications. Locations of each histone modification is as labeled in the figure. Arrow head represents positive effect, while flat head represents negative effect (Bannister and Kouzarides 2011). Reprinted by permission from Springer Nature Customer Service Centre GmbH.

1.1.2.1 H3K9me2/3

H3K9 can be methylated with attaching 1/2/3 methyl groups. Different levels of methylated H3K9, as well as the genomic locations, have distinct functional outcomes. H3K9me1 abundant at promoters exert as a transcriptional activator, while H3K9me2 and me3 enriched in silent region/gene bodies exert as a transcriptional repressor, and are proposed as conserved heterochromatin marks (Zhang, Cooper et al. 2015).

The methylation on H3K9 is loaded step-wise and is dependent on different histone methyltransferases (HMTs). In *Caenorhabditis elegans*, single deletion of MET2, the orthologue of human SETB1, results in reduced H3K9me1/2/3 (10~30% left), and single deletion of SET-25 results in lack of H3K9me3 but wild type level of H3K9me1/2. Deletions of both genes resulted in the elimination of all H3K9 methylation marks. It indicates that H3K9 is mono-/di- methylated by MET2 and subsequently methylated by SET-25 for the third methyl group. This step-wise methylation is also involved in the process of a continuous self-reinforcing movement to perinuclear landing, suggesting potential alternative roles of H3K9me2 and me3 (Towbin, González-Aguilera et al. 2012). In *S.pombe*, Clr4 is the only HMT on H3K9 methylation. *In vitro* binding assay has shown the most binding affinities of its chromodomain to H3K9me3 compared to unmodified H3, H3K9me1/2 (Zhang, Mosch et al. 2008). Point mutations F449Y in the catalytic SET domain of Clr4 dramatically increased H3K9me2 level but almost lose H3K9me3 level at pericentromeric of fission

yeas. It reduces the enrichment of both me2 and me3, and a consequent silencing defect in the subtelomeric heterochromatin region. Surprisingly, the transcription of the pericentromeric region is active with enriched H3K9me2. It suggests distinct roles of H3K9me2 and me3 in RNAi machinery, and only H3K9me3 provides the real transcriptional gene silencing (Jih, Iglesias et al. 2017). However, in the wild type, how this step-wise methylation is regulated is still unclear.

Study in human cells revealed that H3K9me2 demethylation is mediated by the Jumonji domain-containing 1A (JMJD1A) dimers also via two steps (Goda, Isagawa et al. 2013). In fission yeast, a higher rate of histone turnover that replaces modified histone by newly synthesized histones, is important for demethylation on H3K9. JimC-domain protein Epe1 and RNA PolII associated complex PafC promote histone turnover in heterochromatin regions (Aygün, Mehta et al. 2013, Oya, Durand-Dubief et al. 2019).

1.1.2.2 PRC complex

Generally, polycomb proteins construct two complexes: Polycomb Repressive Complex 1 (PRC1) and Polycomb Repressive Complex 2 (PRC2). PRC2 complex consists of core members, including Embryonic Ectoderm Development factor 2 (EED2), zinc finger protein SUZ12, histone methyltransferase EZH1/2, and other proteins, including histone deacetylase HDAC, histone chaperones RbAp46/48. Both EZH1 and EZH2 are responsible for tri-methylation on H3K27 (Margueron and Reinberg 2011, Aranda, Mas et al. 2015). Besides the core members, PCL, EPOP, and PALI1/2, as additional components, form non-canonical PRC2.1, while Jumonji family members associated with histone demethylation JARID2 and zinc finger protein AEBP2 constitute the non-canonical PRC2.2. The non-canonical PRC2 cooperate to affect global SUZ12 recruitment(Healy, Mucha et al. 2019).

Canonical PRC1 complex is constituted of five subset proteins, including RING1A/B, PCGF, PHC, CBX, and SCM. Various non-canonical PRC1 have been reported. Beside RINGA/B and alternative PCGF proteins, non-canonical PRC complex harbor Ring1B-Yy1 binding protein RYBP or its homolog YAF2 together with distinct components (Figure 5) (Aranda, Mas et al. 2015).

Ubiquitination on lysine 119 of H2A is not a conserved HTM in eukaryotes. Mono-ubiquitinated H2AK119, known as a transcriptionally repressive mark, is accomplished by two E3 ubiquitin ligases: 2A-HUB, which is a RING finger protein, and RING1A/B, which is the core member of PRC1. However, it is reported that the repression caused by these two enzymes targets different genes. 2A-HUB can mono-ubiquitinate H2A

both *in vivo* and *in vitro*. Together with the N-Cor complex, 2A-HUB negatively regulates the transcription of a specific subset of chemokine genes in macrophages (Zhou, Zhu et al. 2008). H2AK119ub at the promoter-proximal regions prevents the recruitment of facilitates chromatin transcription (FACT) complex and blocks the RNA PolII release at the beginning of elongation (Van Kruijsbergen, Hontelez et al. 2015). PRC mediated H2AK119ub enforces an uncommon conformation at silent bivalent genes in embryonic stem cells. (Stock, Giadrossi et al. 2007).

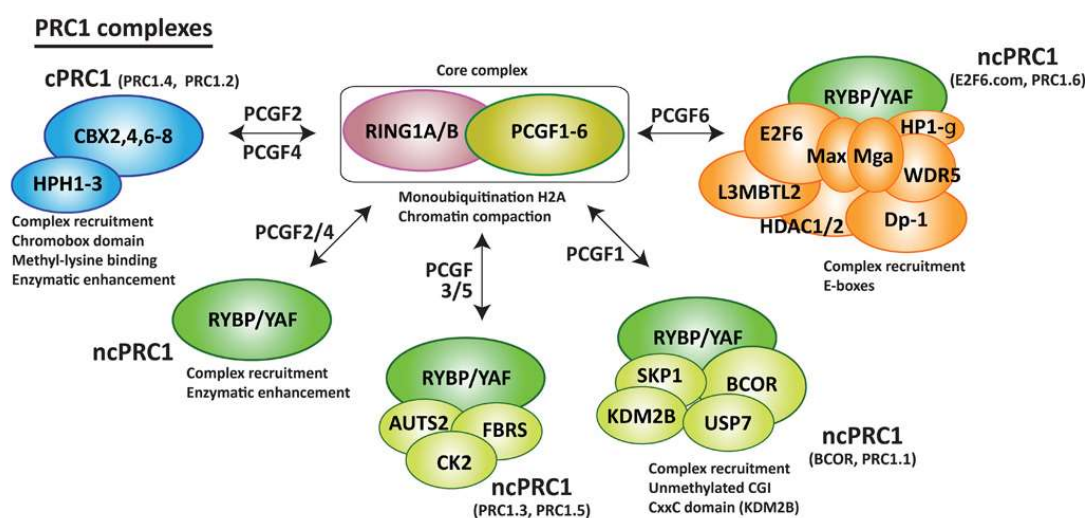


Figure 5 Components of canonical PRC1 complex (cPRC1) and non-canonical PRC1 complex (ncPRC1). The core complex can associate with distinct PCGF proteins to incorporate with alternative members, as illustrated. PCGF2 and PCGF4 in cPCR1/ncPRC1 recruit RYBF/YAF. PCGF3 and PCGF5 are for PRC1.3 and PRC1.5, while PCGF1 is for PRC1.1 and PCGF6, is for PRC1.6. Reprinted with permission from AAAS (Aranda, Mas et al. 2015).

The classical model of polycomb mediated repression is: PRC2 firstly introduces H3K27me3 at the target promoter region. CBX recognizes H3K27me3 to recruit PRC1 that subsequently catalyze H2AK119ub. Moreover, methylation on H3K27 prohibits the acetylation in the same loci. In mammalian cells, the RNA of *Xist* in X chromosome initiates the recruitment of PRC2 and finalize the X chromosome inactivation. Chromatin enriched of PRC1 and PRC2 is scattered in the euchromatin but not heterochromatin in an overlapped or non-overlapped way (Sugiyama, Cam et al. 2005, Vidal and Starowicz 2017). The ncPRC regulates gene transcription more in an H3K27me3 independent way. For instance, histone demethylase KDM2B in PRC1.1, containing a DNA binding CxxC domain, can recognize the unmethylated CpG islands of active genes and consequently guides the recruitment of PRC1.1 (van den Boom,

Maat et al. 2016). A recent study showed that cPRC1 but not ncPRC1 potentially control the repression inheritance through genome replication and cell division (Moussa, Bsteh et al. 2019).

The PRC complexes repress homeotic genes and play crucial roles in embryonic development, stem cell differentiation, as well as hematopoiesis. ncPRC1 has been shown to dynamically accumulate during neural cell development. For instance, PRC1.6 is most abundant in the embryonic stem cells (EMCs), and decreases following the differentiation to neural progenitor cells (NPCs). PRC1.5 exhibits the opposite abundance trend. In humans, the misregulation of the PRC complex occurs in many cancers (Loubiere, Martinez et al. 2019).

1.1.3 ATP dependent chromatin remodelers

The chromatin remodelers are enzymes that can hydrolyze ATP to regulate chromatin structure through several mechanisms: Nucleosome unwrapping, nucleosome repositioning/spacing, nucleosome ejection, histone eviction or exchange of histone variants (Lorch, Maier-Davis et al. 2010). Most chromatin remodelers share a conserved ATPase domain harbored by RNA/DNA helicase superfamily 2. According to the non-catalytic domains and functional analysis, chromatin remodelers have been categorized as four subfamilies: imitation switch (ISWI), chromodomain helicase DNA-binding (CHD), switch/sucrose non-fermentable (SWI/SNF) and INO80 (Figure 6) (Clapier, Iwasa et al. 2017). Remodelers in SWI/SNF family contain a HSA (Helicase-SANT) domain located at N-terminal and a pair of bromodomain at the C-terminal. The HAS domain is predicted to bind DNA, and the bromodomain, as mentioned, recognizes acetylated histone tails. ISWI protein contains a HAND-SANT-SLIDE (HSS) domain located at C-terminal, which binds to unmodified H3 and linker DNA. CHD family is characterized by the two tandem chromodomains presenting at N terminal of protein. A regulatory coupling region that can negatively regulate remodeling function of enzyme, resides adjacent ATPase domain. Similar to the SWI/SNF family, Ino80 also owns an HSA domain at N-terminal, while the large insertion between the two sub-domains of ATPase is various at length through species. INO80 promotes the exchange of H2A-H2B canonical dimers by variant H2A.Z-H2B, mediated by SWI2/SNF2-related protein p400, SWR1 chromatin remodeling complex, and Snf2-related CBP activator protein (SRCAP) complex (Clapier and Cairns 2009, Clapier, Iwasa et al. 2017).

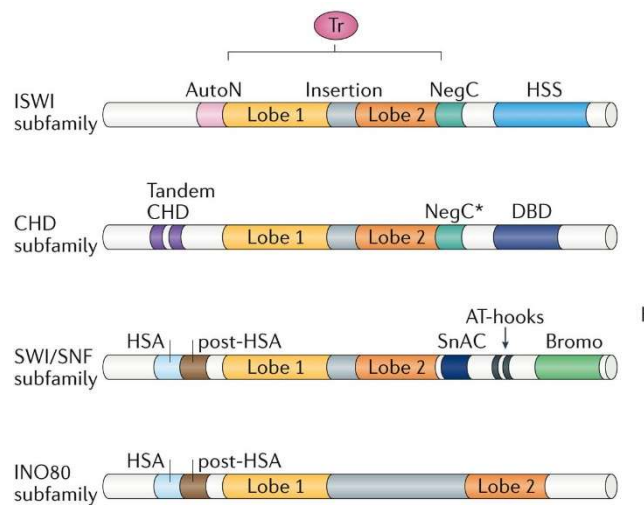


Figure 6. Domain components of four chromatin remodeler families. All four families shared catalytic ATPase-Translocase domain (Tr) contains two-lobe subdomains with small or large insertions. Remodelers can be classified into four subfamilies based on their domain organization. Domains marked here are as explained in the above text section. NegC* in CHD family is structurally similar to the ISWI negative regulator of coupling (NegC) in ISWI (Clapier, Iwasa et al. 2017). Reprinted by permission from Springer Nature Customer Service Centre GmbH.

1.1.3.1 CHD chromatin remodeling family

The CHD remodelers, existing in all eukaryotic species, facilitate chromatin compaction and positive or negative gene transcription. Hence the remodelers are essential for many fundamental cellular processes, such as DNA repair, cell proliferation, and cell development. The loss or malfunction of CHD remodelers presents in many developmental diseases and cancers (Riedmann 2012).

Members of CHD family can be further categorized into three classes, including CHD1-2 for class I, Mi2/NURD (CHD3-5) for class II and CHD6-9 for class III (Murawska and Brehm 2011, Mills 2017). ScCHD1 (*S.cerevisiae* CHD1) is the only CHD remodeler in the budding yeast, while fission yeast has three: Mit1, Helicase-Related protein 1 and 3(Hrp1 and Hrp3) (Längst 2013). CHD1 remodeler is featured by an extra DNA binding domain at C-terminal to interact with AT-rich DNA sequences CHD3-5 belongs to the Mi2/NURD subfamily and contain two particular PHD (plant homeodomain) zinc finger located at N-terminal side of chromodomain, which can bind to methylated histones as mentioned before. Class II CHD remodelers are also featured

by domains at C-terminal for unknown function (DUF). Class III subfamily member contains a SANT motif for DNA binding and a tandem BRK (Braham and Kismet) domain at the C-terminal (Clapier, Iwasa et al. 2017).

1.1.3.1.1 CHD1 and CHD2

Human CHD1 is recruited to the active chromatin via interacting H3K4me_{2/3} with its chromodomain. H3K4me_{2/3} abounds in the transcriptional start site (TSS) of the active gene, as well in the coding region. The CHD1 binds to H3K4me₃ enriched region to attach SNF2H, the FACT complex, the transcriptional elongation associated PAF complex as well as the U2 snRNP complex, and hence enhance the pre-mRNA splicing and transcriptional elongation (Sims III, Millhouse et al. 2007). The function of CHD2 is highly correlated to CHD1. The coupled recruitment of CHD1 and CHD2 regulates the chromatin accessibility and histone H3/H3.3 occupancy at active chromatin regions (Siggens, Cordeddu et al. 2015). CHD1 is required for embryonic stem cell ECS differentiation, while CHD2 determines myogenic cell fate via deposition of H3.3. It has been shown that inactivation of CHD1 and CHD2 occurs in prostate cancer and leukemia, respectively (Mills 2017).

CHD1, together with ISW1, is required to maintain the regular nucleosome spacing around promoters in budding yeast. ScCHD1 lacks several conserved residues in chromodomain and hence cannot bind H3K4me₃. It recruits to transcriptionally active genes via interacting with elongation factors Spt4-Spt5 proteins, Paf1, and FACT (Mills 2017, Lin, Du et al. 2020). Both human CHD1 and ScCHD1 regulate cohesion and hence affect the chromatin compaction (Boginya, Detroja et al. 2019). Some studies showed that CHD1 is a component of conserved HAT complex SAGA-SLIK that favors H3 acetylation and H2B de-ubiquitination (Pray-Grant, Daniel et al. 2005). The chromodomains in ScCHD1 block their ability to use DNA as substrate in order to maintain the targeting preference of nucleosome (Hauk, McKnight et al. 2010). A small region (named coupling region) between ATPase and the DNA binding domain affect the nucleosome spacing function of ScCHD1 but not nucleosome assembly. This observation offers the hypothesis that the remodeling process of ScCHD1 is divided into two sequential steps, promoting nucleosome assembly and exerting nucleosome spacing, both of which are ATP dependent (Torigoe, Patel et al. 2013). In the fission yeast, human CHD class I has two orthologues Hrp1 and Hrp3, and both of them promote the nucleosome positioning in the coding region and inhibit the cryptic transcription (Pointner, Persson et al. 2012, Touat - Todeschini, Hiriart et al. 2012).

1.1.4 Mechanisms of nucleosome assembly and remodeling

In vitro, nucleosomes can be spontaneously assembled onto a DNA molecule with supplied histone octamers. This process is based on the contract charge properties of DNA and protein. *In vivo*, two significant factors are involved in the nucleosome assembly/disassembly in eukaryotic cells:

1) Histone chaperone, such as nucleosome assembly protein 1 (Nap1) in *S.pombe* (Andrews, Chen et al. 2010)

2) ATP dependent motor proteins, which do not only promote nucleosome assembly via wrapping DNA around the histones but also remodel chromatin by altering the positions of nucleosomes along with the DNA, such as CHD1 (Torigoe, Patel et al. 2013).

Although the remodelers variously affect the nucleosome/chromatin, they still share some standard features related to their conserved snf2-like ATPase domain that contains two RecA-like lobes (DE_{xx}, lobe 1; HELIC_c, lobe 2). They all prefer to use nucleosomes as substrates rather than DNA. Based on the conserved catalytic domain, one shared DNA translocation mechanism was discovered: Both RecA-like lobes (translocase domain) in ATPase domain bind superhelical location 2 (SHL2) of DNA in a nucleosome (two DNA helical turns away from the nucleosome dyad). Once bound to the SHL2, the translocase domain performs a DNA translocation with direction 3' to 5' by dragging in DNA from the proximal side of the nucleosome (the DNA entry site, ~50 bp from the translocase) and pushing it towards the distal side (the DNA exit site, ~97 bp from the translocase). DNA translocation exerts this process via creating loops on the naked DNA strands with superhelical torsion at both sides of the translocase domain. The translocase domain keeps on attaching to the octamer during DNA translocation, probably through a histone-binding domain (HBD). In SWI/SNF, the HBD domain is known as Snf2 ATP coupling (SnAC). In ISWI and CHD, the HBD domain resides within the ATPase domain (Deindl, Hwang et al. 2013, Clapier, Iwasa et al. 2017).

The shared DNA translocation mechanism is regulated by alternative non-catalytic domains in remodelers and associated proteins to achieve different outcomes. For instance, the actin-related protein (ARP) module, as HAS and post HAS domains in SWI/SNF, facilitates nucleosome ejection. The position of the neighbor nucleosome is important for nucleosome spacing in the ISWI family that determines the length of the linker DNA by HSS domain (Struhl and Segal 2013, Clapier, Iwasa et al. 2017). Recent electron microscope (EM) studies revealed the architecture of the nucleosome-bound CHD1 structure, suggesting that CHD1 binds to nucleosomes through the DBD domain on linker DNA and ATPase domain on SHL2 (Lin, Du et al. 2020). It creates a twisted

DNA translocation in ATP-bound states. These studies explain the previous observation of nucleosome sliding towards the center position by CHD remodelers *in vitro* assays. This property finally generates the nucleosome array on chromatin (McKnight, Jenkins et al. 2011).

1.2 Fission yeast *Schizosaccharomyces pombe*

Fission yeast has been utilized in the lab since the 1950s as a model organism for investigating various eukaryotic cellular and molecular functions. The studies based on *S.pombe* are driven by its conserved biological processes, some of which have been lost in budding yeast *Saccharomyces cerevisiae*. Fission yeast is a unicellular eukaryotic organism that is ~7–14 μm long and ~4 μm wide. It proliferates via medial cell fission. The cell cycle of wild type fission yeast is rapid with generation time in vegetative growth around 2 hours at normal culturing temperature 30°C in complex and minimal media. Fission yeast cells produce mating types with relevant genotypes: h⁺ and h⁻ cells, which produce P factor and M factor respectively. Both of P and M factors are mating pheromone that can stimulate associated downstream pathways in order to induce the sexual agglutination and crossing (Seike, Nakamura et al. 2013). When there is limited nutrition in the environment, yeast cells will arrest in the G1 phase of the mitotic cell cycle. h⁺ and h⁻ cells conjugate to form a diploid zygote. If the nutrition/nitrogen is re-supplied in this step, the diploid zygote will go back to the mitotic cell cycle. Otherwise, it will continue with the meiotic cell cycle (Hayles and Nurse 1989, Hayles and Nurse 2018).

S.pombe has a small genome around 14MB, consisting of three chromosomes that harbor a total of 2510 protein-coding genes with 5300 introns (Chikashige and Hiraoka 2002). Genome organization of *S.pombe* shares several conserved features with higher eukaryotes compare to budding yeast, such as regional centromere, and similar telomere. Subtelomeric regions reside at both ends side of Chromosome I and II, but not chromosome III (rDNA). As we discussed above, heterochromatin in *S.pombe* is assembled and maintained via RNAi dependent and independent machinery, which is also similar to human. RNA splicing complex and process is also more similar to human compared to budding yeast. Because of the small genome, the mating phenomenon, and rapid growth, fission yeast cells are easy to genetically manipulate for required methods and studies (Muers 2011, Fair and Pleiss 2017, Hayles and Nurse 2018).

1.3 Hematopoietic development

1.3.1 Hematopoiesis

Hematopoiesis is the developmental process of continually producing differentiated blood cells from hematopoietic stem cells (HSCs) with various functions, such as oxygen transferring and immune defense. In adults, HSCs are found in the bone marrow (BM), and are characterized by self-renewal capacity. It generates all lineages of blood cells. The differentiation of HSCs follows two major lineages—Myeloid and lymphoid. HSCs produce common myeloid progenitors (CMP) and common lymphoid progenitors (CLP). Myelopoiesis initiates from CMP and produces megakaryocytes, erythrocytes, mast cells, and myeloblasts that latter differentiate into monocytes/macrophages and granulocytes- basophil, neutrophil, and eosinophils. Granulocytes are a group of white blood cells in the immune system and characterized by the presence of granules in their cytoplasm and polymorphic nuclear. Lymphopoiesis initiates from CLP and finally differentiates into matured lymphocytes that mainly locate in lymph, including natural killer cells, T cells, and B cells (Figure 7). However recent single-cell omics profiling reveals heterogeneity of HSCs and progenitors (Ceredig, Rolink et al. 2009, Zhang, Gao et al. 2018).

The developmental fate and lineage choice in hematopoiesis are influenced by diverse sets of cytokines, chemokines, receptors, and intracellular signaling molecules. Some transcriptional factors can regulate the development of hematopoietic cells, such as GATA-1 for erythropoiesis and PU.1 for myelopoiesis (Burda, Laslo et al. 2010). Transcriptional factors BACH 1/2 are associated with the lineage direction of erythroid-myeloid progenitors and lymphoid-myeloid progenitors as a response to the environmental changes (Kato and Igarashi 2019). Presence of different CD (cluster of differentiation) markers on the cell surface are used to characterize the cell fates. Taking myeloid lineage as an example: CD34⁺/38⁻ for HSCs, CD34⁺/127⁺ for CMPs, CD41⁺/42⁺ for megakaryocytes, and CD11B⁺/13⁺/16⁺/18⁺ for matured granulocytes (Altiindağ and Baykan 2017).

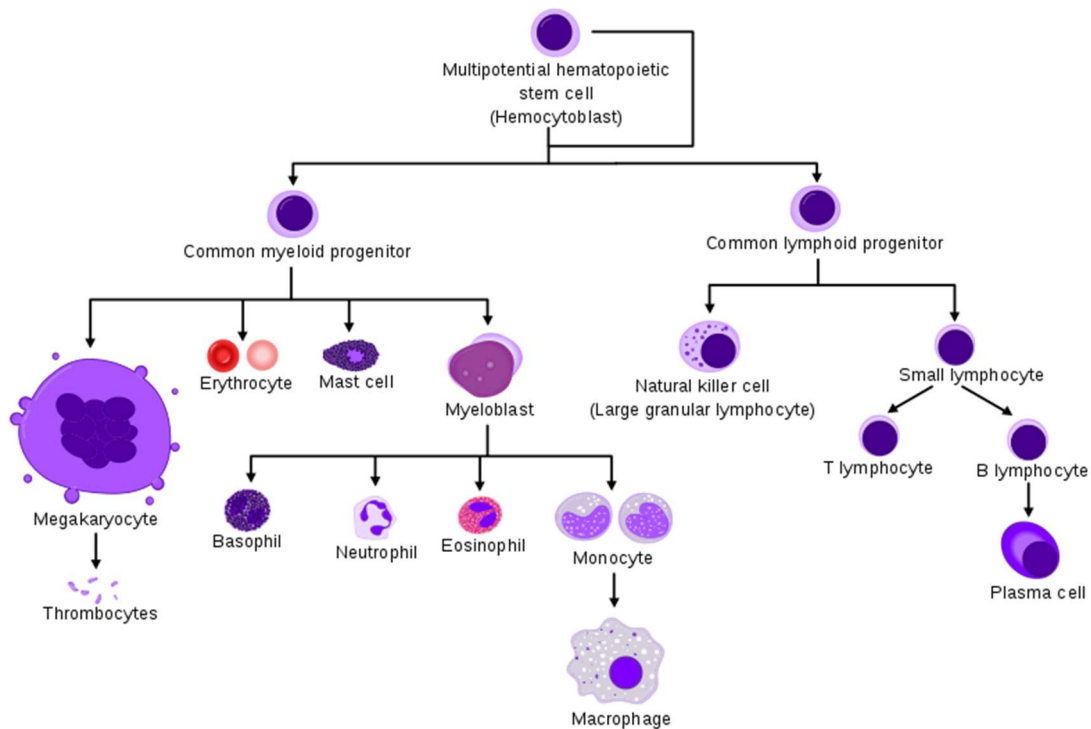


Figure 7. Classical hematopoietic hierarchy. Blood cell differentiation initiates from multi-potential hematopoietic stem cells and evolves into two major lineages-myeloid and lymphoid. Hematopoietic stem cell generates common myeloid progenitors, which differentiate into mature megakaryocytes, erythrocytes, basophils, neutrophils, eosinophils, monocytes /macrophages, and common lymphoid progenitors, which differentiate into mature nature killer cells, T cells and B cells. Reuse permission from A. Rad and M. Häggström. CC-BY-SA 3.0 license.

1.3.2 Epigenetics of hematopoietic development

Epigenetics are changes that can switch on/off gene transcription without altering the DNA sequences. Several epigenetic mechanisms have been described, including DNA methylation, histone modification, chromatin remodeling by remodeling enzymes, and RNA interference (Weinhold 2006).

In general, global DNA methylation changes dynamically during hematopoietic cell development. Overall, the methylation level increase during lymphoid differentiation, while erythroid development is more associated with reduced global methylation (Ji, Ehrlich et al. 2010, Farlik, Halbritter et al. 2016). CpG methylation at developmental regulatory genes' promoters is the critical epigenetic regulation in both lineage-choice and differentiation within hematopoiesis. For instance, DNA methylation/demethylation patterns present for activating lineage-specific genes also exists to suppress transcription of other lineages (Hodges, Molaro et al. 2011). DNA

methylation also involves in regulating the sensitivity to differentiation factor through methylation in the downstream regulatory sites of crucial transcriptional factors, such as the binding site of GATA-1, RUNX1 (Suzuki, Shimizu et al. 2017). DNA methylation in human is carried out by sets of DNA methyltransferases: DNMT1, DNMT3A, and DNMT3B. DNMT3A and DNMT3B are responsible for methylated CpG while Dnmt1 is responsible for maintaining the existing DNA methylation (Okano, Bell et al. 1999). DNMT3A is ubiquitously expressed throughout the differentiation. Loss of DNMT3A in HSC results in increased self-renewal capacity and decreased differentiation capacity, but fewer changes are caused by loss of DNMT3B. The loss of DNMT1 disrupts the self-renewal and multiple differentiation potency in long term HSC that has extensive self-renewal capacity (Trowbridge, Snow et al. 2009, Sashida and Iwama 2012).

The DNA oxidation also regulates global DNA methylation patterns via TET enzymes. TET1/2/3 catalyze 5-methyl-cytosine (5mC) conversion to 5-hydroxymethyl-cytosine (5hmC), and to further oxidation products. This process finally leads to DNA demethylation through the thymine DNA glycosylase (TDG) - base excision repair (BER) pathways. Normally, TET1 and 3 promote 5hmC at promoters and prohibit DNMT activity (Rasmussen and Helin 2016). TET1 is highly expressed in ESCs, TET2 and TET3 are more expressed in differentiated myeloid cells. In mice, disruption of TET2 results in enhanced self-renewal and proliferation ability. The differentiation without TET2 in HSCs is also towards myeloid lineage. TET2 and DNMT3A may co-function to limit the expression of HSC genes but to activate the expression of the lineage-specific gene, such as KLF4 for erythroid differentiation (Sashida and Iwama 2012, Goyama and Kitamura 2017).

Histone modifications regulate transcription of lineage-specific genes. For instance, differentiation from CMP is found coupled with HDAC1 repression by CCAAT/enhancer-binding proteins during myeloid differentiation and HDAC1 activation by GATA binding protein GATA-1 during erythro-megakaryocytic differentiation (Wada, Kikuchi et al. 2009). Double deletion of H3K9 methyltransferase SUV39H1 and SUV39H2 in HSC results in reduced stem cell function and drives the differentiation towards myeloid development with reduced lymphoid output (Keenan, Iannarella et al. 2020). PRC1 and PRC2 facilitate hematopoiesis and self-renewal for HSCs (Sashida and Iwama 2012).

Chromatin remodelers are also involved in hematopoiesis regulation. For example, the chromatin remodeling subunit Baf200 facilitates normal hematopoiesis and suppresses leukemogenesis, through regulation erythropoiesis- and hematopoiesis-associated genes (Liu, Wan et al. 2018).

1.3.3 Disorders of hematopoietic development

Hematologic malignancies can occur at any stage of hematopoiesis and can produce dysfunctional blood cells and defects of the immune system or susceptibility to uncontrolled bleeding. Disruption of hematopoietic differentiation can produce three main types of blood cancer: leukemia, lymphoma, and myeloma. For leukemia, abnormal white blood cells or poorly differentiated cells are massively produced in the bone marrow, resulting in the accumulation of immature dysfunctional leukemic cells in the blood. According to the lineages of the neoplastic cells, leukemia is categorized as acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL) and chronic lymphoid leukemia (CLL) (Hu and Shilatifard 2016).

1.3.3.1 Epigenetic changes in AML

Acute myeloid leukemia (AML) is a common blood malignant disease of the myeloid lineage of hematopoietic cells. It is distinguished by the block of myeloid lineage differentiation and abnormal accumulation of immature cells. Genetic mutations of epigenetic modifiers that can affect myeloid differentiation is one of the core aberrances leading to the disease. These epigenetic factors are involved in the mechanisms of DNA methylation, histone modification, chromatin remodeling enzyme or associated factors, and chromatin structure factor such as cohesion (DiNardo and Cortes 2016).

DNMT3A is frequently mutated in many different types of hematopoietic diseases. Over 20% *de novo* AML harbor this mutation. Most frequent DNMT3A mutation in AML is R882H or R882C, which abolishes enzyme activity and DNA binding. It results in a particular pattern of abnormal DNA methylation, which consequently damages the function of HSCs, enhances self-renewal, and blocks differentiation (Huang, Ma et al. 2013). DNMT3A dysfunction co-appears with a mutation on nucleophosmin (NPM1), FLT3-ITD or isocitrate dehydrogenase 1 (IDH1). Inactivation of TET2, resulting in increased 5mC levels but low levels of 5hmC, is present in 10~20% AML, but in over 50% in CML (Pratcorona, Abbas et al. 2012, DiNardo and Cortes 2016, Goyama and Kitamura 2017, Koya and Kurokawa 2018).

The mutations of IDH1-R132, IDH2-R140, and IDH2-R172 occur in ~20% of AML with increased occurrence with age. Isocitrate dehydrogenase 1 and 2 (IDH1/2) proteins exert the oxidative decarboxylation of isocitrate to α -ketoglutarate. Mutant IDH proteins convert α -ketoglutarate into the oncometabolite 2-hydroxyglutarate, which limits α -ketoglutarate dependent enzymes, including TET2 for DNA hydroxymethylation, histone demethylase such as H3K9 demethylase

KDM4C (Geisbrecht and Gould 1999, Ward, Patel et al. 2010, Hu and Shilatifard 2016, Goyama and Kitamura 2017).

EZH2 facilitates in leukemogenesis. Mutation of *EZH2*, as well as mutations on other PRC2 members, caused lack of H3K27me3 and repression defect on the target genes, *EZH2* mutations have been frequently reported in Myelodysplastic syndrome (MDS) and T cell acute lymphoid leukemia (T-ALL). Last year, *EZH2* mutations were reported as a new prognosis marker together with FLT3 and IDH2 mutations in AML patients (Mechaal, Menif et al. 2019). Gain-function-mutation or overexpression of EZH2, causing hypermethylation on H3K27, has been reported in natural killer/T cell (NKT) or B cell lymphoma(Lund, Adams et al. 2014). Epigenetic factor ASXL1 is associated with PRC mediated gene repression via recruiting PRC2 to the target locus. Mutation of *ASXL1* in AML, usually occurred as C-terminal truncation, promotes myeloid transformation through disrupting PRC2 mediated gene repression (Eriksson, Lennartsson et al. 2015, DiNardo and Cortes 2016)

In AML, mutations are also found in genes of chromatin remodelers and structure factors that regulate downstream gene transcription through altering nucleosome/chromatin structures. BAF250A is one of the central members of the chromatin remodeling BAF complex. Mutant BAF250A has been reported in ~0.5% AML (Network 2013). Another chromatin structure modifier is cohesin, which is the protein complex facilitating sister chromatid cohesion, homologs recombination. It can form the chromatin loop together with CTCF and regulate associated gene expression (Wutz, Várnai et al. 2017). Mutation of cohesion has been reported in 5%-13% AML (Eriksson, Lennartsson et al. 2015).

Cytogenetic abnormality (chromosomal rearrangement), such as RUNX1-RUNX1T1, MLLT3-MLL, MLL-AF9, is observed in over 50% the AML patients. The fused histone methyltransferases MLL proteins catalyze on wrong target genes and consequently cause aberrant gene transcription (Wang, Lin et al. 2009, Yang and Ernst 2017).

2. Aim of The Thesis

The thesis aims to explore the chromatin organization and related transcription in models of the fission yeast and human hematopoietic development.

Study I: Investigate the novel role of Abol in the silencing of different types of heterochromatin in *S.pombe* and understanding the mechanism of H3K9me_{2/3} establishment in heterochromatin regions

Study II: Identify functional role of non-catalytic domain of chromatin remodeler in chromatin remodeling process via *in vitro* assays.

Study III: Study the usage of alternative TSS causing protein domain exclusion and the functional consequences in hematopoietic cells.

Study IV: Identify novel roles of epigenetic regulators in hematopoietic differentiation.

3. Methods

In this thesis, multiple approaches were applied to explore the chromatin structure and functional consequences in the models fission yeast and human hematopoietic cell lines. Here we discuss some of the used methodologies. The specific conditions used in each method and experiment are shown in manuscripts.

3.1 Cell culture

Two models were applied for culturing in the studies for this thesis: fission yeast *Schizosaccharomyces pombe* and human hematopoietic cell lines.

The fission yeast has been established and utilized as a great laboratory model to study chromatin structures. The yeast cells were cultured in the full nutrition medium (YES medium) with the components of yeast extract (5 g/l), glucose (20 g/l), and supplements: 225 mg/l adenine, histidine, leucine, uracil, and lysine hydrochloride. Special conditions were introduced according to required experiments, such as antibiotics/chemicals for strain selection with particular genotypes, limited nitrogen media for inducing crossing to generate the target genotype. The typical growing temperature for yeast culture is 30°C, while the heat and cold induction to examine the environmental stress response are performed at 37°C and 25°C. Fission yeasts with various genotypes were applied in study I and II

Hematopoietic human cell lines used in the studies include the Jurkat cell line (ATCC® TIB-152™) and the K-562 cell line (ATCC® CCL-243™), which were applied in study III and IV. K562 cell line is derived from the bone marrow of a 53-year-old female chronic myelogenous leukemia (CML) patient in blast crisis. It is a suspension cell line cultured in Iscove's Modified Dulbecco's Medium (IMDM) with 10% fetal bovine serum. The K-562 cells were characterized as a multi potential leukemia cell line similar to the early-stage of erythrocytes, granulocytes, and monocytes. K-562 cells can be induced to megakaryocytic differentiation by phorbol 12-myristate 13-acetate PMA treatment (Huang, Zhao et al. 2014). Jurkat cell line is derived from the peripheral blood of a 14-year-old boy with T-cell leukemia. It is a suspension cell line cultured in RPMI-1640 medium with 10% fetal bovine serum.

3.2 Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) is a method widely used for the identification of genome-wide locations of binding of various transcriptional factors,

histones, and other chromatin interacted proteins *in vivo*. Generally, DNA and interacting protein are cross-linked by a cross-linking agent such as formaldehyde. The cross-linked chromatin is then sonicated into segments approximately 300-500bp. The fragments bound the target protein are captured by a specific antibody that is then pulled down with protein A/G coated beads. Unspecific binding is washed away using different washing buffers. The cross-link of the antibody-protein-DNA complex is then reversed by incubation at 65°C together with a proteinase. With additional clean-up steps, the ChIP DNA is eluted and analyzed by different methods, such as qPCR or Next-generation sequencing.

ChIP-sequencing (ChIP-seq) is a well-established and popular method to analyze the genome-wide enrichment of a chromatin-binding protein. Briefly, after the ChIP experiment, adaptors with index sequences are ligated to both ends of the eluted DNA fragments. Adaptor ligated fragments are subsequent amplified to prepare the ChIP DNA library. Hybridization happens in a flowcell between the library fragments and immobilized oligos with complementary sequences to adaptor regions. It triggers the bridge amplification and cluster generation. Next, fragments are sequenced by DNA synthesis with fluorescent-tagged nucleotides. Distinct fluorescent signals represent the read sequences. Mapped sequences to the reference genome reflect locations for the target protein. Together comparison with the enrichment of other factors, such as epigenetic marks, it makes ChIP-seq an excellent method to understand potential function and mechanism of the target protein (Buermans and Den Dunnen 2014). In our studies, eluted ChIP DNA was sent to our core facility BEA (<http://www.bea.ki.se/>), for library preparation and sequencing.

3.3 Overall gene expression analysis

In our studies, different methods were used to explore the overall gene expression, including RNA-microarray, RNA-sequencing, and cap analysis of gene expression (CAGE).

RNA-microarray

A microarray is a laboratory tool containing a slide with a fixed microscopy DNA probe that can hybridize with nucleotide sequences of the target. Total RNA molecules are extracted, reverse-transcribed, and labeled with a fluorescent dye. The complementary hybridization between cDNA fragment and the fixed probe can produce colored signals on the microarray slide. After hybridization, the microarray is scanned to measure the signals reflecting the expression of each gene printed on the slide. These slides, referred as gene chips or DNA chips, are utilized to detect gene expressions --

transcriptome or the set of messenger RNA (mRNA) transcripts expressed by a group of genes (Sealfon and Chu 2011). Each chip allows measuring more than one sample by labeling the cDNA with distinct dyes, which make it valuable for comparing the expression of the same gene in different cells/conditions. In study I, for *S.pombe*, we applied RNA microarray in the multiple samples (different genotypes with alternative culturing conditions) by using the GeneChip 1.0FR array from Affymetrix. It is a tiling array, which means the probes cover the whole fission yeast genome, even the silent centromere region. The length of the probe is 25bp, with an overlap of 5bp.

RNA-sequencing

RNA sequencing (RNA-seq) is a high-throughput technique to examine the quantity and sequences of extracted RNA molecules by using next-generation sequencing (NGS). It is now another common method in the lab to analyze the overall transcriptome (Wang, Gerstein et al. 2009). In study III and V, after purification of total RNA from cells. Illumina TruSeq Stranded RNA assays were applied, which includes mRNA isolation, cDNA synthesis, ligations of adapters, and amplification of index libraries. The libraries were sequenced using Illumina Nextseq 550, generating 75bp single-end reads. Furthermore, the output reads were aligned to the human genome Refseq (hg38/GRCH38). After removing the repetitive and fuzzy regions, mapped reads offered the informative over-all gene expression. RNA sequencing is favored for its high dynamic range, detectability of RNA splicing, and single nucleotide polymorphism identification. However, the disadvantages are also very obvious for its high cost, the complexity of analysis (Altındağ and Baykan 2017).

CAGE

In study III, we aim to study the distribution of alternative TSS usage over a wide range of primary cells. Therefore, data obtained with Cap Analysis of Gene Expression (CAGE) that only measure a small fragment from the 5'-end of mRNA is perfect for this study.

Cap Analysis of Gene Expression (CAGE), developed by RIKEN in Japan, is a high throughput method to analyze transcriptome via measuring the sequence tags 5' ends of mRNA at the cap sites and thereby identifying the transcriptional starting site. Briefly, RNA is extracted from cells and then reversed transcribed into first-strand cDNA with an oligo dT and random primer mix. The full-length cDNA is then picked out by biotinylated cap-trapper. The linker sequence contains restriction enzyme digestion sites of XhoI or I-CeuI or XmaJI, and MmeI. Additional upper single-stranded overhang oligonucleotide GN5 (GNNNNN) is also designed in the linker sequence. GN5 can subsequently be ligated to the single-strand full-length cDNA and is used to

synthesis of the second strand. The double-stranded cDNA is cleaved by enzyme MmeI, which creates a 2bp overhang at the cleavage site. The second linker sequence, which contains the XbaI site, ligates to the 2bp overhang. Streptavidin beads separate the ligation product via linking to the biotin at the 5' end of the fragment. PCR amplifies the purified 5' end tag. The tags are then sequenced and matched to the reference genome (Figure 8) (Shiraki, Kondo et al. 2003). CAGE shows accurate gene expression through detecting TSS but not gene body, and consequently offer information for prediction of transcriptional factor binding motifs. It is also can be used for detection of short enhancer RNA (eRNA) that usually expresses at every low level.

In the FANTOM 5 project (<https://fantom.gsc.riken.jp/5/>), researchers have systemically investigated the TSSs using the CAGE method in ~400 distinct cell types. In study III, we used the CAGE data from FANTOM 5 project to study the alternative TSS usage in different cell types.

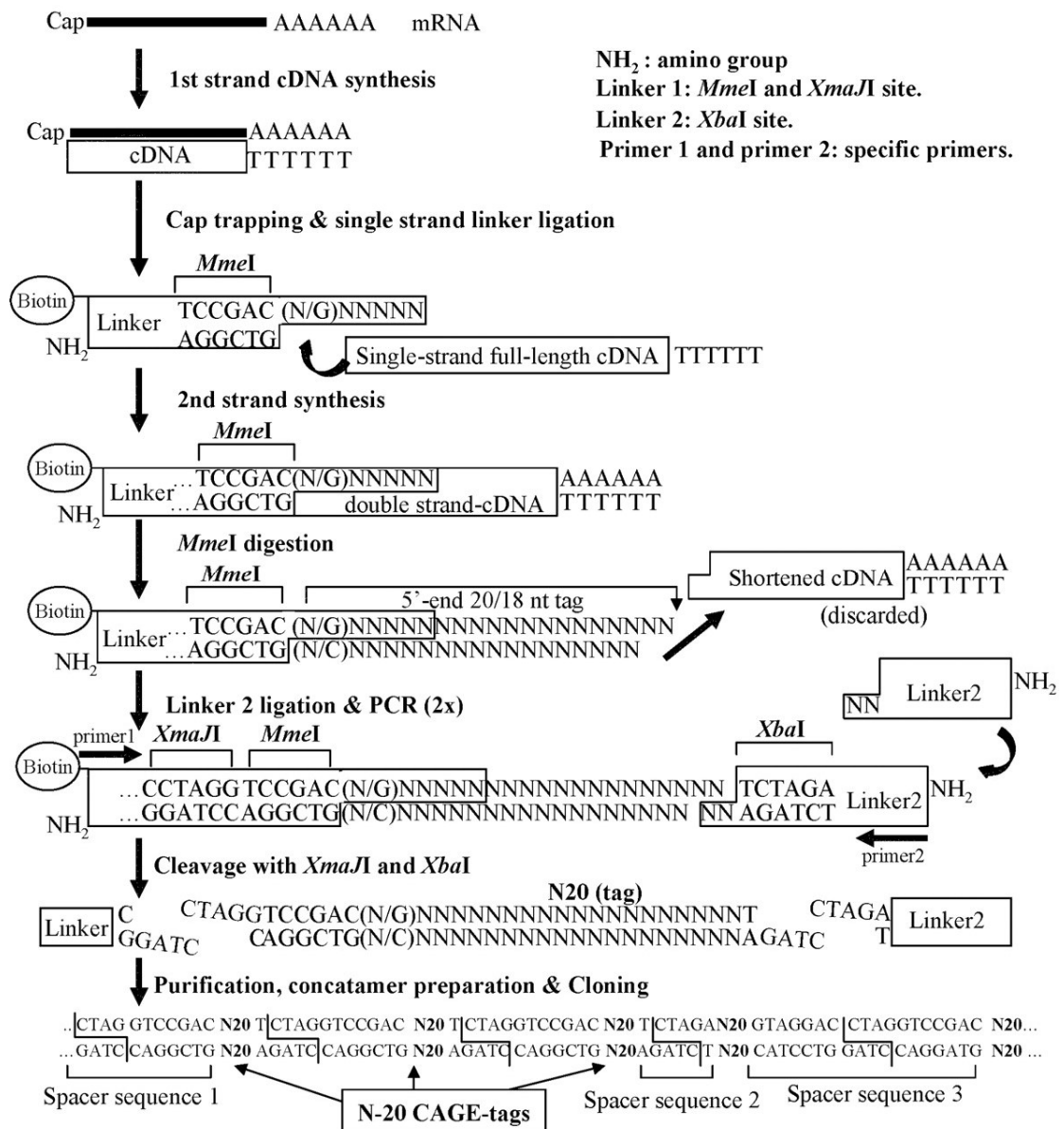


Figure 8. Schematic procedure of the CAGE protocol as detailed in Methods (Shiraki, Kondo et al. 2003). Copyright (2020) National Academy of Sciences

3.4 Biochemical assays *in vitro*

In study II, a series of *in vitro* biochemical assays were performed in fission yeast to identify the functional roles of specific domains in our protein of interest. The *in vitro* assays include histone protein expression and purification, histone octamer reconstitution, nucleosome reconstitution, affinity purification and *in vitro* ATPase hydrolyzing activity. Methods performed for these *in vitro* assays such as plasmid construction, inclusion body extraction, gel filtration, DNA amplification, and High-performance liquid chromatography (HPLC). HPLC is a technique for separating a

mixture of compounds (proteins in our study) by pumping the mixture-dissolved solvent (mobile phase) at a high-pressure condition through a column filled with solid packing materials (stationary phase). Different components in the mixture interact differently with the stationary phase, which results in distinct flow rates and consequent components separation. The ATPase activity assay was performed with isotope Pi labeled ATP. The percentage of released labeled Pi represented the capacity of ATP hydrolysis with various substrates. Details of these biochemical assays were described in the manuscript.

3.5 siRNA knocking down

Gene knockdown is a common technique that decreases the expression of one gene (or several genes) via modifications on DNA or RNA level. RNA interference knocking down is the method of reducing gene transcription by introducing the small double-stranded siRNA. Once this exogenous siRNA is transfected into cells, it will complementarily bind to the target mRNA and trigger the recruitment of RNA-induced silencing complex. Consequently, it leads to the degradation of mRNA. This method is widely utilized in the lab to study gene function. In our study III, we used siRNA was transfected by using NeonTM Transfection system to knockdown different potential transcripts to study the functional consequences caused by potential alternative transcripts from alternative TSS usage.

4. Results and Discussion

4.1 Study I: Abo1 is required for the H3K9me2 to H3K9me3 transition in heterochromatin

Constitutive heterochromatin and facultative heterochromatin, which are featured by di-/tri- methylated H3K9 and associated heterochromatin protein HP1, are relied on different silencing machinery. In study I, we investigated the role of the conserved bromodomain AAA-ATPase Abo1 in the heterochromatin regions of the fission yeast model, *Shizosaccharomyces pombe*.

In this study, we first investigated the involvement of Abo1 in different aspects of heterochromatin assembly through performing Synthetic Genetic Array (SGA) assay, in which a small library containing 711 single gene deletion strains crossed with *abo1Δ* strain. The strongest negatively genetic interactions were observed between Abo1 and heterochromatin factors, including Clr3, Clr4, and Swi6. Interestingly, transcriptomic analysis reveals significant changes at heterochromatin regions between wild type and *abo1*-depleted mutant. Deletion of *abo1* caused transcription silencing defect at both pericentromeric and subtelomeric heterochromatin regions. We subsequently examined the H3K9me2 and H3K9me3 heterochromatin marks in *abo1Δ* cells compared with wild type. Deletion of *abo1* caused an increased H3K9me2 and reduced H3K9me3 at pericentromeric heterochromatin region. Meanwhile, in the subtelomeric region, Abo1 deletion results in decreased H3K9me2 and H3K9me3 marks. RT-qPCR and ChIP-qPCR validated these observations on different heterochromatin regions. Facultative heterochromatin regions, known as “determinant of selective removal” (DSR) islands and containing meiotic genes, were also investigated. We found that *abo1Δ* mutant compared to wild type, displayed a reduced enrichment of H3K9me2 and H3K9me3 marks in DSR islands without any effect on gene expression indicating that Abo1 is required for the establishment of heterochromatin and contributes to the transition of H3K9me2 to me3 at DSR islands. To search for the heterochromatin assembly mechanism involving Abo1, we analyzed published H3K9me2 ChIP-seq data from several different mutant strains. Interestingly, *abo1* deletion shows similar H3K9me2 regulation patten in different heterochromatin regions to the H3K9 methyltransferase Clr4 point mutation Clr4W31G that inhibits the transition from H3K9me2 to H3K9me3 through disturbing the Clr4 self-recruitment by the chromodomain.

ChIP-qPCR at exemplated genes for each heterochromatin regions revealed that the Clr4 occupancy decreased in *abo1Δ* at subtelomeric region and pericentromeric region, which is consistent to the reduced H3K9me3 and the silencing defect in the same loci.

Clr4 enrichment in DSR islands was also significantly decreased with deletion of *abo1*. These results supported the role of Abo1 to stabilize Clr4 recruitment to allow the H3K9me2-H3K9me3 transition at different heterochromatin regions. By examining histone occupancy by H3 ChIP as well as nucleosome positioning data from previous published papers, we did not find significant changes in subtelomeric, pericentromeric and DSR heterochromatin regions, suggesting that H3K9me2 and H3K9me3 changes in *abo1Δ* cells is not induced by nucleosome dynamics or occupancy defects (Gal, Murton et al. 2016).

Based on these findings, this work has identified Abo1 as a new factor involved in the H3K9 methylation process in fission yeast that we summarize in a simplified mode

In the constitutive pericentromeric region or cen-like region, silencing machinery is divided into two steps: 1) RNAi co-transcriptional gene silencing (RNAi-CTGS) followed by transcriptional gene silencing (RNAi-TGS). siRNA from the RNAi-CTGS activate RITE complex and help to recruit Clr4 to establish H3K9me2. In this step, H3K9me2 and H3ac present at the same time and still allow the transcription of dg-dh repeats. 2) In the RNAi-TGS step, H3K9 is tri-methylated. Swi6 is recruited to the H3K9me3 to establish the real transcriptional silencing (Jih, Iglesias et al. 2017). Deacetylase Clr3 may also involve in this process. In the facultative heterochromatin region, without RNAi-CTGS, Abo1 may help to recruit Clr4 promoting both H3K9me2 and H3K9me3, leading to TGS

In this study, we uncover a role for Abo1 in stabilizing directly or indirectly Clr4 recruitment to allow the H3K9me2 to H3K9me3 transition in heterochromatin in *S. pombe*. However, many questions about Abo1 in *S. pombe* still need to be answered in the future study. The recruitment of Abo1 to heterochromatin and Clr4 and the functional role of its non-canonical bromodomain are still unclear. The ATPase domain in Abo1 indicates its capacity of ATP hydrolysis. The functional role of ATPase domain in heterochromatin assembly, even in regulating genome-wide histone occupancy as a histone chaperone, are still not clear. Its human homolog ATAD2 has been found to overexpress in several types of cancer and regulates transcription of several key factors including Myc, and EZH2, as well as crosstalk with P53/P21 pathways (Altintas, Shukla et al. 2012, Lu, Chua et al. 2015, Morozumi, Boussouar et al. 2016). Since the exact function of the human Abo1 homolog ATAD2 in both cancer and normal cells remains unclear, this work opens new understanding of the role of the conserved bromodomain AAA-ATPase heterochromatin assembly

4.2 Study II: The role of non-catalytic domains of Hrp3 in chromatin remodeling

Chromatin is the fundamental molecular structure for packing DNA in chromosomes. The basic unit forming chromatin structure is a nucleosome that is consisted of ~147bp linear DNA wrapped around a histone octamer. ATPase dependent chromatin remodelers regulate chromatin structure through nucleosome sliding, nucleosome assembly, nucleosome unwrapping, histone eviction, and histone variants exchange (Cooper 2000). CHD1 is a conserved ATP dependent chromatin remodeling enzyme regulating H3.3 turnover and maintaining an open chromatin state in the active gene (Siggens, Cordeddu et al. 2015). A study in CHD1 in *S.cerevisiae* revealed a small region between the ATPase catalytic domain and DNA binding domain, coupling the processes of nucleosome assembly and nucleosome spacing (Torigoe, Patel et al. 2013). SWI/SNF remodelers share a feature of specific taking nucleosome as substrate but not DNA. Hrp3, as one of the two the homologs of CHD1/2 remodeler in *S.pombe*, has been previously reported to act more significate role in maintaining nucleosome occupancy (Pointner, Persson et al. 2012). In this study, we aim to explore the functional roles in chromatin remodeling for the non-catalytic domains of CHD1/2 remodeler homolog Hrp3 in *S.pombe*.

In this study, we generated a series of mutant strains lacking different non-catalytic domain of Hrp3 that is TAP tagged at its C-terminus. Affinity purification of wild type and mutant Hrp3_TAP was performed and found that loss of non-catalytic domains significantly affect the co-purification of histones. To perform *in vitro* studies, we expressed four canonical *S. pombe* histone proteins H3, H4 H2A, and H2B from optimally plasmids and *E.coli* strains. Overexpression of proteins in *E.coli* cells formed inclusion bodies. We extracted inclusion bodies and purified histone proteins from the extracts through high-performance liquid chromatography. The almost equal ratios of histone proteins were mixed and dialyzed to refold histone octamer. The dialyzed histone mix was applied to gel filtration colomun to purify the histone octamer complex. 210 bp DNA containing '601' nucleosome positioning sequence along with 70 bp of extra-nucleosomal DNA was amplified. It further constituted with histone octamer into a sp70N0 nucleosome. With affinity purification, we determined the compromised histone association of mutant Hrp3 *in vivo* compared to wild type Hrp3, through anti-H3 western blot. With this observation, *in vitro* assay was applied to analyze the ATP hydrolyzing capacity of mutant Hrp3 compared to wild type. Using DNA and sp70N0 nucleosome as substrate, we aimed to identify the domains that effect on catalytic activity. Most of the mutant Hrp3 exhibited different degrees of compromised ATPase activity. SANT and SLIDE sub-domains are composed of DNA binding domain in Hrp3. They may exert distinct functional roles, proposed from their distinct effects from

domain deletions on *in vivo* nucleosome association and ATP hydrolysis capacity. We also observed that Hrp3 with SANT domain and coupling region deletions exhibited the similar ATPase activity level by using DNA and nucleosome as substrate. It indicated that Hrp3 enzyme loose the preference of taking nucleosome without SANT domain and the coupling region. Taken together, all our data revealed that the non-catalytic domain of Hrp3 can affect the enzymatic activity and could further affect the chromatin remodeling process. However, future experiments, such as the *in vitro* nucleosome binding assay, nucleosome sliding assay, still need to be done to figure out how these non-catalytic domain effect in remodeling process.

In this study, we have down lots of works to generate the *in vitro* assays and established a platform for further exploration.

4.3 Study III: Investigation of protein coding sequence exclusion by alternative transcription start site usage across the human body

In mammalian cells, multiple protein isoforms or protein variants are transcribed from the same gene. This is a widespread phenomenon. Protein isoforms are produced by several different mechanisms, including alternative transcription initiation, alternative translational initiation, alternative splicing, and alternative poly A-tail. Multi-mechanisms can also co-functionalize to produce various protein isoforms. In this study, we used CAGE data from the FANTOM 5 project to analyze 890 CAGE libraries in 176 different primary cell types, aiming to explore the distribution of alternative TSSs. Furthermore we investigated whether their usage causes exclusion of coding sequences, and consequently potentially functional consequences in regulating biological processes.

Firstly, we merged the detected TSSs located around the same loci of the same strand as Tag cluster (TC) to categorize detected tag clusters (TC) and their expression. All TCs with an expression range of at least 1 or 10 tags per million (TPM) in any of CAGE library were hierarchical classified. Analysis of TCs were defined into subtypes, including final regions from 5'UTR, 500bp upstream region of TSS to 3'UTR. In one primary cell as an example, most TCs could be mapped to RefSeq genes. Within this group, most TCs could be mapped to the same strand of genes, of which most (over 70%) were characterized as unknown novel TSS. Within TC group of unknown TSSs, most TCs (96%) could be mapped to the coding genes but not to the annotated TSS of the gene, which may induce potentially exclusively protein-coding. Combining the hierarchical classification in all CAGE-libraries, we further identified that annotated TSSs of protein-coding gene and ncRNA are ubiquitously expressed, while the

expression of TSS located in intergenic regions, antisense strand and within coding region turns out more cell-specific.

We then re-examined this hierarchical classification analysis of all TCs expressed in each CAGE-library and clustered the analysis for each specific primary cell type, aiming to identify whether the distribution patterns across the classification tree were different between cell types. Surprisingly, the analysis revealed a stable fraction of each TCs category throughout cell types. Hematopoietic cells, especially monocytes with different inductions were outliers with less annotated TSS usage of transcript genes and more intragenic TSS usage. Then we narrowed down the analysis in hematopoietic cells with percentages of usage of various types of TCs. The hematopoietic cells include progenitors, myeloid and lymphoid cells. Interestingly, TCs in annotated TSS of protein-coding gene and TCs within 5'UTR were more preferred in hematopoietic progenitor cells, while TCs within the protein-coding region was more favored in matured myeloid cells. Lymphoid cells exhibited preference of TCs between progenitors and myeloid cells.

TCs within the protein-coding region represents the TSSs that potentially generate coding sequences truncated protein and may change the functions of the expressed protein. In our analysis, 7.8% of total TCs mapped to the Refseq protein-coding transcripts belong to this kind of TSS. Over half of the genes have this kind of TSS that has been previously annotated and further supported by CAGE data in our study. We also analyzed the expression pattern (with a cutoff 10TPM) of these TCs potentially leading truncated proteins. The TCs mapped to annotated TSS leading truncated protein were more cell-specific compared to the TCs mapped to annotated TSS leading to no truncations. However, no significant functional clustering of genes mapped by the TCs of coding sequence exclusion was found. However, considering cell type, we observed a subgroup of immune cells, including blood cells.

In the next step, we aimed to define the main and alternative TSSs. Usually, the most upstream TSS is commonly viewed as the main TSS. Nevertheless, this definition is not based on expression level. Only 33% of genes exhibited the most upstream TSS, and most expression at the same time. Over half of the genes, harboring annotated or all coding sequence exclusive TSS, showed the higher expression of coding sequence exclusive TSS than other TSSs. Our analysis suggested that the most upstream TSSs of coding genes are not always the most expressed TSSs, but are more universal used in different cell types. Therefore, we still kept the common view of main and alternative TSS in the following analysis.

Furthermore, we set out filters of TCs to explore the cell-specific expression of alternative TSS leading protein domain loss. Within TCs mapped to annotated coding TSS, 78 protein domains from 36 genes could be excluded in a cell-specific manner. Within all TCs, this number of genes increased to 286, with 715 protein domains. In hematopoietic cells, we identified a total of 60 genes that have potential alternative TSS leading to domain exclusion according to specific cell type or specific differentiation lineage. This observation was further validated by RT-qPCR of examples PRDM1, KDM2B, RERE in different sorted hematopoietic cells, suggesting the different usage of alternative TSS of genes may result in distinct functional consequences in hematopoiesis.

To explore the functional changes caused by alternative TSS usage in different hematopoietic cells, we took the H3K4 and H3K36 demethylase KDM2B as an example for further studies. KDM2B can regulate lineage commitment in normal and malignant hematopoiesis in mice (Andricovich, Kai et al. 2016). Studies in mice also showed the presence of two isoform proteins of KDM2B: the full-length one and the short one that lacks JmjC catalytic domain (He, Shen et al. 2013). The CAGE analysis revealed three TSS in KDM2B. The most expressed TSS1 and TSS3 matched the reported isoforms in mice. They showed different expression levels in lymphoid and myeloid cells. We speculated that the short isoform might participate more specifically in the PRC1.1 complex to locate unmethylated CpG islands. To investigate consequent functional changes with the usage of alternative TSS of KDM2B, we knocked down the TSS1 and alternative TSS3 in Jurkat cells and performed analysis on RNA-seq and the PRC1.1 target H2AK119ub, with ChIP-seq. Our analysis showed the differences between knocking down these two TSS. The more significant changes in both RNA-seq and ChIP-seq were present in TSS1 knockdown cells, while no significant changes were observed for knocking down both TSS1 and TSS3 compared to the control, which contradicts our hypotheses.

We performed CAGE analysis with 16 human time course to identify protein domain loss during differentiation. A total 76 genes were identified harboring different TSS usage to express different isoforms, which some of them were unknown before.

In this study, we characterized alternative TSS usage of genes, especially in hematopoietic cells. We further explored how the isoform regulation is associated with differentiation or cell type. We discovered two kinds of domain loss according to usage of TSS within coding region: varying numbers repeated domains such as NFLX1, or functional domain loss such as MYO10. The whole analysis and methods were based on RNA level. We still lack the evidence of whether these unannotated alternative TSS

usage leading to potential domain exclusion can be transcribed into real proteins. We could not confirm our functional hypothesis of KDM2B alternative TSS expression due to technical problems.

4.4 Study IV: A regulatory role for CHD2 in myelopoiesis

Epigenetic regulators play a crucial role in regulating hematopoiesis via different biological processes as cell proliferation, differentiation, and self-renewal. In this study, we aimed to study the role of chromatin factors in hematopoietic development. Stable K-562-Cas9 cells were generated and transduced with a unique CRISPR guide library that targets 1092 epigenetic regulators and 320 control genes with four sgRNAs for each. The transduced cells were 72 hours treated by phorbol 12-myristate 13-acetate PMA for megakaryocytic differentiation, or DMSO for control.

After the PMA treatment, megakaryocyte differentiation was observed by morphology changes. Furthermore, treated cells were grouped and examined through the expression of surface marker for megakaryocyte differentiation (CD41 and CD61) with flow cytometry. With the set gating, we collected undifferentiated population harboring both negative CD61-/CD41- (P1), differentiated population harboring both positive CD61+/CD41+ (P2), and differentiated population only positive for CD61+ (P3). For each population, cells are sequenced to identify the guide sequences in each population. The top 10% overlapped genes for two biological-replicates for enriched sgRNA, and >0.2 mean log fold change (average of all four guides) were chosen for further analysis. In P1, 14 candidate genes were suggested a role of driving the myeloid differentiation. In P2 and P3, 13 and 30 candidate genes were suggested to inhibit the differentiation, respectively. Several members of the CHD chromatin remodeler family have been found to be involved in pluripotency and myeloid leukemia (Gaspar-Maia, Alajem et al. 2009, Heshmati, Türköz et al. 2018). Therefore, CHD2 from the P3 group was selected for further validation and characterization.

To validate the observation of CHD2 from CRISPR screening, we used CRISPR-Cpf1 to knockout CHD2 in K-562 cells with four sgRNA located in exons 3, 7, 14, and 28 of CHD2. Empty Py095 vector was transduced as control. Cells were sorted and collected by GFP expression. The single sorted cells were expanded as mono clones. We confirmed the CHD2 KO via western blot and Sanger sequencing of the sgRNA sites for indel formation. Instead of 72 hours, 24 hours of PMA/DMSO treatments were applied to mono clones to observe the early effect of differentiation. Significantly increased enrichment in the P2 population for DMSO control was observed without

PMA induction, suggesting spontaneous differentiation in CHD2 KO cells. PMA induction further enhanced the differentiation, suggested a consistent role of CHD2 in inhibiting myeloid differentiation. To identify if the effect for CHD2 KO in differentiation was coupled with cell proliferation, proliferation analysis of both control cells and CHD2 KO cells was performed for four days. The results showed that CHD2 KO cells had a low proliferation rate at low cell density. In addition, colony-forming assay showed a reduced colony-forming capacity of CHD2 KO cells. CHD2 has been shown to be recruited at the TSS region by RNA PolII, and to regulate nucleosome disassembly (Siggens, Cordeddu et al. 2015). Through analyzing CHD2 enriched genes in K-562 cells from ENCODE project and overall transcriptome in K-562 cells from FANTOM 5 CAGE data, we revealed that CHD2 target genes participated in multiple cellular processes and were highly transcribed. This suggests that CHD2 is associated with active transcription. This conclusion was subsequently validated by RNA-seq in CHD2 KO clones, where CHD2 target genes were significantly down-regulated in comparison with CHD2 non-target genes. We also found that the CHD2 co-expressed genes in AML patients overlapped with CHD2 target genes in K-562 cells. Together, the results suggested that CHD2 also positively regulates transcription in AML patients.

In conclusion, we utilized a CRISPR-cas9 screen as an efficient method to study the role of epigenetic regulators (1092 factors in the library for this study) in hematopoiesis. Of those factors 5% exhibited potential regulation in megakaryocyte differentiation. CHD2, a chromatin remodeler, has been reported to regulate muscle differentiation. This regulation may depend on its promotion of H3.3 deposition at myogenic loci together with interaction with MyoD (Harada, Okada et al. 2012). Previous study in our group revealed that CHD2 is involved H3.3 deposition in myeloid cells (Siggens, Cordeddu et al. 2015). CHD2 has been shown to be ubiquitously expressed in hematopoiesis (Prasad, Rönnerblad et al. 2014). Thus, suggesting a general regulation of CHD2 in all hematopoietic cells. Our finding of CHD2 for prohibition of differentiation and promotion of cell growth confirms its importance of CHD2 in myeloid hematopoiesis, especially megakaryocytic differentiation.

5. Acknowledgement

I want to express my great gratitude to all the people who helped me during my Ph.D. study and contributed to this work.

Firstly, I would like to give my appreciation to my supervisor **Andreas Lennartsson** for having me as your Ph.D. student. You are always supportive and considerate. I enjoy the time that we discussed the projects. You were always willing to let us try our hypothesis with experiments, and even they were not always working. You have been patient and helpful, especially when I faced problems or when I was upset. Thank you, Andreas, for supervising me to explore the hematopoiesis world. I admire your networks for collaboration and scientific perspectives.

To my co-supervisor, **Karl Ekwall**, thank you for having me in your group first for the master project and continue to the Ph.D. study. You showed me how to think and read like a scientist at the very beginning of my Ph.D. time. Without your support, this work would not start. Thank you for all the help in scientific discussion, problem-solving, and knowledge sharing. To my co-supervisor, **Mickaël Durand-dubief**, it's my great honor and pleasure to collaborate with you. I enjoy the time we work together for the Abo1 project. Discussion with you always inspires me with ideas. Without you, I would not finish the Abo1 paper. I also learn a lot from you for being more positive. To my co-supervisor, **Punit Prasad**, you are the person who guided me to enter this yeast field. You taught me from the most basic culturing and plating to all these biochemical assays. I want to thank you for your selfless supervision and help. I miss the kitchen class you made us in Novum.☺

My special thanks to **Peter Svensson**, for the scientific discussion on the troubleshooting. I admire your help and contribution to the Abo1 project. To **Ling Han**, you are such a warmhearted person. Chatting with you always made me calmed and relaxed, especially when I was upset. I hope to see you in China in the future. To my supervisor in China, **Hongmin Li**, without you, I cannot start this journey. Thank you for introducing me to the world of science.

I would like to give a deep appreciation to all the members of the **ALE** group! I am so lucky to work with all of you as colleagues in the same group. Because of you guys, we can have such an open-minded working environment. **Anna** - You were always willing to give advice and help when I faced problems. I admire your help on my thesis writing and your knowledge sharing. **Sophia** – You are a smart person with great passion.

Thank you for sharing your experience not only for the experiments but also for the daily life. I enjoy the time we share in the office and fika time ☺ **Feri**- It's so lovely to collaborate with you in the alternative TSS and CHD2 projects. Thank you for being so kind and considerate to me. I will miss the time we chatted, laughed, and shared chocolate in the office. Good luck with your job! **Sylvain**- Thanks for the help on the computer! **Xiangfu**- Thank you for sharing exciting news and information. Good luck with your Ph.D. defence. I hope you will enjoy your time in this group as I did. **Wlaa and Elizabeth**- It was nice to talk and discuss with you in the office, and good luck for your future life! **Birgitta**- Thank you for helping me with all the orders! Even some time I need it urgently, you can still get the order super-fast!

I would like to thank members of **KEK** and **VMB** groups for the time we shared in Novum, and the time we had brunches together. **Yasaman**- Thank you for the hard work of FACS in the Abo1 project! Good luck with your Ph.D. study. **Eriko**- Thanks a lot for the ChIP work and also for the Japanese snacks you shared with me. **Lina**- Thank you for helping me with all the lab orders and experimental staffs. **Shengyuan**- I like your amazing homemade cookies! Good luck with your Ph.D. study. **Marc**- I hope you have a wonderful time in the KEK group. **Alex**- You are always kind to share experimental staff when I need it urgently. Good luck with your papers. **Marjan**- I admire your passion for both science and life. I hope you will have good results in all your future experiments.

I would also like to thank members of **Sören's groups** in HERM and Uppsala for all the valuable discussion in our joint group meetings and lab retreats. **Albin, Christer** and **Anna** - Thank you for sharing the AML knowledge from the clinic. **Huthyfa, Sophia** and **Naomi**- I wish you guys all my best for your future works. **Anne**- I appreciate the discussion from you about RACE. I hope you have a wonderful time for the rest of your Ph.D. studies.

To my friends in Neo, thank you for always be caring for me. I will miss the time we laugh, chat, and party together. My friends **Yumei, Ning, Zhiqiang, Rongrong, Jianjiang, Ting, Jian, Haidong, Xiaoyan** - I will always remember the time we spent together for eating out, drinking beer, and playing cards. I wish you all my best for your future life. **Xue, Feifei, Zuoneng**- good luck with your Ph.D. study. **Huan, Dandan** and **Dan**-You girls are so sweet. I cherish the time we hang out for hotpot, shopping, and even bubble tea. Keep in touch. Good luck with your papers. My neighbors in Neo **Hui, Xiaofei, Chunli, Likun, Yanhong** and **Jonathan**- Thank you for sharing the fruit in the office. Good luck with all your works.

I would like to give my gratitude to my friends in Sweden for being part of my life here. **Ying**- I admire your attitude towards being a scientist and a mother. **Olga**- I will not forget the time we shared in the same office in Novum. You are a biochemical expert and a super mom. **Vlad**- Since both of us are night persons. So we always stayed in the lab super late. Thank you for your collaboration on histone octamer reconstitution and purification. I promise I will bring you another bottle of Chinese wine when we meet next time. My dear friends in Uppsala, **Li, Sisi, Jing, Pengcheng, Xin, Tong**- I will miss the party time we had together in Uppsala! I wish you guys the best luck in your future.

I cannot miss two little cuties **Kirara** and **Cola**, thank you for helping me release my stress and melting my heart :)

衷心感谢我的家人，谢谢**爸爸妈妈，姥姥姥爷**，感谢你们这么多年来对我的爱与养育，也感谢你们这么多年来对于我的理解和支持。是你们对我的言传身教，才让我有追逐和探索这个世界的理想，勇气和信心。感谢你们包容了我的所有自我和任性，永远爱你们。谢谢我的其他**所有的家人**，不论相隔多远，我都能感受到你们的关心和爱护。谢谢**吴和平与李翠英**，感谢你们一直以来对我的包容和理解，能成为你们的家人是我的福气。最后感谢亲爱的**吴华星**同学，你是我这一段人生中最大的收获，感谢你所有的爱与陪伴，包容与尊重，唯愿白首不相离。

6. References

- Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter (2002). Chromosomal DNA and its packaging in the chromatin fiber. Molecular Biology of the Cell. 4th edition, Garland Science.
- Aldrup-Macdonald, M. E. and B. A. Sullivan (2014). "The past, present, and future of human centromere genomics." Genes (Basel) **5**(1): 33-50.
- Allfrey, V., R. Faulkner and A. Mirsky (1964). "Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis." Proceedings of the National Academy of Sciences **51**(5): 786-794.
- Allshire, R. C. and H. D. Madhani (2018). "Ten principles of heterochromatin formation and function." Nature Reviews Molecular Cell Biology **19**(4): 229.
- Altındağ, E. and B. Baykan (2017). "Discover the world's research." Turk J Neurol **23**: 88-89.
- Altintas, D., M. S. Shukla, D. Goutte-Gattat, D. Angelov, J. Rouault, S. Dimitrov and J. Samarut (2012). "Direct cooperation between androgen receptor and E2F1 reveals a common regulation mechanism for androgen-responsive genes in prostate cells." Molecular endocrinology **26**(9): 1531-1541.
- Andrews, A. J., X. Chen, A. Zevin, L. A. Stargell and K. Luger (2010). "The histone chaperone Nap1 promotes nucleosome assembly by eliminating nonnucleosomal histone DNA interactions." Mol Cell **37**(6): 834-842.
- Andricovich, J., Y. Kai, W. Peng, A. Foudi and A. Tzatsos (2016). "Histone demethylase KDM2B regulates lineage commitment in normal and malignant hematopoiesis." The Journal of clinical investigation **126**(3): 905-920.
- Annunziato, A. (2008). "DNA packaging: nucleosomes and chromatin." Nature Education **1**(1): 26.
- Aranda, S., G. Mas and L. Di Croce (2015). "Regulation of gene transcription by Polycomb proteins." Science advances **1**(11): e1500737.
- Audry, J. and K. W. Runge (2019). Telomere Formation Systems in Budding and Fission Yeasts. Telomerase and non-Telomerase Mechanisms of Telomere Maintenance, IntechOpen.
- Aygün, O., S. Mehta and S. I. Grewal (2013). "HDAC-mediated suppression of histone turnover promotes epigenetic stability of heterochromatin." Nature structural & molecular biology **20**(5): 547.
- Babu, A. and R. S. Verma (1987). "Chromosome structure: euchromatin and heterochromatin." Int Rev Cytol **108**: 1-60.
- Bandaria, J. N., P. Qin, V. Berk, S. Chu and A. Yildiz (2016). "Shelterin Protects Chromosome Ends by Compacting Telomeric Chromatin." Cell **164**(4): 735-746.
- Bannister, A. J. and T. Kouzarides (2011). "Regulation of chromatin by histone modifications." Cell research **21**(3): 381-395.
- Biscotti, M. A., A. Canapa, M. Forconi, E. Olmo and M. Barucca (2015). "Transcription of tandemly repetitive DNA: functional roles." Chromosome Research **23**(3): 463-477.
- Black, B. E., L. E. Jansen, P. S. Maddox, D. R. Foltz, A. B. Desai, J. V. Shah and D. W. Cleveland (2007). "Centromere identity maintained by nucleosomes assembled with

histone H3 containing the CENP-A targeting domain." *Mol Cell* **25**(2): 309-322.

Boginya, A., R. Detroja, A. Matityahu, M. Frenkel-Morgenstern and I. Onn (2019). "The chromatin remodeler Chd1 regulates cohesin in budding yeast and humans." *Scientific reports* **9**(1): 1-10.

Britten, R. J. and D. E. Kohne (1968). "Repeated sequences in DNA. Hundreds of thousands of copies of DNA sequences have been incorporated into the genomes of higher organisms." *Science* **161**(3841): 529-540.

Brown, S. W. (1966). "Heterochromatin." *Science* **151**(3709): 417-425.

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

Buermans, H. and J. Den Dunnen (2014). "Next generation sequencing technology: advances and applications." *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* **1842**(10): 1932-1941.

Burda, P., P. Laslo and T. Stopka (2010). "The role of PU. 1 and GATA-1 transcription factors during normal and leukemogenic hematopoiesis." *Leukemia* **24**(7): 1249-1257.

Buscaino, A. (2019). "Chromatin-Mediated Regulation of Genome Plasticity in Human Fungal Pathogens." *Genes* **10**(11): 855.

Ceredig, R., A. G. Rolink and G. Brown (2009). "Models of haematopoiesis: seeing the wood for the trees." *Nat Rev Immunol* **9**(4): 293-300.

Chikashige, Y. and Y. Hiraoka (2002). "[Genome sequence of Schizosaccharomyces pombe]." *Tanpakushitsu Kakusan Koso* **47**(9): 1215-1220.

Chikashige, Y., N. Kinoshita, Y. Nakaseko, T. Matsumoto, S. Murakami, O. Niwa and M. Yanagida (1989). "Composite motifs and repeat symmetry in S. pombe centromeres: direct analysis by integration of NotI restriction sites." *Cell* **57**(5): 739-751.

Clapier, C. R. and B. R. Cairns (2009). "The biology of chromatin remodeling complexes." *Annu Rev Biochem* **78**: 273-304.

Clapier, C. R., J. Iwasa, B. R. Cairns and C. L. Peterson (2017). "Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes." *Nature reviews Molecular cell biology* **18**(7): 407-422.

Collins, K. and J. R. Mitchell (2002). "Telomerase in the human organism." *Oncogene* **21**(4): 564-579.

Cooper, G. M. (2000). "Chromosomes and Chromatin." *The Cell: A Molecular Approach*. 2nd ed. Sunderland, MA: Sinauer Associates.

Cubiles, M. D., S. Barroso, M. I. Vaquero-Sedas, A. Enguix, A. Aguilera and M. A. Vega-Palas (2018). "Epigenetic features of human telomeres." *Nucleic acids research* **46**(5): 2347-2355.

Deindl, S., W. L. Hwang, S. K. Hota, T. R. Blosser, P. Prasad, B. Bartholomew and X. W. Zhuang (2013). "ISWI Remodelers Slide Nucleosomes with Coordinated Multi-Base-Pair Entry Steps and Single-Base-Pair Exit Steps." *Cell* **152**(3): 442-452.

Deng, Z., J. Norseen, A. Wiedmer, H. Riethman and P. M. Lieberman (2009). "TERRA RNA binding to TRF2 facilitates heterochromatin formation and ORC recruitment at telomeres." *Molecular cell* **35**(4): 403-413.

Dillon, N. and R. Festenstein (2002). "Unravelling heterochromatin: competition between positive and negative factors regulates accessibility." TRENDS in Genetics **18**(5): 252-258.

DiNardo, C. D. and J. E. Cortes (2016). "Mutations in AML: prognostic and therapeutic implications." Hematology 2014, the American Society of Hematology Education Program Book **2016**(1): 348-355.

Diotti, R. and D. Loayza (2011). "Shelterin complex and associated factors at human telomeres." Nucleus **2**(2): 119-135.

Elgin, S. C. and G. Reuter (2013). "Position-effect variegation, heterochromatin formation, and gene silencing in *Drosophila*." Cold Spring Harbor perspectives in biology **5**(8): a017780.

Eriksson, A., A. Lennartsson and S. Lehmann (2015). "Epigenetic aberrations in acute myeloid leukemia: early key events during leukemogenesis." Experimental hematology **43**(8): 609-624.

Fair, B. J. and J. A. Pleiss (2017). "The power of fission: yeast as a tool for understanding complex splicing." Curr Genet **63**(3): 375-380.

Farlik, M., F. Halbritter, F. Müller, F. A. Choudry, P. Ebert, J. Klughammer, S. Farrow, A. Santoro, V. Ciaurro and A. Mathur (2016). "DNA methylation dynamics of human hematopoietic stem cell differentiation." Cell stem cell **19**(6): 808-822.

Filion, G. J., J. G. van Bommel, U. Braunschweig, W. Talhout, J. Kind, L. D. Ward, W. Brugman, I. J. de Castro, R. M. Kerkhoven, H. J. Bussemaker and B. van Steensel (2010). "Systematic protein location mapping reveals five principal chromatin types in *Drosophila* cells." Cell **143**(2): 212-224.

Gal, C., H. E. Murton, L. Subramanian, A. J. Whale, K. M. Moore, K. Paszkiewicz, S. Codlin, J. Bahler, K. M. Creamer, J. F. Partridge, R. C. Allshire, N. A. Kent and S. K. Whitehall (2016). "Abo1, a conserved bromodomain AAA-ATPase, maintains global nucleosome occupancy and organisation." EMBO Rep **17**(1): 79-93.

Gaspar-Maia, A., A. Alajem, F. Polesso, R. Sridharan, M. J. Mason, A. Heidersbach, J. Ramalho-Santos, M. T. McManus, K. Plath and E. Meshorer (2009). "Chd1 regulates open chromatin and pluripotency of embryonic stem cells." Nature **460**(7257): 863-868.

Geisbrecht, B. V. and S. J. Gould (1999). "The human PICD gene encodes a cytoplasmic and peroxisomal NADP(+)-dependent isocitrate dehydrogenase." J Biol Chem **274**(43): 30527-30533.

Goda, S., T. Isagawa, Y. Chikaoka, T. Kawamura and H. Aburatani (2013). "Control of histone H3 lysine 9 (H3K9) methylation state via cooperative two-step demethylation by Jumonji domain containing 1A (JMJD1A) homodimer." Journal of Biological Chemistry **288**(52): 36948-36956.

Gottschling, D. E., O. M. Aparicio, B. L. Billington and V. A. Zakian (1990). "Position effect at *S. cerevisiae* telomeres: reversible repression of Pol II transcription." Cell **63**(4): 751-762.

Goyama, S. and T. Kitamura (2017). "Epigenetics in normal and malignant hematopoiesis: An overview and update 2017." Cancer science **108**(4): 553-562.

Harada, A., S. Okada, D. Konno, J. Odawara, T. Yoshimi, S. Yoshimura, H. Kumamaru, H. Saiwai, T. Tsubota and H. Kurumizaka (2012). "Chd2 interacts with H3. 3 to

determine myogenic cell fate." The EMBO journal **31**(13): 2994-3007.

Hauk, G., J. N. McKnight, I. M. Nodelman and G. D. Bowman (2010). "The chromodomains of the Chd1 chromatin remodeler regulate DNA access to the ATPase motor." Mol Cell **39**(5): 711-723.

Hayles, J. and P. Nurse (1989). "A review of mitosis in the fission yeast *Schizosaccharomyces pombe*." Exp Cell Res **184**(2): 273-286.

Hayles, J. and P. Nurse (2018). "Introduction to Fission Yeast as a Model System." Cold Spring Harb Protoc **2018**(5).

He, J., L. Shen, M. Wan, O. Taranova, H. Wu and Y. Zhang (2013). "Kdm2b maintains murine embryonic stem cell status by recruiting PRC1 complex to CpG islands of developmental genes." Nature cell biology **15**(4): 373-384.

Healy, E., M. Mucha, E. Glancy, D. J. Fitzpatrick, E. Conway, H. K. Neikes, C. Monger, G. Van Mierlo, M. P. Baltissen and Y. Koseki (2019). "PRC2. 1 and PRC2. 2 synergize to coordinate H3K27 trimethylation." Molecular cell **76**(3): 437-452. e436.

Heitz, E. (1928). Das heterochromatin der moose, Borträger.

Heshmati, Y., G. Türköz, A. Harisankar, S. Kharazi, J. Boström, E. K. Dolatabadi, A. Krstic, D. Chang, R. Månsson and M. Altun (2018). "The chromatin-remodeling factor CHD4 is required for maintenance of childhood acute myeloid leukemia." haematologica **103**(7): 1169-1181.

Hodges, E., A. Molaro, C. O. Dos Santos, P. Thekkat, Q. Song, P. J. Uren, J. Park, J. Butler, S. Rafii and W. R. McCombie (2011). "Directional DNA methylation changes and complex intermediate states accompany lineage specificity in the adult hematopoietic compartment." Molecular cell **44**(1): 17-28.

Hu, D. and A. Shilatifard (2016). "Epigenetics of hematopoiesis and hematological malignancies." Genes & development **30**(18): 2021-2041.

Huang, R., L. Zhao, H. Chen, R.-H. Yin, C.-Y. Li, Y.-Q. Zhan, J.-H. Zhang, C.-h. Ge, M. Yu and X.-M. Yang (2014). "Megakaryocytic differentiation of K562 cells induced by PMA reduced the activity of respiratory chain complex IV." PloS one **9**(5).

Huang, X., D. Ma, W. Dong, P. Li, T. Lu, N. He, T. Tian, N. Liu, Y. Du and C. Ji (2013). "Gene expression profiling of the DNMT3A R882 mutation in acute leukemia." Oncol Lett **6**(1): 268-274.

Hubner, M. R., M. A. Eckersley-Maslin and D. L. Spector (2013). "Chromatin organization and transcriptional regulation." Curr Opin Genet Dev **23**(2): 89-95.

Hyun, K., J. Jeon, K. Park and J. Kim (2017). "Writing, erasing and reading histone lysine methylations." Experimental & molecular medicine **49**(4): e324-e324.

Iglesias, N., J. A. Paulo, A. Tatarakis, X. Wang, A. L. Edwards, N. V. Bhanu, B. A. Garcia, W. Haas, S. P. Gygi and D. Moazed (2020). "Native Chromatin Proteomics Reveals a Role for Specific Nucleoporins in Heterochromatin Organization and Maintenance." Molecular Cell **77**(1): 51-66. e58.

Janssen, A., S. U. Colmenares and G. H. Karpen (2018). "Heterochromatin: guardian of the genome." Annual review of cell and developmental biology **34**: 265-288.

Ji, H., L. I. Ehrlich, J. Seita, P. Murakami, A. Doi, P. Lindau, H. Lee, M. J. Aryee, R. A. Irizarry and K. Kim (2010). "Comprehensive methylome map of lineage commitment from haematopoietic progenitors." Nature **467**(7313): 338-342.

Jih, G., N. Iglesias, M. A. Currie, N. V. Bhanu, J. A. Paulo, S. P. Gygi, B. A. Garcia and D. Moazed (2017). "Unique roles for histone H3K9me states in RNAi and heritable silencing of transcription." Nature **547**(7664): 463-+.

Job, G., C. Brugger, T. Xu, B. R. Lowe, Y. Pfister, C. Qu, S. Shanker, J. I. B. Sanz, J. F. Partridge and T. Schalch (2016). "SHREC silences heterochromatin via distinct remodeling and deacetylation modules." Molecular cell **62**(2): 207-221.

Kalverda, B., M. D. Röling and M. Fornerod (2008). "Chromatin organization in relation to the nuclear periphery." FEBS letters **582**(14): 2017-2022.

Kanoh, J. and F. Ishikawa (2001). "spRap1 and spRif1, recruited to telomeres by Taz1, are essential for telomere function in fission yeast." Curr Biol **11**(20): 1624-1630.

Kanoh, J., M. Sadaie, T. Urano and F. Ishikawa (2005). "Telomere binding protein Taz1 establishes Swi6 heterochromatin independently of RNAi at telomeres." Current biology **15**(20): 1808-1819.

Kato, H. and K. Igarashi (2019). "To be red or white: lineage commitment and maintenance of the hematopoietic system by the "inner myeloid"." haematologica **104**(10): 1919-1927.

Keenan, C. R., N. Iannarella, G. Naselli, N. G. Bediaga, T. M. Johanson, L. C. Harrison and R. Allan (2020). "Extreme disruption of heterochromatin is required for accelerated haematopoietic aging." Blood Journal: blood. 2019002990.

Kleinjan, D. A. and L. A. Lettice (2008). "Long-range gene control and genetic disease." Adv Genet **61**: 339-388.

Kouzarides, T. (2007). "Chromatin modifications and their function." Cell **128**(4): 693-705.

Koya, J. and M. Kurokawa (2018). "Functional role of DNMT3A mutation in acute myeloid leukemia." [Rinsho ketsueki] The Japanese journal of clinical hematology **59**(5): 602-610.

Lan, F., M. Zaratiegui, J. Villén, M. W. Vaughn, A. Verdel, M. Huarte, Y. Shi, S. P. Gygi, D. Moazed and R. A. Martienssen (2007). "S. pombe LSD1 homologs regulate heterochromatin propagation and euchromatic gene transcription." Molecular cell **26**(1): 89-101.

Längst, L. M. a. G. (2013). Chromatin Remodelers and Their Way of Action. Chromatin Remodelling, Dr. Danuta Radzioch (Ed.).

Lawrence, M., S. Daujat and R. Schneider (2016). "Lateral thinking: how histone modifications regulate gene expression." Trends in Genetics **32**(1): 42-56.

Lee, Y. C. G., Y. Ogiyama, N. M. Martins, B. J. Beliveau, D. Acevedo, C.-t. Wu, G. Cavalli and G. H. Karpen (2020). "Pericentromeric heterochromatin is hierarchically organized and spatially contacts H3K9me2 islands in euchromatin." PLoS Genetics **16**(3): e1008673.

Lin, A., Y. Du and W. Xiao (2020). "Yeast chromatin remodeling complexes and their roles in transcription." Current genetics.

Lippman, Z., A.-V. Gendrel, M. Black, M. W. Vaughn, N. Dedhia, W. R. McCombie, K. Lavine, V. Mittal, B. May and K. D. Kasschau (2004). "Role of transposable elements in heterochromatin and epigenetic control." Nature **430**(6998): 471-476.

Liu, L., X. Wan, P. Zhou, X. Zhou, W. Zhang, X. Hui, X. Yuan, X. Ding, R. Zhu and G.

Meng (2018). "The chromatin remodeling subunit Baf200 promotes normal hematopoiesis and inhibits leukemogenesis." Journal of hematology & oncology **11**(1): 27.

Locke, S. and R. Martienssen (2009). Epigenetic Silencing of Pericentromeric Heterochromatin by RNA Interference in *Schizosaccharomyces pombe*. Epigenomics, Springer: 149-162.

Lorch, Y., B. Maier-Davis and R. D. Kornberg (2010). "Mechanism of chromatin remodeling." Proc Natl Acad Sci U S A **107**(8): 3458-3462.

Loubiere, V., A. M. Martinez and G. Cavalli (2019). "Cell fate and developmental regulation dynamics by polycomb proteins and 3D genome architecture." BioEssays **41**(3): 1800222.

Lu, W.-J., M.-S. Chua and S. K. So (2015). "Suppression of ATAD2 inhibits hepatocellular carcinoma progression through activation of p53-and p38-mediated apoptotic signaling." Oncotarget **6**(39): 41722.

Lund, K., P. Adams and M. Copland (2014). "EZH2 in normal and malignant hematopoiesis." Leukemia **28**(1): 44-49.

Margueron, R. and D. Reinberg (2011). "The Polycomb complex PRC2 and its mark in life." Nature **469**(7330): 343-349.

Matsuda, A., H. Asakawa, T. Haraguchi and Y. Hiraoka (2017). "Spatial organization of the *Schizosaccharomyces pombe* genome within the nucleus." Yeast **34**(2): 55-66.

Matsuda, A., Y. Chikashige, D.-Q. Ding, C. Ohtsuki, C. Mori, H. Asakawa, H. Kimura, T. Haraguchi and Y. Hiraoka (2015). "Highly condensed chromatins are formed adjacent to subtelomeric and decondensed silent chromatin in fission yeast." Nature communications **6**(1): 1-12.

Mechaal, A., S. Menif, S. Abbes and I. Safra (2019). "EZH2, new diagnosis and prognosis marker in acute myeloid leukemia patients." Advances in medical sciences **64**(2): 395-401.

Mills, A. A. (2017). "The chromodomain helicase dna-binding chromatin remodelers: family traits that protect from and promote cancer." Cold Spring Harbor perspectives in medicine **7**(4): a026450.

Mondoux, M. and V. Zakian (2006). "Telomere position effect: silencing near the end." Cold Spring Harbor Monograph Series **45**: 261.

Morozumi, Y., F. Boussouar, M. Tan, A. Chaikuad, M. Jamshidikia, G. Colak, H. He, L. Nie, C. Petosa and M. De Dieuleveult (2016). "Atad2 is a generalist facilitator of chromatin dynamics in embryonic stem cells." Journal of molecular cell biology **8**(4): 349-362.

Motamedi, M. R., A. Verdel, S. U. Colmenares, S. A. Gerber, S. P. Gygi and D. Moazed (2004). "Two RNAi complexes, RITS and RDRC, physically interact and localize to noncoding centromeric RNAs." Cell **119**(6): 789-802.

Moussa, H. F., D. Bsteh, R. Yelagandula, C. Pribitzer, K. Stecher, K. Bartalska, L. Michetti, J. Wang, J. A. Zepeda-Martinez and U. Elling (2019). "Canonical PRC1 controls sequence-independent propagation of Polycomb-mediated gene silencing." Nature communications **10**(1): 1-12.

Muers, M. (2011). "Evolutionary genomics: fission yeast compared and contrasted."

Nat Rev Genet **12**(6): 381.

Muller, H. J. (1930). "Types of visible variations induced by X-rays in *Drosophila*." Journal of Genetics **22**(3): 299-334.

Murawska, M. and A. Brehm (2011). "CHD chromatin remodelers and the transcription cycle." Transcription **2**(6): 244-253.

Nakayama, J., J. C. Rice, B. D. Strahl, C. D. Allis and S. I. Grewal (2001). "Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly." Science **292**(5514): 110-113.

Network, C. G. A. R. (2013). "Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia." New England Journal of Medicine **368**(22): 2059-2074.

Nicholson, T. B., N. Veland and T. Chen (2015). Writers, readers, and erasers of epigenetic marks. Epigenetic Cancer Therapy, Elsevier: 31-66.

Nishibuchi, G. and J. Déjardin (2017). "The molecular basis of the organization of repetitive DNA-containing constitutive heterochromatin in mammals." Chromosome Research **25**(1): 77-87.

Nonaka, N., T. Kitajima, S. Yokobayashi, G. Xiao, M. Yamamoto, S. I. Grewal and Y. Watanabe (2002). "Recruitment of cohesin to heterochromatic regions by Swi6/HP1 in fission yeast." Nat Cell Biol **4**(1): 89-93.

O'Sullivan, R. J. and J. Karlseder (2010). "Telomeres: protecting chromosomes against genome instability." Nat Rev Mol Cell Biol **11**(3): 171-181.

Oberdoerffer, P. and D. A. Sinclair (2007). "The role of nuclear architecture in genomic instability and ageing." Nature reviews Molecular cell biology **8**(9): 692-702.

Okano, M., D. W. Bell, D. A. Haber and E. Li (1999). "DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development." Cell **99**(3): 247-257.

Oya, E., M. Durand-Dubief, A. Cohen, V. Maksimov, C. Schurra, J.-i. Nakayama, R. Weisman, B. Arcangioli and K. Ekwall (2019). "Leo1 is essential for the dynamic regulation of heterochromatin and gene expression during cellular quiescence." Epigenetics & chromatin **12**(1): 45.

Pointner, J., J. Persson, P. Prasad, U. Norman-Axelsson, A. Stralfors, O. Khorosjutina, N. Krietenstein, J. P. Svensson, K. Ekwall and P. Korber (2012). "CHD1 remodelers regulate nucleosome spacing in vitro and align nucleosomal arrays over gene coding regions in *S. pombe*." Embo Journal **31**(23): 4388-4403.

Prasad, P., M. Rönnerblad, E. Arner, M. Itoh, H. Kawaji, T. Lassmann, C. O. Daub, A. R. Forrest, A. Lennartsson and K. Ekwall (2014). "High-throughput transcription profiling identifies putative epigenetic regulators of hematopoiesis." Blood, The Journal of the American Society of Hematology **123**(17): e46-e57.

Pratcorona, M., S. Abbas, M. A. Sanders, J. E. Koenders, F. G. Kavelaars, C. A. Erpelinck-Verschueren, A. Zeilemakers, B. Lowenberg and P. J. Valk (2012). "Acquired mutations in ASXL1 in acute myeloid leukemia: prevalence and prognostic value." Haematologica **97**(3): 388-392.

Pray-Grant, M. G., J. A. Daniel, D. Schieltz, J. R. Yates and P. A. Grant (2005). "Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation." Nature **433**(7024): 434-438.

Rasmussen, K. D. and K. Helin (2016). "Role of TET enzymes in DNA methylation, development, and cancer." Genes & development **30**(7): 733-750.

Riedmann, E. (2012). "CHD chromatin remodelers and the transcription cycle." Nucleus-Austin **3**(1): 1-1.

Saksouk, N., E. Simboeck and J. Déjardin (2015). "Constitutive heterochromatin formation and transcription in mammals." Epigenetics & chromatin **8**(1): 3.

Saksouk, N., E. Simboeck and J. Dejardin (2015). "Constitutive heterochromatin formation and transcription in mammals." Epigenetics Chromatin **8**: 3.

Sanchez, R. and M.-M. Zhou (2009). "The role of human bromodomains in chromatin biology and gene transcription." Current opinion in drug discovery & development **12**(5): 659.

Sashida, G. and A. Iwama (2012). "Epigenetic regulation of hematopoiesis." International journal of hematology **96**(4): 405-412.

Sealfon, S. C. and T. T. Chu (2011). RNA and DNA microarrays. Biological microarrays, Springer: 3-34.

Seike, T., T. Nakamura and C. Shimoda (2013). "Distal and proximal actions of peptide pheromone M-factor control different conjugation steps in fission yeast." PLoS One **8**(7).

Shiraki, T., S. Kondo, S. Katayama, K. Waki, T. Kasukawa, H. Kawaji, R. Kodzius, A. Watahiki, M. Nakamura and T. Arakawa (2003). "Cap analysis gene expression for high-throughput analysis of transcriptional starting point and identification of promoter usage." Proceedings of the National Academy of Sciences **100**(26): 15776-15781.

Siggens, L., L. Cordeddu, M. Rönnerblad, A. Lennartsson and K. Ekwall (2015). "Transcription-coupled recruitment of human CHD1 and CHD2 influences chromatin accessibility and histone H3 and H3. 3 occupancy at active chromatin regions." Epigenetics & chromatin **8**(1): 4.

Sims III, R. J., S. Millhouse, C.-F. Chen, B. A. Lewis, H. Erdjument-Bromage, P. Tempst, J. L. Manley and D. Reinberg (2007). "Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription postinitiation factors and pre-mRNA splicing." Molecular cell **28**(4): 665-676.

Smurova, K. and P. De Wulf (2018). "Centromere and pericentromere transcription: roles and regulation... in sickness and in health." Frontiers in genetics **9**: 674.

Steglich, B., A. Strålfors, O. Khorosjutina, J. Persson, A. Smialowska, J.-P. Javerzat and K. Ekwall (2015). "The Fun30 chromatin remodeler Fft3 controls nuclear organization and chromatin structure of insulators and subtelomeres in fission yeast." PLoS genetics **11**(3).

Stock, J. K., S. Giadrossi, M. Casanova, E. Brookes, M. Vidal, H. Koseki, N. Brockdorff, A. G. Fisher and A. Pombo (2007). "Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells." Nature cell biology **9**(12): 1428-1435.

Strachan, T. and A. Read (2003). "Human molecular genetics. Garland science." Edition, Kapitel 13: 418.

Struhl, K. and E. Segal (2013). "Determinants of nucleosome positioning." Nature Structural & Molecular Biology **20**(3): 267-273.

Sugiyama, T., H. Cam, A. Verdel, D. Moazed and S. I. Grewal (2005). "RNA-dependent RNA polymerase is an essential component of a self-enforcing loop coupling heterochromatin assembly to siRNA production." Proc Natl Acad Sci U S A **102**(1): 152-157.

Sugiyama, T., H. P. Cam, R. Sugiyama, K.-i. Noma, M. Zofall, R. Kobayashi and S. I. Grewal (2007). "SHREC, an effector complex for heterochromatic transcriptional silencing." Cell **128**(3): 491-504.

Suzuki, T., Y. Shimizu, E. Furuhashi, S. Maeda, M. Kishima, H. Nishimura, S. Enomoto, Y. Hayashizaki and H. Suzuki (2017). "RUNX1 regulates site specificity of DNA demethylation by recruitment of DNA demethylation machineries in hematopoietic cells." Blood advances **1**(20): 1699-1711.

Tashiro, S., Y. Nishihara, K. Kugou, K. Ohta and J. Kanoh (2017). "Subtelomeres constitute a safeguard for gene expression and chromosome homeostasis." Nucleic acids research **45**(18): 10333-10349.

Torigoe, S. E., A. Patel, M. T. Khuong, G. D. Bowman and J. T. Kadonaga (2013). "ATP-dependent chromatin assembly is functionally distinct from chromatin remodeling." Elife **2**.

Touat-Todeschini, L., E. Hiriart and A. Verdel (2012). "Nucleosome positioning and transcription: fission yeast CHD remodellers make their move." The EMBO journal **31**(23): 4371-4372.

Towbin, B. D., C. González-Aguilera, R. Sack, D. Gaidatzis, V. Kalck, P. Meister, P. Askjaer and S. M. Gasser (2012). "Step-wise methylation of histone H3K9 positions heterochromatin at the nuclear periphery." Cell **150**(5): 934-947.

Trojer, P. and D. Reinberg (2007). "Facultative heterochromatin: is there a distinctive molecular signature?" Molecular cell **28**(1): 1-13.

Trowbridge, J. J., J. W. Snow, J. Kim and S. H. Orkin (2009). "DNA methyltransferase 1 is essential for and uniquely regulates hematopoietic stem and progenitor cells." Cell stem cell **5**(4): 442-449.

Valenzuela, L. and R. T. Kamakaka (2006). "Chromatin insulators." Annu Rev Genet **40**: 107-138.

van den Boom, V., H. Maat, M. Geugien, A. R. López, A. M. Sotoca, J. Jaques, A. Z. Brouwers-Vos, F. Fusetti, R. W. Groen and H. Yuan (2016). "Non-canonical PRC1. 1 targets active genes independent of H3K27me3 and is essential for leukemogenesis." Cell reports **14**(2): 332-346.

van Emden, T. S., M. Forn, I. Forné, Z. Sarkadi, M. Capella, L. M. Caballero, S. Fischer-Burkart, C. Brönnner, M. Simonetta and D. Toczyski (2019). "Shelterin and subtelomeric DNA sequences control nucleosome maintenance and genome stability." EMBO reports **20**(1).

Van Kruijsbergen, I., S. Hontelez and G. J. C. Veenstra (2015). "Recruiting polycomb to chromatin." The international journal of biochemistry & cell biology **67**: 177-187.

Vanrobays, E., M. Thomas and C. Tatout (2018). "Heterochromatin Positioning and Nuclear Architecture." Annual Plant Reviews online: 157-190.

Verdel, A., S. Jia, S. Gerber, T. Sugiyama, S. Gygi, S. I. Grewal and D. Moazed (2004). "RNAi-mediated targeting of heterochromatin by the RITS complex." Science

303(5658): 672-676.

Vidal, M. and K. Starowicz (2017). "Polycomb complexes PRC1 and their function in hematopoiesis." Experimental hematology **48**: 12-31.

Volpe, T. A., C. Kidner, I. M. Hall, G. Teng, S. I. Grewal and R. A. Martienssen (2002). "Regulation of heterochromatic silencing and histone H3 lysine-9 methylation by RNAi." Science **297**(5588): 1833-1837.

Wada, T., J. Kikuchi, N. Nishimura, R. Shimizu, T. Kitamura and Y. Furukawa (2009). "Expression levels of histone deacetylases determine the cell fate of hematopoietic progenitors." Journal of Biological Chemistry **284**(44): 30673-30683.

Wang, J., S. T. Lawry, A. L. Cohen and S. Jia (2014). "Chromosome boundary elements and regulation of heterochromatin spreading." Cellular and Molecular Life Sciences **71**(24): 4841-4852.

Wang, J., X. Tadeo, H. Hou, P. G. Tu, J. Thompson, J. R. Yates and S. Jia (2013). "Epe1 recruits BET family bromodomain protein Bdf2 to establish heterochromatin boundaries." Genes & development **27**(17): 1886-1902.

Wang, P., C. Lin, E. R. Smith, H. Guo, B. W. Sanderson, M. Wu, M. Gogol, T. Alexander, C. Seidel, L. M. Wiedemann, K. Ge, R. Krumlauf and A. Shilatifard (2009). "Global analysis of H3K4 methylation defines MLL family member targets and points to a role for MLL1-mediated H3K4 methylation in the regulation of transcriptional initiation by RNA polymerase II." Mol Cell Biol **29**(22): 6074-6085.

Wang, Z., M. Gerstein and M. Snyder (2009). "RNA-Seq: a revolutionary tool for transcriptomics." Nature reviews genetics **10**(1): 57-63.

Ward, P. S., J. Patel, D. R. Wise, O. Abdel-Wahab, B. D. Bennett, H. A. Collier, J. R. Cross, V. R. Fantin, C. V. Hedvat, A. E. Perl, J. D. Rabinowitz, M. Carroll, S. M. Su, K. A. Sharp, R. L. Levine and C. B. Thompson (2010). "The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate." Cancer Cell **17**(3): 225-234.

Wei, G. H., D. P. Liu and C. C. Liang (2005). "Chromatin domain boundaries: insulators and beyond." Cell Res **15**(4): 292-300.

Weinhold, B. (2006). Epigenetics: the science of change, National Institute of Environmental Health Sciences.

Wutz, G., C. Várnai, K. Nagasaka, D. A. Cisneros, R. R. Stocsits, W. Tang, S. Schoenfelder, G. Jessberger, M. Muhar and M. J. Hossain (2017). "Topologically associating domains and chromatin loops depend on cohesin and are regulated by CTCF, WAPL, and PDS5 proteins." The EMBO journal **36**(24): 3573-3599.

Wynford-Thomas, D. and D. Kipling (1997). "The end-replication problem." Nature **389**(6651): 551-551.

Yang, W. and P. Ernst (2017). "SET/MLL family proteins in hematopoiesis and leukemia." International journal of hematology **105**(1): 7-16.

Yun, M., J. Wu, J. L. Workman and B. Li (2011). "Readers of histone modifications." Cell research **21**(4): 564.

Zhang, D., Z. Tang, H. Huang, G. Zhou, C. Cui, Y. Weng, W. Liu, S. Kim, S. Lee and M. Perez-Neut (2019). "Metabolic regulation of gene expression by histone lactylation." Nature **574**(7779): 575-580.

Zhang, K., K. Mosch, W. Fischle and S. I. Grewal (2008). "Roles of the Clr4 methyltransferase complex in nucleation, spreading and maintenance of heterochromatin." Nature structural & molecular biology **15**(4): 381.

Zhang, T., S. Cooper and N. Brockdorff (2015). "The interplay of histone modifications—writers that read." EMBO reports **16**(11): 1467-1481.

Zhang, Y., S. Gao, J. Xia and F. Liu (2018). "Hematopoietic Hierarchy - An Updated Roadmap." Trends Cell Biol **28**(12): 976-986.

Zhou, W., P. Zhu, J. Wang, G. Pascual, K. A. Ohgi, J. Lozach, C. K. Glass and M. G. Rosenfeld (2008). "Histone H2A monoubiquitination represses transcription by inhibiting RNA polymerase II transcriptional elongation." Molecular cell **29**(1): 69-80.

Zofall, M., S. Yamanaka, F. E. Reyes-Turcu, K. Zhang, C. Rubin and S. I. Grewal (2012). "RNA elimination machinery targeting meiotic mRNAs promotes facultative heterochromatin formation." Science **335**(6064): 96-100.