Targeting of proteins to chromatin in *Drosophila melanogaster*

von

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Preamble

This cumulative PhD thesis presents two original research articles on the targeting mechanism of the dosage compensation complex to the male X chromosome and its transfer to target genes in *Drosophila melanogaster*. The supplementary files of each research article are included. Furthermore, one manuscript for an original research article submitted to a peer-reviewed journal and deposited on a public preprint server on the targeting mechanism and function of the chromosomal kinase JIL-1 in *D. melanogaster* is presented. The contributions of all co-authors to each research article are stated here and before each article presented in the chapter 'Results'. The lists of references and abbreviations for the chapter 'Introduction' can be found at the end of this thesis.

List of publications

Albig, C., Tikhonova, E., Krause, S., Maksimenko, O., Regnard, C., and Becker, P.B. (2019). Factor cooperation for chromosome discrimination in *Drosophila*. Nucleic acids research *47*, 1706-1724, DOI 10.1093/nar/gky1238.

Schauer, T., Ghavi-Helm, Y., Sexton, T., Albig, C., Regnard, C., Cavalli, G., Furlong, E.E., and Becker, P.B. (2017). Chromosome topology guides the *Drosophila* Dosage Compensation Complex for target gene activation. EMBO Reports *18*, 1854-1868, DOI 10.15252/embr.201744292.

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Declaration of contributions

Authors' contributions to 'Factor cooperation for chromosome discrimination in *Drosophila*':

C.A., C.R. and P.B.B. conceived the study; C.A. performed all experiments except for co-immunoprecipitation and Yeast Two-Hybrid experiments; S.K. expressed proteins and performed co-immunoprecipitation experiments; E.T. and O.M. conceived, performed and analyzed Yeast Two-Hybrid experiments; C.A. performed bioinformatics analyses; All authors analyzed data; C.R. and P.B.B. provided feedback and supervision; C.A., C.R. and P.B.B. wrote the manuscript; P.B.B. secured funding.

Authors' contributions to 'Chromosome topology guides the *Drosophila* Dosage Compensation Complex for target gene activation':

The 4C-seq, ChIP-seq and RNA-seq experiments with Kc cells were performed by T.Sc.; 4C-seq was designed and supervised by Y.G.-H. and E.E.M.F.; Hi-C was performed by T.Se. and supervised by G.C.; ChIP-seq on H3K36me3 was carried out by C.A.; RNA-seq in S2 cells was performed by C.R.; Datasets were analyzed and visualized by T.Sc.; The study was conceptualized and supervised by P.B.B.; T.Sc. and P.B.B. wrote the manuscript with contributions from all authors.

Authors' contributions to 'JASPer controls interphase histone H3S10 phosphorylation by chromosomal kinase JIL-1 in *Drosophila*':

C.R. conceived this study and performed experiments; C.A. performed MNase and sonication ChIP-seq experiments and all bioinformatics analysis also with support from T.S.; C.W. generated and characterized the cw2 mutant line with help from J.G., W.C. did the LacO-LacI targeting experiments with support from Y.L., and J.J. and K.M.J. supervised the work and secured funding; G.P.D. performed mononucleosome library experiments and F.W. generated the mononucleosomes and arrays for the kinase assays in T.W.M.'s lab; S.K. prepared recombinant proteins for all *in vitro* assays, RNA-seq experiments under the supervision of C.R. and spike-in ChIP-seq experiments under supervision of C.A.; All authors analyzed data; C.R. and C.A. wrote the manuscript with contributions from all authors; P.B.B. secured funding and established collaborations.

Summary

Dosage compensation of sex chromosomes in *Drosophila melanogaster* is an excellent model system to study various aspects of targeting of protein factors to chromatin. Dosage compensation prevents male lethality by up-regulating transcription from the single male X chromosome in the ~2-fold range to match the two active X chromosomes in females [reviewed in e.g. (Ferrari et al., 2014; Kuroda et al., 2016; Samata and Akhtar, 2018)]. This up-regulation is facilitated by the male-specific-lethal (MSL) dosage compensation complex (DCC). The DCC binds selectively to ~300 high affinity sites (HAS) on the X chromosome, containing a low-complexity GAGA-rich sequence motif, the MSL recognition element (MRE) (Alekseyenko et al., 2008; Straub et al., 2008). However, the DCC neglects thousands of other similar sequences in the genome outside of HAS. The DNA-binding subunit MSL2 alone can enrich X chromosomal MREs *in vitro*, although MSL2 misses most MREs within HAS (Villa et al., 2016). The Chromatin-Linked Adaptor for MSL Proteins (CLAMP) binds thousands of MREs genome-wide and contributes to DCC targeting to HAS (Kaye et al., 2018; Soruco et al., 2013). The role of CLAMP in facilitating MSL2 targeting to HAS was investigated by several approaches. Monitoring MSL2 chromatin binding *in vivo* by chromatin immunoprecipitation with high-throughput sequencing (ChIP-seq) showed the requirement of CLAMP for HAS targeting. Next, the interplay between CLAMP and MSL2 in genome-wide *in vitro* DNA binding was studied by DNA immunoprecipitation with high-throughput sequencing (DIP-seq) (Gossett and Lieb, 2008; Liu et al., 2005; Villa et al., 2016). The data revealed mutual recruitment of both factors to each other's binding sites and cooperative binding to novel sites. This DNA binding cooperativity extended each other's binding repertoire to facilitate robust binding of MREs located within HAS, although increased binding to other non-functional sites was observed. Both factors interacted directly with each other in co-IP experiments, providing an explanation for cooperative DNA binding. Whether CLAMP and MSL2 are required for keeping HAS nucleosome-free was studied by assay for transposase accessibly chromatin with high-throughput sequencing (ATAC-seq) (Buenrostro et al., 2013; Buenrostro et al., 2015). Both factors cooperate to stabilize each other's binding and to compete with nucleosome positioning at HAS.

After successful binding of the DCC to HAS, it interacts with neighboring target genes, which are marked by trimethylation of histone H3K36 (H3K36me3). There, the DCC catalyzes acetylation of H4K16 (H4K16ac) to boost transcription (Akhtar and Becker, 2000; Gelbart et al., 2009; Larschan et al., 2007; Prestel et al., 2010). The DCC employs the chromosome 3D organization, which seems to be invariant between males and females, to transfer from HAS to active genes (Ramirez et al., 2015; Ulianov et al., 2016). The contribution of HAS to the chromosome interaction network was studied by using different chromosome conformation

capture techniques. Hi-C analysis on sex-sorted embryos showed that, H4K16ac and H3K36me3 correlate well with the active compartments (Sexton et al., 2012). Interestingly, compartment switching on the X chromosome between males and females was correlated with H4K16ac and therefore attributed to dosage compensation. The involvement of the Pioneering sites on the X (PionX), a special sub-class of HAS, in chromosome architecture was studied by high-resolution 4C-seq in male and female cells. Chromosomal segments containing PionX made frequent contact with many loci within the active compartment and even looped over large domains of the inactive compartment (Ghavi-Helm et al., 2014). These long-range interactions between PionX with other PionX/HAS were more robust in males compared to females, indicating that the dosage compensation machinery reinforced them. Moreover, *de novo* induction of DCC assembly in female cells showed that the DCC uses long-range interaction within the active compartment to transfer from PionX to target genes marked by H3K36me3 for up-regulation of transcription.

The chromosomal kinase JIL-1, which catalyzes phosphorylation of histone H3S10, localizes also to actively transcribed genes marked by H3K36me3 and is two-fold enriched on the male X chromosome (Jin et al., 2000; Regnard et al., 2011; Wang et al., 2001). JIL-1 is implicated in maintaining overall chromosome organization and preventing the spreading of heterochromatin into the euchromatic part of the X chromosome in both sexes (Cai et al., 2014; Ebert et al., 2004; Jin et al., 1999). Furthermore, JIL-1 localizes to the non-LTR retrotransposon arrays of the telomeres to positively regulate their expression (Andreyeva et al., 2005; Silva-Sousa and Casacuberta, 2013; Silva-Sousa et al., 2012). The role of JIL-1 in regulating gene expression was studied using various methods. JIL-1 formed a stable complex with the novel PWWP domain-containing protein, JIL-1 Anchoring and Stabilizing Protein (JASPer). The JIL-1-JASPer (JJ)-complex specifically enriched H3K36me3 modified nucleosomes *in vitro* via JASPer's PWWP domain from a nucleosome library containing 115 different nucleosome types. Consistently, ChIP-seq experiments showed that the JJ-complex localizes to H3K36me3 chromatin at active gene bodies and at telomeric transposons *in vivo*. As previously described, the JJ-complex is also enriched on the male X chromosome relative to autosomes. Loss of JIL-1 resulted in loss of JASPer enrichment, a small increase in H3K9me2 and a decrease in H4K16ac on the X chromosome shown by spike-in ChIP-seq. Gene expression analysis by RNA-seq showed that the JJ-complex positively regulates expression of genes, in particular of genes from the male X chromosome, and of telomeric transposons. Furthermore, the JJ-complex associated with the Set1/COMPASS complex and with other remodelling complexes as shown by co-IP coupled to mass spectrometry analysis.

Zusammenfassung

Dosiskompensation der Geschlechtschromosomen in *Drosophila melanogaster* ist ein exzellentes Modelsystem um verschiedene Aspekte der gezielten Bindung von Proteinfaktoren an Chromatin zu studieren. Dosiskompensation verhindert die Letalität in Männchen, durch Hochregulierung der Transkription des einfachen männlichen X Chromosoms im ungefähr zweifachen Bereich um es den beiden aktiven X Chromosomen der Weibchens anzupassen [rezensiert in z.B. (Ferrari et al., 2014; Kuroda et al., 2016; Samata and Akhtar, 2018)]. Diese Hochregulation wird durch den männerspezifisch letalen (MSL) Dosiskompensationskomplex (DCC) bewerkstelligt. Der DCC bindet selektiv an ~300 hochaffine Stelle (HAS) auf dem X Chromosom, welche ein niederkomplexes, GAGA-reiches Sequenzmotiv beinhalten, das MSL Erkennungselement (MRE) (Alekseyenko et al., 2008; Straub et al., 2008). Jedoch missachtet der DCC tausende ähnliche Sequenzen im Genom, die außerhalb von HAS liegen. Die DNA bindende Untereinheit MSL2 kann *in vitro* alleine X chromosomale MREs anreichern, wenngleich MSL2 die meisten MREs in HAS auslässt (Villa et al., 2016). Der chromatingebundene Adapter für MSL Proteine (CLAMP) bindet tausende MREs genomweit und trägt zur gezielten Bindung des DCC an HAS bei (Kaye et al., 2018; Soruco et al., 2013). Die Beitrag von CLAMP zur MSL2 Bindung an HAS wurde durch mehrere Ansätze untersucht. Messung der MSL2 Chromatinbindung *in vivo* durch Chromatin-Immunopräzipitation mit Hochdurchsatzsequenzierung (ChIP-seq) zeigte, dass CLAMP für die HAS-Bindung benötigt wird. Danach wurde das Zusammenspiel von CLAMP und MSL2 bei der genomweiten DNA-Bindung *in vitro* durch DNA-Immunopräzipitation mit Hochdurchsatzsequenzierung (DIP-seq) sudiert (Gossett and Lieb, 2008; Liu et al., 2005; Villa et al., 2016). Die Daten zeigten eine gegenseitige Anreicherung beider Faktoren an den jeweils anderen Bindestellen und eine kooperative Binding von neuen Stellen. Diese DNA-Bindekooperativität vergrößerte das Bindungsrepertoire des jeweils Anderen um eine robuste Bindung der MREs in HAS zu ermöglichen, obgleich eine verstärkte Bindung von andere nicht-funktionelle Stellen beobachtet wurde. Beide Faktoren interagierten direkt mit einander in Ko-IP-Experimenten, was eine Erklärung für die kooperative DNA-Bindung liefert. Ob CLAMP und MSL2 benötigt werden um HAS nukleosomfrei zu halten wurde durch den Assay für tranposasezugängliches Chromatin mit Hochdurchsatzsequenzierung (ATAC-seq) studiert. (Buenrostro et al., 2013; Buenrostro et al., 2015). Beide Faktore kooperieren um ihre Bindung gegenseitig zu stabilisieren und mit Nukleosompositionierung an HAS zu konkurrieren.

Nach erfolgreicher Bindung des DCC an HAS geht dieser zu benachbarten Zielgenen über, welche durch Trimethylierung von Histon H3K36 (H3K36me3) markiert sind. Dort katalysiert der DCC die Acetylierung von H4K16 (H4K16ac) um die Transkription zu erhöhen (Akhtar and Becker, 2000; Gelbart et al., 2009; Larschan et al., 2007; Prestel et al., 2010). Der DCC bedient sich dabei dem Chromosomeninteraktionsnetzwerk um von HAS zu aktiven Genen

zu gelangen, welches invariant zwischen Männchen und Weibchen zu seien scheint (Ramirez et al., 2015; Ulianov et al., 2016). Der Beitrag der HAS zum Chromosomeninteraktionsnetzwerk wurde durch die Benutzung verschiedene Chromosomenkonformationserfassungstechniken studiert. Hi-C-Analysen von nach Geschlechtern sortierten Embryos zeigte, dass H4K16ac und H3K36me3 gut mit dem aktiven Kompartiment korrelieren (Sexton et al., 2012). Interessanterweise ist ein Kompartimentwechsel auf dem X Chromosom zwischen Männchen und Weibchen mit H4K16ac korreliert und wird deshalb der Dosiskompensation zu geschrieben. Die Beteiligung der Pionierstellen auf dem X (PionX), einer besonderen Subklasse der HAS, zur Chromosomenarchitektur wurde durch hochauflösendes 4C-seq in männlichen und weiblichen Zellen studiert. Dies zeigte, dass PionX-beinhaltende Chromosomensegmente häufigeren Kontakt zu zahlreichen anderen Loci innerhalb des aktiven Kompartiments haben und sogar über große Domänen des inaktiven Kompartiments hinweg Schleifen bilden. (Ghavi-Helm et al., 2014). Diese weit reichenden Interaktionen zwischen PionX mit anderen PionX/HAS sind robuster in Männchen verglichen zu Weibchen, dies deutet darauf hin, dass diese durch die Dosiskompensationsmaschinerie verstärkt werden. Darüberhinaus zeigte *de novo* Induktion der DCC-Assemblierung in weiblichen Zellen, dass der DCC weitreichende Interaktionen innerhalb des aktiven Kompartimentes nutzt um von PionX zu Zielgenen zu transferieren, welche durch H3K36me3 markiert sind, zur Hochregulierung der Transkription.

Die chromosomale Kinase JIL-1, welche die Phosphorylierung von Histone H3S10 katalysiert, lokalisiert auch an aktiv transkribierte Gene, die durch H3K36me3 markiert sind, und ist ungefähr zweifach auf dem männlichen X Chromosom angereichert (Jin et al., 2000; Regnard et al., 2011; Wang et al., 2001). Generell trägt JIL-1 zur Aufrechterhaltung der allgemeinen Chromosomenorganisation bei und verhindert die Ausbreitung von Heterochromatin in den euchromatischen Teil des X Chromosoms in beiden Geschlechtern (Cai et al., 2014; Ebert et al., 2004; Jin et al., 1999). Zudem lokalisiert JIL-1 an die nicht-LTR Retrotransposonansammlung der Telomere um deren Expression positive zu regulieren (Andreyeva et al., 2005; Silva-Sousa and Casacuberta, 2013; Silva-Sousa et al., 2012). Die Rolle von JIL-1 bei der Genexpressionsregulation wurde unter Benutzung verschiedener Methoden studiert. JIL-1 formt mit dem neuen Protein JIL-1 verankerndes und stabilisierendes Protein (JASPer), das eine PWWP-Domäne enthält, einen stabilen Komplex, der JIL-1-JASPer (JJ)-Komplex. Der JJ-Komplex erkennt spezifisch H3K36me3 modifizierte Nucleosome *in vitro* über JASPer's PWWP-Domäne, was durch die Benutzung einer Nucleosomenbibliothek gezeigt wurde, die 115 verschieden modifizierte Nucleosome enthält. Konsistenter weise lokalisiert der JJ-Komplex an H3K36me3-Chromatin an aktive Genkörper und telomerische Transposons *in vivo* und ist auf dem männlichen X Chromosom

angereichert, dies wurde mit ChIP-seq analysiert. Die Anreicherung hängt direkt von JIL-1 ab, was durch beigespickten ChIP-seq gezeigt wurde. Verlust von JIL-1 führt zu einer kleinen Zunahme von H3K9me2 und Abnahme von H4K16ac auf dem männlichen X Chromosom. Genexpressionsanalyse durch RNA-seq zeigte, dass der JJ-Komplex die Genexpression positiv reguliert, insbesondere vom X Chromosom, und von telomerischen Transposons. Außerdem assoziiert der JJ-Komplex mit dem Set1/COMPASS-Komplex und mit anderen Remodelulierungskomplexen, was durch Ko-IP gekoppelt an Massenspektrometrieanalyse gezeigt wurde.

1 Introduction

Reproduction within all kingdoms of life requires inheritance of genetic information encoded in the genomic deoxyribonucleic acid (DNA) between generations (Avery et al., 1944). In eukaryotic cells, a rather small nucleus accommodates long DNA molecules, the chromosomes, in the form of chromatin. While most cells within a higher multicellular organism harbor the identical genetic information, many functional different tissues and cell types must be formed. This cell type specific information is stored in the epigenome [reviewed in e.g. (Rivera and Ren, 2013; Yadav et al., 2018)]. Sequencing of the human genome in the 1990s and early 2000s was still heavily time and cost consuming (International Human Genome Sequencing Consortium, 2004; International Human Genome Sequencing Consortium et al., 2001; Venter et al., 2001). Recent advances in sequencing technologies gave rise to time and cost effective next generation sequencing (NGS) approaches allowing the sequencing of millions of DNA molecules in parallel in a high-throughput format [reviewed in e.g. (Goodwin et al., 2016; Levy and Myers, 2016)]. Approaches by big consortia like the '1000 Genomes Project', 'Encode', and 'modEncode' projects sequenced thousands of human genomes and localized numerous chromatin marks and proteins (Celniker et al., 2009; Genomes Project et al., 2015; Stamatoyannopoulos et al., 2012; Sudmant et al., 2015; The Encode Project Consortium et al., 2012). Most recently, the 'Human cell atlas' project set out to generate a comprehensive map of all human cells using the latest single-cell genomic approaches (Regev et al., 2017). NGS provides a powerful tool to study various genome- and chromatin-linked aspects on a genome-wide scale.

1.1 Chromatin

At the end of the 19th century, chromatin and its basic building blocks DNA and histones were first described [reviewed in (Cremer and Cremer, 1988; Doenecke and Karlson, 1984; Olins and Olins, 2003)]. In 1869, Friedrich Miescher isolated a phosphorus-rich substance named *Nuklein* from pus leukocytes in the laboratory of Felix Hoppe-Seyler in Tübingen (Miescher, 1871). A few years later, Alfred Kossel, another former student of Felix Hoppe-Seyler, described a basic compound isolated from avian erythrocyte nuclei and termed it *Histon* (Kossel, 1884). In 1889, Richard Altman discovered that the non-protein portion of the *Nuklein* has acidic properties and named it therefore *Nukleinsäure* (= nucleic acid) (Altmann, 1889). At the same time, Walther Flemming described a stainable substance in the nucleus, which he named *Chromatin* (Flemming, 1882). In 1888, Wilhelm Waldeyer used first the word *Chromosom* to describe the chromatin structures in the

nucleus (Waldeyer, 1888). Only a century later in the 1970s, the basic structural and functional units of chromatin, the nucleosomes called 'ν bodies' at that time were independently described by Olins and Olins (Olins and Olins, 1974) and Woodcock et al. (Woodcock et al., 1976) visualized by electron microscopy as 'beads on a string' [reviewed in (Olins and Olins, 2003)]. Around the same time, Hewish and Burgoyne could show by limited nuclease digestion of chromatin leading to distinctly sized DNA fragments that chromatin is organized in a regular repeating structure (Hewish and Burgoyne, 1973). In 1974, Roger Kornberg suggested that these repeating units consist of a ~200 base pairs (bp) DNA fragment and two of each of the four main histones (Kornberg, 1974; Kornberg and Thomas, 1974). Finally, in 1975, Oudet et al. confirmed the proposed model of Roger Kornberg and the fundamental chromatin unit was by then termed 'nucleosome' (Oudet et al., 1975).

By now, numerous factors contributing to chromatin organization and chromatin plasticity have been described [reviewed in e.g. (Rivera and Ren, 2013; Yadav et al., 2018)]. At a glance, besides the canonical histones many different histone variants exist, their exchange is facilitated by histone chaperones and nucleosome remodelers, which can also reposition nucleosome along the DNA. Histones can be post-transcriptionally modified, e.g. methylation or acetylation of lysine residues and phosphorylation of serine residues, by histone modifying enzymes, so called 'writers'. These post-transcriptional modifications (PTMs) can be recognized by PTM binding proteins, the 'readers'. The DNA itself can also be bound by transcription factors and modified by DNA-modifying enzymes, most prominent the methylation of cytosines. In addition, the chromatin fibre is organized in higher-order structures to reach further compaction involving chromatin looping and forming of topologically associating domains (TADs). Thereby, all chromatin-related processes, such as gene expression, transcription, DNA replication and DNA repair, are regulated at various levels.

1.1.1 The nucleosome and higher-order chromatin structures

The canonical nucleosome core particle builds up from a central histone octamer core, containing two of each of the canonical histones H2A, H2B, H3 and H4, entwined with 147 bp DNA in 1.65 left-handed superhelical turns (Figure 1) (Davey et al., 2002; Luger et al., 1997) [reviewed in e.g. (Andrews and Luger, 2011; Cutter and Hayes, 2015; Khorasanizadeh, 2004; Luger et al., 2012; Pepenella et al., 2014)]. Each histone possesses a globular domain, formed by three α-helices arranged into the so-called 'histone fold', and unstructured N- and C-terminal regions, the 'histone tails'. By forming a 'handshake' structure, the histones dimerize giving rise to H2A-H2B and H3-H4 heterodimers, while the

later ones further dimerize to form a H3-H4 tetramer. To form the histone octamer, a H3-H4 tetramer associates with two H2A-H2B dimers on opposite sides of the tetramer. The globular domains of the histones within the octamer form a disc-like structure, where 147 bp of DNA are wrapped around to form the canonical nucleosome core particle. Basic histone residues contact the DNA giving rise to 14 weak protein-DNA interactions, however, summed up these interactions stably position the nucleosome. The histone tails emerge between the DNA to form an accessible hub for PTMs. *In vivo*, various histone chaperones are implicated in nucleosome formation.

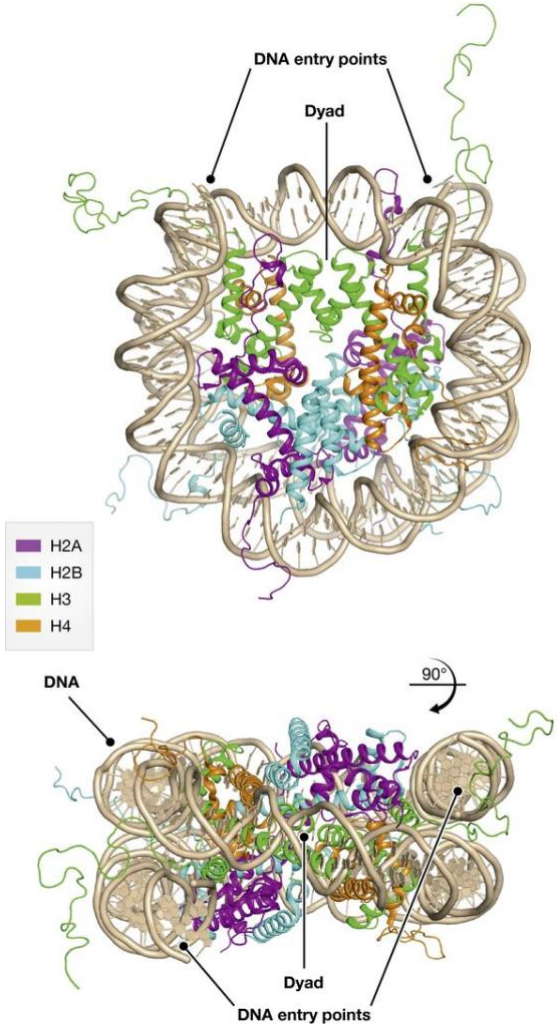


Figure 1. Structure of the canonical core nucleosome.

Representations of the X-ray crystal structure of the core nucleosome at 1.9 Å resolution (Davey et al., 2002). Cartoon of the nucleosome structure with H2A in purple, H2B in cyan, H3 in green, H4 in orange and DNA in wheat. Figure is reproduced from (Speranzini et al., 2016) with permission from John Wiley and Sons.

As *in vivo* DNA fragments have the size of chromosomes with tens to thousands of million base pairs, numerous nucleosomes are positioned on them forming the basis of chromatin [reviewed in e.g. (Bian and Belmont, 2012; Li and Reinberg, 2011; Luger et al., 2012;

Robinson and Rhodes, 2006; Tremethick, 2007; Woodcock et al., 2006)]. Therefore, neighboring nucleosome core particles are separated by short DNA stretches, the so-called 'linker DNA', to form nucleosome arrays, which have a diameter of 10 nm. The precise average linker length (mostly 20 – 40 bp) varies between organisms, but also between individual cell types and genomic locations. Binding of the linker histone H1 (or avian histone H5) at the nucleosome dyad and contacting both linker-DNAs lead to further compaction (Bednar et al., 2017). Histone H1 has an unstructured N-terminal region, a globular internal domain with a winged helix DNA-binding fold for binding the nucleosome dyad and a basic C-terminal domain. Further compaction of the primary nucleosomal array can be achieved by folding into a secondary structure, the 30 nm fiber. The extent to which the 30 nm fiber forms *in vivo* is still under debate. Further compaction is proposed to be alternatively achieved through interdigitated 10 nm fibers (Maeshima et al., 2016) [reviewed in e.g. (Ohno et al., 2018)]. However, based on various *in vitro* data two major models for the 30 nm fiber have been proposed (Figure 2). In the first model, the 'one-start' solenoid model, consecutive nucleosome form a super-helical path with 6 – 8 nucleosomes per turn involving bending of the linker DNA (Robinson et al., 2006). In the second model, the 'two-start' zigzag model, two nucleosomal rows fold into a two-start helix with straight linker DNA whereby very second next nucleosome interact with each other (Schalch et al., 2005). Recently, the special nucleosome positioning and orientation in chromosomes *in vivo* was studied by high-throughput chromosome conformation capture (Hi-C) resolving individual nucleosomes combined with simulated annealing-molecular dynamics in yeast (Ohno et al., 2019). The study showed that nucleosomes can fold into two secondary structures, either an α-tetrahedron or a β-rhombus fold, and the 3D nucleosome fold can be further altered through epigenetic marks at individual genomic loci. Generally, the secondary chromatin structure can be further compacted by folding into a tertiary structure. The highest compaction degree is achieved in metaphase chromosomes requiring condensin dependent loop formation (Gibcus et al., 2018). *In vivo*, higher-order chromatin structures are modulated and stabilized through binding of additional proteins to the chromatin fiber, including methyl-CpG-binding protein 2, heterochromatin protein 1 (HP1), high mobility group (HMG) proteins, Polycomb-group proteins. In the cell nucleus two general types of chromatin can be distinguished: euchromatin, which is more open and transcriptionally active, and heterochromatin, which is more compacted and transcriptionally inactive [reviewed in e.g. (Becker et al., 2016; Elgin and Reuter, 2013)].

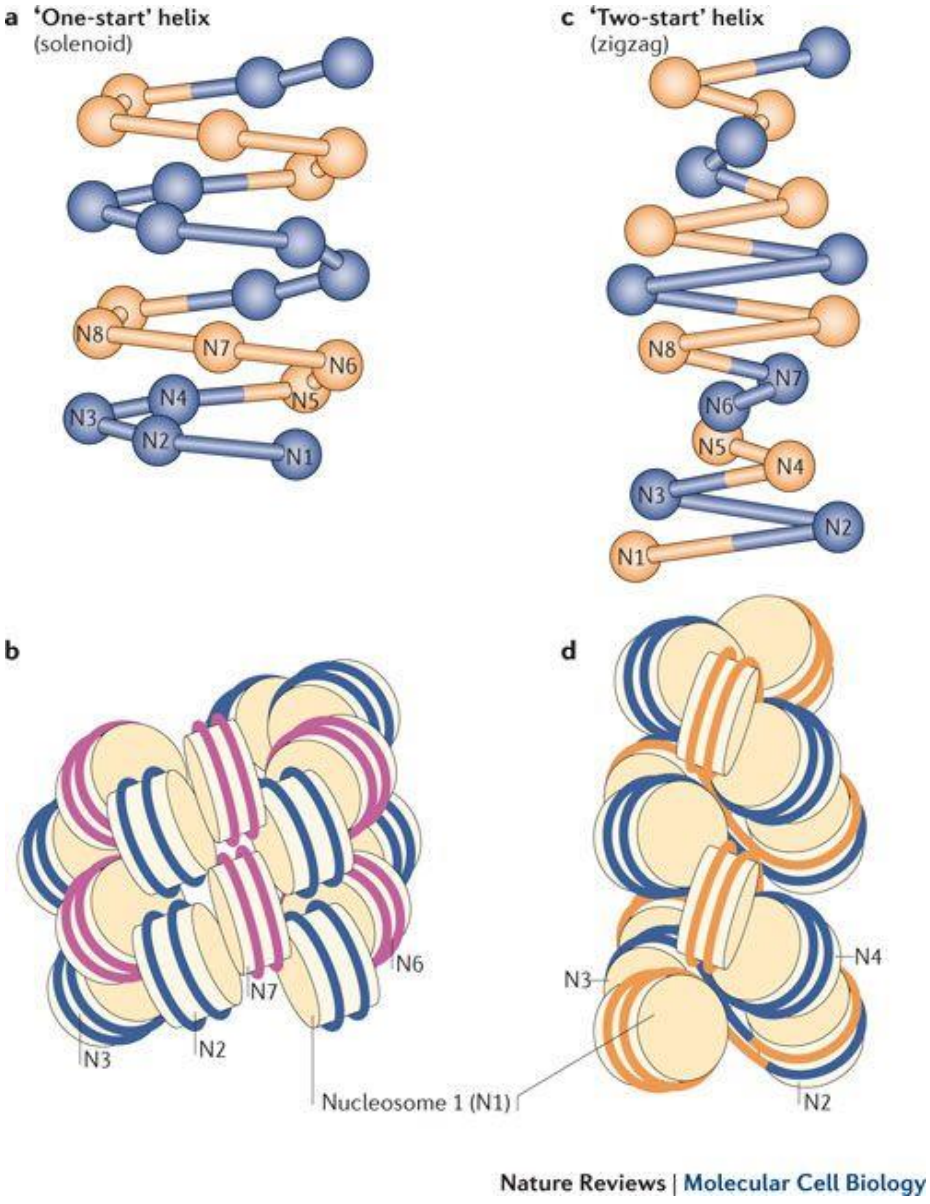


Figure 2. Models for 30 nm chromatin fibre.

Schematic representation of the two major structural models for chromatin secondary structure: left side, 'one-start' solenoid model, consecutive helical gyres are marked in blue and magenta/orange and right side, 'two-start' zigzag model, consecutive nucleosome pairs are marked in blue and orange. Figure is reproduced from (Luger et al., 2012) with permission of Elsevier.

Regulation apart from chromatin compaction is also achieved through positioning of individual nucleosomes along the DNA [reviewed in e.g. (Becker and Workman, 2013; Clapier and Cairns, 2009; Clapier et al., 2017; Luger et al., 2012; Radman-Livaja and Rando, 2010)]. Besides the intrinsic DNA sequence also adenosine triphosphate (ATP)-dependent nucleosome remodelers actively alter the nucleosome position and DNA-binding factors create local boundaries contributing to the precise positioning of nucleosomes. ATP-dependent nucleosome remodelers perform diverse functions to alter the chromatin structure such as nucleosome sliding, histone variant exchange and nucleosome eviction.

ATP-dependent nucleosome remodelers can be divided into four subfamilies differing in protein composition of the remodeling complexes. However, all remodeling complexes possess an ATPase 'motor' subunit that translocate DNA within the nucleosome.

1.1.2 Histone variants and modifications

The chromatin structure can be further modified through the exchange of canonical histones within the nucleosome with replacement histone variants [reviewed in e.g. (Gurard-Levin et al., 2014; Happel and Doenecke, 2009; Henikoff and Smith, 2015; Izzo and Schneider, 2016; Maze et al., 2014; Venkatesh and Workman, 2015)]. The genes of the canonical histones are present in multiple copies and clustered in the genome. They are only expressed during S-phase of the cell cycle and incorporated into chromatin in a replication-dependent manner. Their messenger ribonucleic acid (mRNA) lack introns and a polyA tail, instead they have a 3' stem loop structure crucial for regulating mRNA stability and translation. In contrast, histone variant genes are expressed throughout the whole cell cycle and incorporated in a replication independent manner involving specialized nucleosome remodelers and histone chaperones. Their mRNA can contain introns and they possess a 3' polyA tail. Except for histone H4, all canonical histones have replacement variants, which can differ at the protein level from the canonical histone by only few amino acids but my also contain additional non-histone domains. These differences in protein sequence can lead to changes in nucleosome stability, chromatin structure, PTM pattern and interaction with specific interaction partners.

The chromatin landscape can be altered at the level of histones not only by the exchange of histone variants but also by placing PTMs on histones (

Figure 3) [reviewed in e.g. (Andrews et al., 2016; Bannister and Kouzarides, 2011; Gelato and Fischle, 2008; Jenuwein and Allis, 2001; Kouzarides, 2007; Musselman et al., 2012; Rothbart and Strahl, 2014; Taverna et al., 2007)]. PTMs are often small chemical groups added to amino acid residues, e.g. covalent linkage of methyl-groups to lysine and arginine residues, acetyl-groups to lysine residues and phosphate-groups to serine and threonine residues. Probably methylation is the most wide—spread histone PTM, whereby lysine residues can be mono-, di- and trimethylated, while the arginine residues can be mono- and dimethylated. Larger groups like ubiquitin and Small Ubiquitin-related Modifier (SUMO) can also be post-transcriptionally added to lysine residues. In recent years, many novel histone modifications were identified, e.g. adenosine diphosphate (ADP) ribosylation, crotonylation or addition of β-N-acetylglucosamine.

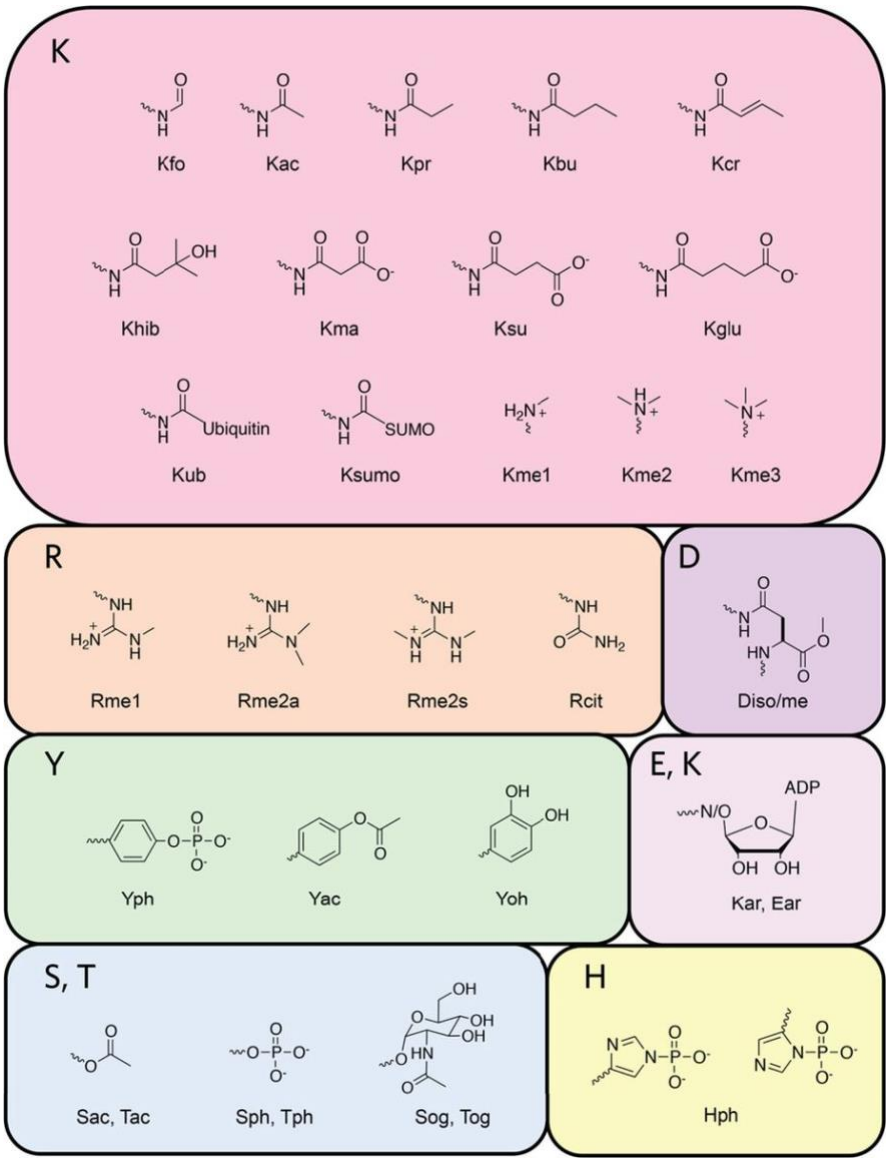


Figure 3. Identified post-transcriptional modifications in histones.

Modifications are grouped according to the modified amino acid, using the abbreviations: ar - ADP ribosylation; cit - citrullination; fo - formylation; glu - glutarylation; ma - malonylation; oh - hydroxylation; og - O-GlcNAcylation; su - succinylation; ub - ubiquitination. Figure is reproduced from (Andrews et al., 2016) with permission from Springer Nature.

Histone PTMs are often placed at specific amino acid residues in the histone tails, still some residues in the globular domain or at the C-terminus can be modified (Figure 4). PTMs are placed by site-specific histone modifying enzymes, the ‘writers’, and the presence of specific PTMs is a dynamic process as most can also be specifically removed by other histone modifying enzymes, the ‘erasers’. Histone modifications can shape the chromatin landscape in different ways. The addition of PTMs can cause direct biophysical changes to the chromatin fiber, as charges can be added to or removed from the histones. While methylation of lysine residues does not alter the positive net charge, acetylation neutralizes the charge, which weakens the interaction of the histone tail with the negatively charged

DNA and causes a more open chromatin state. Additionally, many chromatin binding proteins, the 'readers', can specifically recognize individual PTMs as they have modification-specific binding domains. In many cases, specific PTMs accumulate in distinct genome region where they are associated with specific chromatin processes, e.g. constitutive heterochromatin is marked by trimethylation of histone H3 at lysine 9 (H3K9me3) and active promoters with trimethylation of histone H3 at lysine 4 (H3K4me3). Therefore, the chromatin landscape can be separated into discrete chromatin states due to the prevalent histone marks and associated chromatin factors (Ernst and Kellis, 2010, 2012; Filion et al., 2010; Kharchenko et al., 2011).

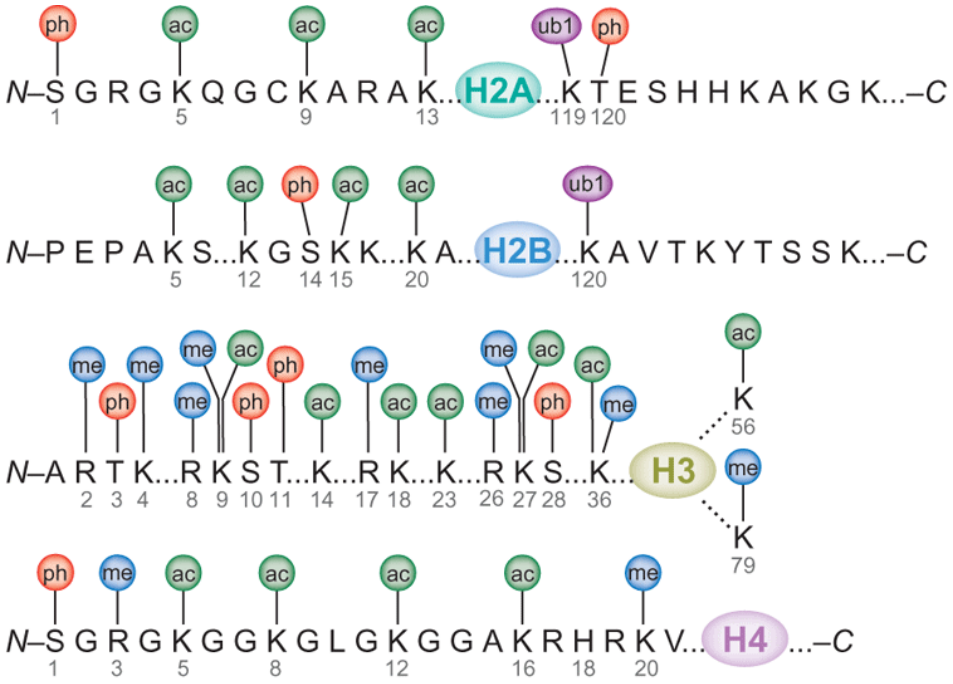


Figure 4. Post-transcriptional modifications of histone tails.

Modifications of specific histone tail residues. Positions are indicated by numbers. Colored ovals represent globular histone domains. Figure is reproduced from (Bhaumik et al., 2007) with permission from Springer Nature.

1.2 Chromatin binding factors

Chromatin binding proteins often recruit and regulate other chromatin modifying, chromatin remodeling, or transcription regulating complexes to target genome loci. Chromatin binders can either directly bind to DNA in a chromatin environment or interact with histone PTMs. Until now, many distinct protein domain families were discovered to recognize and bind specifically to certain histone PTMs [reviewed in e.g. (Musselman et al., 2012; Patel and Wang, 2013; Taverna et al., 2007; Weaver et al., 2018; Yun et al., 2011)]. Probably the most extensively studied group are the binders of methylated lysine residues, they include ATRX-DNMT3-DNMT3L (ADD) domain, ankyrin domain, bromo-adjacent homology domain, chromo-barrel domain, chromodomain, double chromodomain, malignant brain tumor (MBT) domain, plant homeodomain (PHD), PWWP (Pro-Trp-Trp-Pro) domain, tandem Tudor domain, Tudor domain, WD40 domain and zinc finger CW domain. The methyl-lysine binding domains contain an aromatic cage formed by two to four aromatic residues, which specifically recognize the methyl mark. The Tudor domain and WD40 domain are also capable in binding to methylated arginine residues. Substantially fewer protein domains are known to recognize acetylated lysine residues. So far the bromodomain, double PHD finger domain and double pleckstrin homology domain were described to bind acetyl-lysine. Similarly, only a few binding domains were identified to specifically interact with phosphorylated serine and threonine residues of histones, including tandem BRCT domain, BIR domain and the 14-3-3 protein family.

Some of the histone reader domains, including bromodomains, PHD fingers, PWWP domains, chromodomains and Tudor domains, are also described to have additional nucleic acid binding activity [reviewed in (Weaver et al., 2018)]. Binding of reader domains to DNA and/or RNA is largely sequence-independent. Accordingly, nucleic acid binding may be independent of histone binding, or binding to DNA/RNA increases histone binding and the other way around. The additional nucleic acid binding of reader domains can result in increased affinity for chromatin and retention at target sites. Furthermore, multivalent histone and nucleic acid binding can increase the affinity for nucleosomes or enable proper positioning on the nucleosome to increase specificity for certain histone residues. Another mechanism could be that binding of the reader domain to DNA releases DNA-interacting histone tails, to make the histone tail accessible for reader/writer domains.

Besides histone reader domains, numerous domains with sequence specific DNA binding activity are described (Fulton et al., 2009; Vaquerizas et al., 2009) [reviewed in e.g. (Lambert et al., 2018; Luscombe et al., 2000)]. Proteins with specific DNA binding activity are often transcription factors (TF) as they participate in transcription regulation. The major DNA binding domain families include C2H2 zinc finger (ZnF), Homeodomain, basic helix-loop-helix

domain, basic leucine zipper domain, and nuclear hormone receptor domain. DNA binding domains have two DNA recognition modes to readout the DNA sequence. One form of DNA interaction is referred to as ‘direct readout’, whereby amino acid side chains of the DNA binding domain directly contact the base pairs via direct or water mediated hydrogen bonds and hydrophobic interactions. The other form is known as ‘indirect readout’, whereby the binding domain senses the DNA shape, which in turn is a sequence-specific structural feature of the DNA. Sequence-specific DNA binding factors usually recognize a set of hundreds to thousands similar DNA sequence over a range of affinities within the genome [reviewed in e.g. (Inukai et al., 2017; Pan et al., 2010; Rohs et al., 2010; Siggers and Gordan, 2014; Slattery et al., 2014)]. The consensus binding site motif of bound DNA sequences is commonly modelled by position weight matrix (PWM). However, only a subset of DNA sequences which resemble to the binding site motif are bound by the DNA binding factor *in vivo*. Besides the DNA sequence, additional features contribute to binding site selection *in vivo*, including DNA shape, flanking DNA, variable spacing of bipartite binding motifs, DNA methylation/modification, multimeric binding, cofactor interaction, interaction/cooperation with other DNA binding factors, DNA accessibility, nucleosome occupancy and competition with nucleosomes.

1.2.1 Cooperative DNA binding

DNA binding factors are non-uniformly distributed throughout the genome, they rather form clusters or hotspots at distinct loci. Since binding motifs are only slightly enriched within the binding clusters, cooperativity between DNA binding factors through various mechanisms seems to contribute to the enrichment of factors at binding clusters [reviewed in e.g. (Morgunova and Taipale, 2017; Siggers and Gordan, 2014)]. Cooperation can be achieved through direct protein-protein interaction of two DNA binding factors in the absence of DNA. There, the protein complex poses a higher affinity for DNA as the individual factors. Similarly, if the interaction of two factors is too weak to form a stable soluble complex, DNA binding can favor this interaction by bringing both factors in close proximity or introducing conformational changes required for stable interaction, resulting in increased DNA binding affinity. Furthermore, solely DNA-mediated cooperativity between two factors that lack direct protein-protein interaction can still strengthen their DNA binding. Changes in DNA shape or dynamics induced by binding of one factor can result in increased affinity for DNA of another factor. Additionally, an indirect cooperativity mechanism is described that functions through competition for DNA binding between binding factors and nucleosomes allowing binding of several factors next to each other at the created nucleosome free region (NFR).

1.2.2 DNA binding through CXC domains

The Tesmin/TSO1-like CXC domain is a rather rare protein domain, which can be found in the Animalia and Plantae kingdom and is absent in the Fungi kingdom (Marin, 2003). The Tesmin/TSO1-like CXC domains are particularly conserved within the C-terminal part with the consensus formula [C-$(X)_4$-C-(X)-C-$(X)_6$-C-X_4-5-C-$(X)_2$-C], while the N-terminal part is more variable. Three subtypes of Tesmin/TSO1-like CXC domains can be distinguished (Marin, 2003; Mitchell et al., 2019): (a) “CRC domain” found in the human Tesmin protein and in the *Arabidopsis thaliana* TSO1 protein; Many proteins with “CRC domains” have two domains in tandem separated by a RNPXAFXPK linker (Andersen et al., 2007; Song et al., 2000; Sutou et al., 2003); To this class also the LIN-54 homolog family members belong, in *Drosophila melanogaster* this is mip120 (Beall et al., 2002; Marin, 2003; Schmit et al., 2007); (b) “CXC domain” found in the homologs of the polycomb group protein Enhancer of zeste [E(z)] (Abel et al., 1996; Chen et al., 1996; Goodrich et al., 1997; Jones and Gelbart, 1993); (c) “E3 ubiquitin-protein ligase Msl2, CXC domain” found in the homologs of the *D. melanogaster* male-specific-lethal (MSL) 2 protein (Figure 5) (Bashaw and Baker, 1995; Smith et al., 2005; Zhou et al., 1995); However, the “E3 ubiquitin-protein ligase Msl2, CXC domain” is specific to animals (Marin, 2003). The CXC domain seems to be a conserved DNA binding domain as some protein members are shown to bind DNA, including *D. melanogaster* MSL2 (Fauth et al., 2010; Zheng et al., 2014), human LIN54 (Marceau et al., 2016; Schmit et al., 2009) and soybean Cysteine-rich polycomb-like protein 1 belonging to the “CRC domain” family too (Cvitanich et al., 2000). Additionally, the DNA binding mode of *D. melanogaster* MSL2 and human LIN54 were studied using X-ray crystallography and nuclear magnetic resonance (NMR) (Figure 5) (Marceau et al., 2016; Zheng et al., 2014; Zheng et al., 2012). *D. melanogaster* MSL2 harbors a single CXC fold, which is formed by three zinc ions coordinated by nine cysteine residues. The Zn_3Cys_9 cluster is wrapped by flexible loops and a short α-helix in a two turn right-handed helix. When bound to DNA, two CXC domains bind next to each other on opposite strands making protein-protein contacts. Each CXC domain contacts the DNA phosphate backbone by hydrogen bonding and inserts two arginine residues, one into the minor and one into major groove, forming base-specific hydrogen bonds. The human LIN54 harbors two tandem “CRC domains” with independent CXC folds, which are connected by a flexible linker. To form the CXC fold, nine cysteine residues coordinate three zinc ions, which are connected by flexible loops and short α-helix making 1.5 right-handed helical turns. In addition, each fold contains a C-terminal α-helix, which is absent in the MSL2 CXC fold. The tandem “CRC domains” bind to adjacent, opposite sites at the minor grove to the target DNA and the domains make no protein-protein contacts. Each domain makes extensive hydrogen bonding to the DNA phosphate backbone

and base-specific contacts by inserting two tyrosine residues into the minor groove. The tyrosine residues form hydrogen bonds to two adjacent bases from opposite strands.

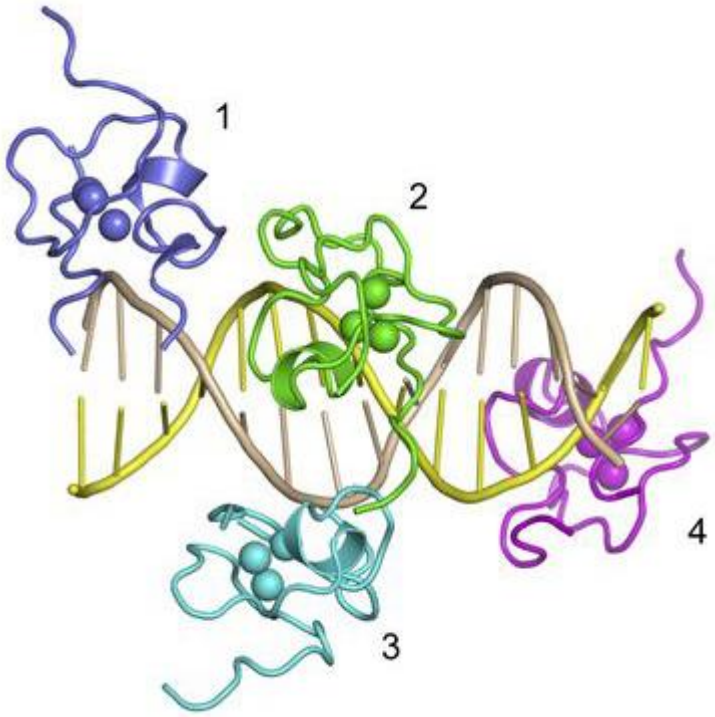


Figure 5. CXC domain binding to DNA.

Representation of NMR assembly of MSL2 CXC domain bound to DNA. Cartoon representations of four CXC domains of MSL2 binding to DNA. Figure is reproduced from (Zheng et al., 2014) with permission of Cold Spring Harbor Laboratory Press.

1.2.3 DNA binding through C2H2 zinc finger domains

Zinc finger domains are found throughout all kingdoms of life and are one of the most abundant protein domains in eukaryotes [reviewed in e.g. (Brayer and Segal, 2008; Brown, 2005; Iuchi, 2001; Razin et al., 2012; Wolfe et al., 2000)]. Various types of ZnF domains exist, which have in common that they use cysteine and/or histidine residues to complex a single zinc ion for stabilizing their fold. The founding member of the ZnF superfamily is the "classical" C2H2 ZnF domain. The C2H2 ZnF domain consists of ~30 amino acid residues with the consensus sequence (F/Y)-X-C-X_{2-5}-C-X_3-(F/Y)-X_5-ψ-X_2-H-X_{3-5}-H, ψ representing hydrophobic residues. The first structure of C2H2 ZnF domain was solved in 1989, showing that the single zinc ion is tetrahedrally coordinated by two cysteine and two histidine residues (Lee et al., 1989; Wolfe et al., 2000). Further, the C2H2 ZnF domain folds into two N-terminal, antiparallel β-sheets with the connecting loop harboring the two cysteine residues and one C-terminal α-helix with the two histidine residues at the C-terminal part of the α-helix (Figure 6). C2H2 ZnF domains are involved in DNA binding, RNA binding and protein-protein

interaction. Many proteins contain patches of multiple C2H2 ZnF domains, some proteins even > 30. Based on the number and distribution of C2H2 ZnF domains within a protein, C2H2 ZnF containing proteins can be categorized into three groups (Iuchi, 2001; Razin et al., 2012): (a) “triple-C2H2 ZnF proteins”, this group includes the Krüppel-like family and SP1-like family of TFs; (b) “multiple-adjacent-C2H2 ZnF proteins” is the largest group and the proteins contain clusters of > 4 closely spaced C2H2 ZnF domains; one well-studied member of this group is the CTCF protein containing a cluster of 11 C2H2 ZnF domains (c) “separated-paired-C2H2 ZnF proteins”, this group comprises the lowest number of TFs, including tramtrack, PRDII-BF1 and basonuclin; This group of ZnF proteins contain one or more pairs of C2H2 ZnF domains, where individual pairs are separated by many intervening amino acid residues. Alternatively, C2H2 ZnF proteins are classified according to additional domains present in the protein, such as the KRAB domain, SCAN domain and BTB/POZ domain.

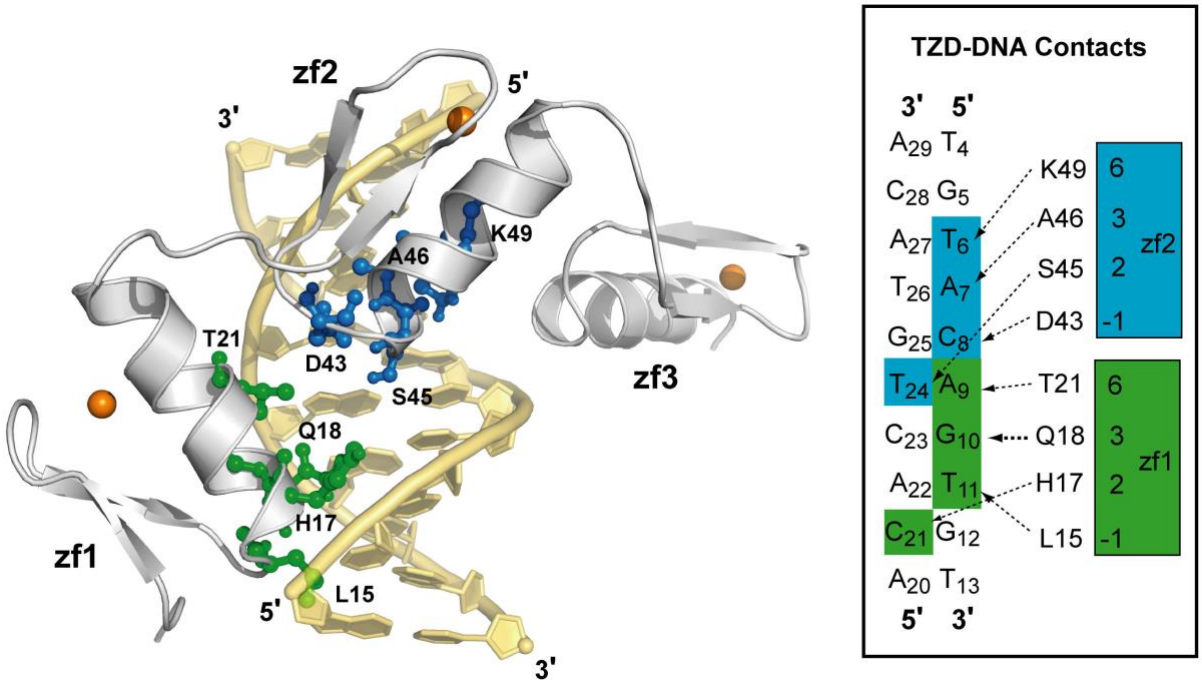


Figure 6. C2H2 zinc finger domain binding to DNA.

Representation of NMR assembly of TZD ZnF domain bound to DNA. Left side, cartoon representation of three C2H2 ZnF domains of TZD binding to DNA, with side chains at position -1, 2, 3 and 6 as sticks and balls. Right side, schematic representation of direct DNA base contact of ZnF domains. Figure is reproduced from (Chou et al., 2017) with permission of PLOS.

C2H2 ZnF domains are mostly described in DNA binding, as many TFs contain multiple C2H2 ZnF domains. Binding of C2H2 ZnF domains to DNA is studied extensively using structural approaches (Elrod-Erickson et al., 1996; Pavletich and Pabo, 1991; Wolfe et al., 2001; Wolfe et al., 2000; Wuttke et al., 1997). For DNA binding, the C2H2 ZnF domain makes numerous contacts to the phosphate back bone and direct base contacts by inserting the α-helix into the major groove. For direct base contact, the amino acid residues at position

-1, 3, and 6 form hydrogen bonds with one DNA strand and the residue at position 2 contacts the complementary strand (Figure 6). C2H2 ZnF domains occur often in clusters and neighboring C2H2 ZnF domains wrap around DNA when bound. Each C2H2 ZnF domain contacts four consecutive bases and adjacent C2H2 ZnF domains are shifted by three base pairs, resulting in the overlap by one base pair. Neighboring C2H2 ZnF domains are most commonly connected by a five amino acid-linker with the consensus sequence TGEKP. The linker contributes to correct spacing of adjacent C2H2 ZnF domains and responsible for capping the C-terminus of the preceding α-helix in a DNA binding-dependent manner (Laity et al., 2000). Capping of the α-helix C-terminus provides rigidity and locks the α-helix into the correct place when bound to DNA, contributing to high affinity binding. While scanning the DNA strand for appropriate binding sites, the linker does not fold onto the α-helix C-terminus providing flexibility between adjacent domains. However, structural studies of GLI and TFIIA as well as biochemical and genomic studies of CTCF showed that proteins with multiple adjacent C2H2 ZnF domains can just use a subset of their C2H2 ZnF domains to bind DNA, allowing certain flexibility and deviation in DNA binding site recognition (Filippova et al., 1996; Filippova et al., 2002; Nakahashi et al., 2013; Nolte et al., 1998; Pavletich and Pabo, 1993; Renda et al., 2007).

1.2.4 Nucleosome binding through PWWP domains

The PWWP domain belongs to the Royal superfamily, which also includes the Tudor domain, chromodomain, and MBT domain [reviewed in e.g. (Qin and Min, 2014; Rona et al., 2016; Weaver et al., 2018)]. Members of the Royal superfamily can bind specifically to methylated lysine and/or arginine residues in histone tails. They share a conserved aromatic cage structure, which is responsible for methyl-lysine/arginine binding, present in a β-barrel fold formed by three to five β-sheets. The PWWP domain is named after the characteristic Pro-Trp-Trp-Pro sequence motif present in the N-terminal part of the domain. However, especially the first and second position of the motif is variable. The PWWP domain was first described in WHSC1 and is conserved within all eukaryotes (Stec et al., 1998). Over the last years, much structural information on PWWP domains and their binding to nucleosomes was obtained (Figure 7) (Eidahl et al., 2013; Laguri et al., 2008; Qiu et al., 2002; Sue et al., 2004; van Nuland et al., 2013; Vezzoli et al., 2010; Wen et al., 2014; Wu et al., 2011a).

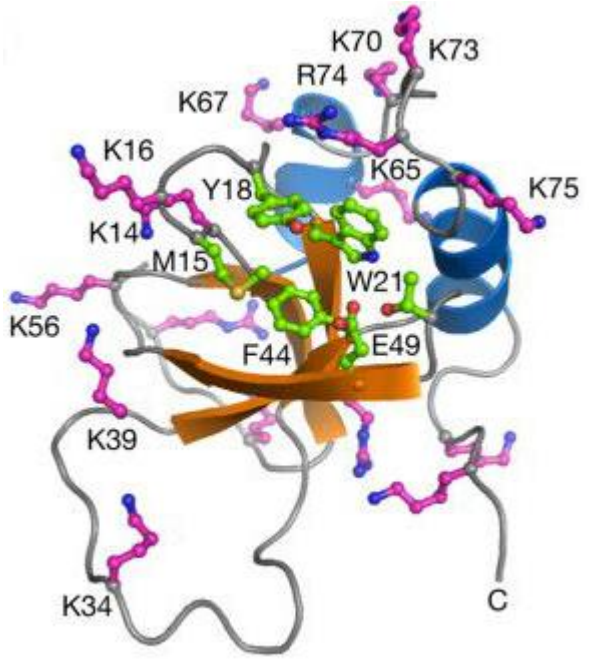


Figure 7. Protein structure of PSIP1 PWWP domain.

Representation of NMR assembly of PSIP1 PWWP domain. Cartoon representation of PSIP1 PWWP domain with aromatic side chains in green balls and sticks and basic side chains in purple. Figure is reproduced from (van Nuland et al., 2013) with permission of BioMed Central.

The PWWP domain is formed by a β-barrel fold of five antiparallel β-sheets and a helix bundle of one to six α-helices. Within the β-barrel, a loop region of variable length and secondary structure between β2 and β3 and often a 3_{10} helix between β4 and β5 are inserted. Furthermore, three aromatic residues located in the loop between β1 and β2, in the PWWP motif and in the β3-strand form an aromatic cage accommodating the methyl-lysine side chain. Most PWWP domains are suggested to bind trimethylated histone H3 at lysine 36 (H3K36me3), also binding to trimethylated histone H3 at lysine 79 (H3K79me3) and mono/trimethylated histone H4 at lysine 20 (H4K20me1/3) were described (Dhayalan et al., 2010; Eidahl et al., 2013; Li et al., 2013a; van Nuland et al., 2013; Vermeulen et al., 2010; Wen et al., 2014; Wu et al., 2011a). A second interaction surface is formed by solvent exposed basic residues mostly located at β1-β2 loop, PWWP motif and β2-strand next to the aromatic cage. This positively charged interaction surface mediates sequence-unspecific DNA binding through interactions with the phosphate backbone (Figure 8). As shown for PWWP domain binding to H3K36me3 nucleosome, the positively charged DNA binding surface and methyl-lysine binding aromatic cage synergize for binding with high affinity (Eidahl et al., 2013; van Nuland et al., 2013). The PWWP domains contacts the phosphate backbone of both DNA gyres next to the H3 tail exit site through its basic surface and the aromatic cage engages with the K36me3 residue.

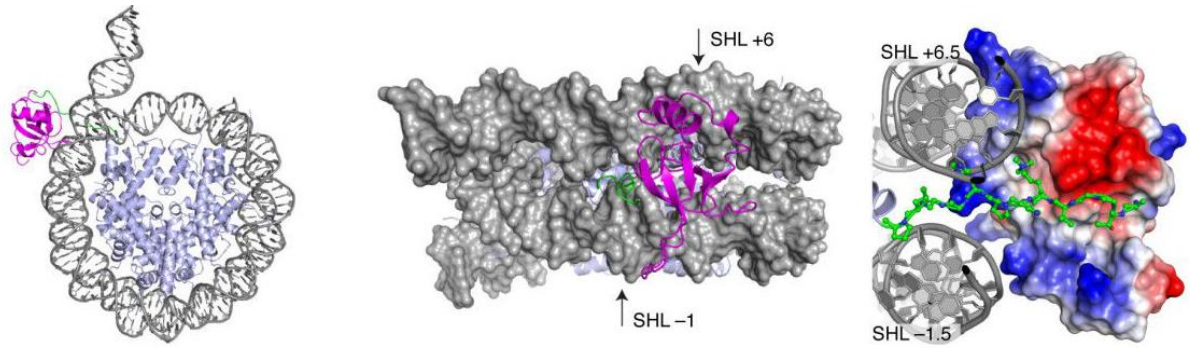


Figure 8. PWWP domain binding to H3K36me3 nucleosome.
Structural model of PSIP1 PWWP domain bound to H3K36me3 nucleosome. Left side and middle, cartoon representation of lowest energy structure of PSIP1 PWWP domain docked to H3K36me3 nucleosome. Right side, detailed view on PSIP1 PWWP domain bound to H3K36me3 nucleosome with residues 31 to 38 of the H3 N-terminal tail as balls and sticks in green, DNA a cartoon representation and electrostatic potential of molecular surface of PWWP domain. Figure is reproduced from (van Nuland et al., 2013) with permission of BioMed Central.

1.3 Sex chromosome dosage compensation

Most metazoans propagate by various means of sexual reproduction. Here, sex determination is genetically encoded by sex chromosomes, which often belong to the XY system. In the XY system, females have two X chromosomes in addition to a diploid set of autosomes, while males have a single X chromosome and an often degenerated, gene-poor Y chromosome [reviewed in e.g. (Chandler, 2017; Disteche, 2012, 2016; Gu and Walters, 2017; Lucchesi, 2018)]. The concept of sex chromosome evolution was already introduced one hundred years ago by Hermann J. Muller, who proposed that reduced recombination between the X and Y chromosome leads to degeneration of the Y chromosome and later he also presented the concept of dosage compensation (Muller, 1914). In 1967, Susumu Ohno extended that idea and proposed that the gene-rich X and degenerated Y chromosome evolved from a normal pair of autosomes that acquired a sex-determining locus (Ohno, 1967). The different number of X chromosomes creates an imbalance of X-linked genes between males and females and between the single male X chromosome and the diploid autosomes. This imbalance is adjusted by a process called dosage compensation, first proposed by Hermann J. Muller (Muller, 1932, 1950). During evolution, various mechanisms for sex chromosome dosage compensation evolved individually, the best studied systems are eutherian mammals (humans/mice), *Caenorhabditis elegans* and *Drosophila melanogaster* (Figure 9).

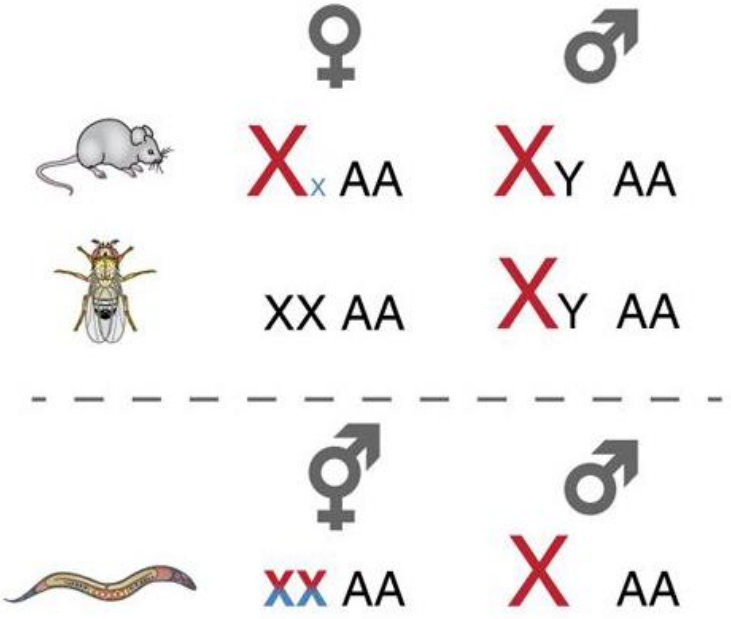


Figure 9. Dosage compensation of sex chromosomes.
Schematic representation of sex chromosome dosage compensation in *Mus musculus*, *Drosophila melanogaster* and *Caenorhabditis elegans*. Figure is reproduced from (Ferrari et al., 2014) with permission of Springer Nature.

In mammalian dosage compensation, one of the female X chromosomes is inactivated by the formation of the heterochromatic Barr body [reviewed in e.g. (Bonora and Disteche, 2017; Brockdorff, 2017; Brockdorff and Turner, 2015; Migeon, 2017; Pinheiro and Heard, 2017; Sahakyan et al., 2017)]. Inactivation of the X chromosome is triggered through the expression of the long non-coding RNA (lncRNA) Xist, which coats the whole chromosome. Xist expression leads to the recruitment of polycomb complexes which repress gene expression by placing heterochromatic histone marks including trimethylated histone H3 at lysine 27 (H3K27me3) and ubiquitylation of histone H2A at lysine 119 (H2AK119ub). Furthermore, X chromosome silencing includes incorporation of the histone variant macroH2A. Stochastic inactivation of one of the two female X chromosomes leaves females with only one active X chromosome like males. This creates an imbalance between X-linked genes and autosomal genes in both sexes. Therefore, the gene expression from the single active X chromosome is up-regulated in both sexes through a mechanism that involves acetylation of histone H4 at lysine 16 (H4K16ac) by males absent on the first (MOF) at promoters and enrichment of paused RNA polymerase II and increased RNA stability (Deng et al., 2013).

In *C. elegans*, males have a single X chromosome and lack a Y chromosome, while hermaphrodites have two X chromosomes. In hermaphrodites, the two X chromosomes are silenced to 50% during dosage compensation. Dosage compensation in worms is facilitated by the dosage compensation complex (DCC), which is a specialized condensin complex

[reviewed in e.g. (Albritton and Ercan, 2018; Strome et al., 2014)]. Binding of the DCC to the two X chromosomes in hermaphrodites leads to reduced expression of X-linked genes through chromosome compaction, reduced H4K16ac level and increased monomethylation of histone H4 at lysine 20, which represses RNA polymerase II binding to promoters. As in mammals, this silencing by half of both X chromosomes results in an imbalance between X-linked and autosomal genes, which is also present in males. Therefore, the expression of X-linked genes is up-regulated in both sexes.

1.3.1 Dosage compensation in *Drosophila melanogaster*

D. melanogaster dosage compensation differs from mammals and *C. elegans* as it lacks inactivation of the female X chromosomes and only the single male X chromosome is upregulated to match the two active female X chromosomes to prevent male lethality (Mukherjee and Beermann, 1965) [reviewed in e.g. (Birchler, 2016; Conrad and Akhtar, 2012; Ferrari et al., 2014; Keller and Akhtar, 2015; Kuroda et al., 2016; Lucchesi and Kuroda, 2015; Maenner et al., 2012; McElroy et al., 2014; Samata and Akhtar, 2018; Straub and Becker, 2007, 2011)]. That male-specific upregulation of X-linked gene expression in the two-fold range is facilitated by the so-called dosage compensation complex (DCC). The DCC is a ribonucleoprotein complex consisting of five male-specific-lethal (MSL) protein subunits and a long, non-coding (lnc) RNA component (Figure 10). The first DCC members, including MSL1, MSL2, MSL3 and maleless (MLE), were discovered by genetic screens or isolated in nature around the early 1980s (Belote and Lucchesi, 1980a, b; Fukunaga et al., 1975; Uchida et al., 1981). Later in the early 1990s, bulk acetylation of H4K16 was described to be restricted to the male X chromosome and depending on the presence of MSL proteins (Bone et al., 1994; Turner et al., 1992). The remaining DCC components were only discovered in the late 1990$_{th}$, including the fifth MSL protein males absent on the first (MOF), the histone acetyltransferase specific for H4K16ac, and the two lncRNA subunits, RNA-on-the-X (roX) 1 and 2 (Amrein and Axel, 1997; Franke and Baker, 1999; Hilfiker et al., 1997; Meller et al., 1997). As indicated by their naming, both RNAs roX1 and roX2 are encoded on the X chromosome and co-localize with the MSL proteins on the male X chromosome (Kelley et al., 1999; Meller et al., 2000). Co-immunoprecipitation (IP) experiments indicated that the MSL proteins form a stable complex together with the roX RNAs (Alekseyenko et al., 2014; Copps et al., 1998; Kelley et al., 1995; Meller et al., 2000; Smith et al., 2000; Wang et al., 2013). Furthermore, the DCC assembles exclusively in males, as translation of MSL2 protein is restricted to males (Gebauer et al., 1999; Gebauer et al., 2003; Grskovic et al., 2003; Hennig et al., 2014; Kelley et al., 1995; Kelley et al., 1997; McDowell et al., 1996; Szostak et al., 2018). The Sex lethal (SXL) protein, which regulates sex determination through sex-specific

splicing, is only functional in females due to female-specific expression and splicing during early embryogenesis. SXL prevents translation of msl2 mRNA by a splicing-independent mechanism through inhibiting ribosome assembly on msl2 mRNA. In males, a non-functional splicing isoform of SXL is expressed allowing msl2 mRNA translation and accumulation of MSL2 protein leading to DCC formation and finally dosage compensation. The mechanism of dosage compensation in the model organism *D. melanogaster* is widely conserved throughout various *Drosophilid* species (Alekseyenko et al., 2013; Quinn et al., 2016; Sural et al., 2008).

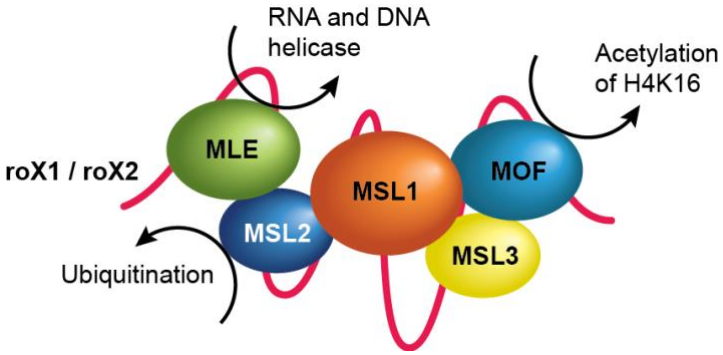


Figure 10. Dosage compensation complex.
Schematic representation of the DCC in *D. melanogaster*. Figure is adapted from (Straub and Becker, 2007) with permission of Springer Nature.

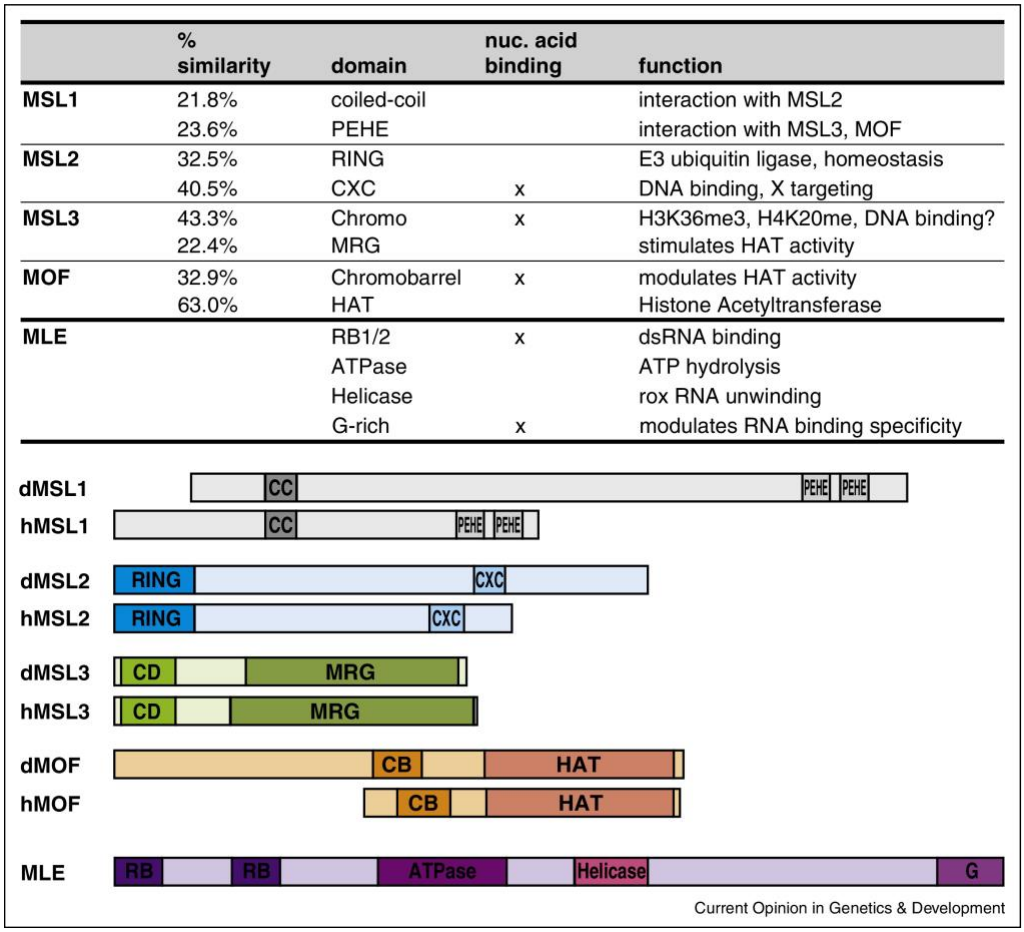
1.3.2 The Dosage Compensation Complex

The DCC forms most likely a dimeric complex through homodimerization of MSL1 via its N-terminal coiled-coil domain (Figure 11) (Hallacli et al., 2012). MSL1 seems to be a rather unstructured protein and in addition to the N-terminal coiled-coil domain it has only a C-terminal PEHE domain. MSL1 serves as a scaffold for the other DCC members, as it interacts via its coiled-coil domain with the really interesting new gene (RING) finger domain of MSL2, via the N-terminal half of the PEHE domain with the histone acetyltransferase (HAT) domain of MOF and via the C-terminal half of the PEHE domain with the MRG domain of MSL3 (Hallacli et al., 2012; Kadlec et al., 2011; Li et al., 2005; Morales et al., 2005; Morales et al., 2004; Scott et al., 2000; Wu et al., 2011b). MSL2 is the male-specific subunit of the complex and it harbors two domains, the N-terminal RING finger domain and a C-terminal CXC domain followed by a proline-rich region including a patch of basic residues. MSL2's RING finger domain possesses an E3 ubiquitin ligase activity besides being required for interaction with MSL1 (Kruse and Gu, 2009; Schunter et al., 2017; Villa et al., 2012; Wu et al., 2011b). MSL2 can ubiquitylate itself and other members of the DCC, including MSL1, MSL3 and MOF. It is thought that MSL2 contributes to DCC homeostasis by ubiquitylating surplus DCC and marking it for proteasome-dependent degradation as DCC levels need to

be tightly regulated (Demakova et al., 2003; Johansson et al., 2011; Kelley et al., 1995; Kelley et al., 1997; Villa et al., 2012). MSL2's CXC domain is a DNA binding domain required for DCC targeting to the X chromosome (Fauth et al., 2010; Villa et al., 2016; Zheng et al., 2014). MSL3 belongs to the MGR15/MSL3 protein family and has, besides the C-terminal MRG domain required for interaction with MSL1, an N-terminal chromodomain (Buscaino et al., 2006; Morales et al., 2005). The chromodomain of MSL3 specifically recognizes the active chromatin mark H3K36me3 and facilitates DCC binding to its target genes (Bell et al., 2008; Larschan et al., 2007; Sural et al., 2008). MSL3's chromodomain is also described to bind DNA and RNA as well as methylated H4K20 (Akhtar et al., 2000; Kim et al., 2010; Moore et al., 2010). Furthermore, MSL3 recruits MOF via the interaction with MSL1 to nucleosomes and triggers acetylation of H4 (Conrad et al., 2012; Morales et al., 2005; Morales et al., 2004). MOF belongs to MYST-family of HATs and specifically catalyzes H4K16ac (Akhtar and Becker, 2000; Feller et al., 2015; Smith et al., 2000). MOF contains a C-terminal HAT domain, which catalyzes H4K16ac and mediates the interaction with MSL1, and a C2HC ZnF domain just N-terminal to the HAT domain required for substrate binding (Akhtar and Becker, 2001). Additionally, MOF has a chromobarrel domain located in the central part of the protein, which binds nucleic acids and stimulates the HAT activity (Akhtar et al., 2000; Conrad et al., 2012; Nielsen et al., 2005).

So far, all DCC subunits are assembled into a protein complex by protein-protein interactions with MSL1. The fifth subunit MLE seems to be more transiently connected to the DCC. MLE is an ATP-dependent RNA/DNA helicase of the DExH box subfamily and remodels the roX RNA for incorporation into the DCC (Ilik et al., 2013; Lee et al., 1997; Maenner et al., 2013; Meller et al., 2000; Militti et al., 2014; Morra et al., 2008; Prabu et al., 2015; Quinn et al., 2014; Richter et al., 1996). MLE has a modular architecture with the central catalytic domain consisting of two RecA ATPase domains and a helicase-associated 2 (HA2) domain. On the N-terminal site, MLE harbors two double-stranded (ds) RNA binding domains, essential for roX binding, and an OB-fold and glycine-rich region at the C-terminus (Ankush Jagtap et al., 2019; Izzo et al., 2008; Lv et al., 2019; Prabu et al., 2015). MLE especially binds and remodels both roX RNAs trough recognition of U-rich roX boxes located in prominent stem loop structures at the 3' end (Ilik et al., 2017; Ilik et al., 2013; Maenner et al., 2013; Park et al., 2007; Quinn et al., 2014; Stuckenholz et al., 2003; Sural et al., 2008). Even though, both roX RNAs share common features, they are very different in sequence composition and size. RoX1 is much longer with ~3.7 kb and roX2 is only ~0.6 kb. However, they appear to be genetically redundant as the individual mutants are viable and only the double mutant is male-specific-lethal (Meller and Rattner, 2002). While the roX RNAs are required for DCC binding to target genes and their up-regulation, the localization of their genes on the X chromosome is dispensable for X chromosome recognition (Deng and Meller, 2006;

Figueiredo et al., 2014; Ilik et al., 2017; Kageyama et al., 2001; Kelley et al., 1999; Meller, 2003; Meller et al., 2000; Meller and Rattner, 2002). Until now, it remains elusive, whether only one roX RNA is part of one DCC particle at any given time or both roX RNAs associate simultaneously. Also their precise function is unknown.



	% similarity	domain	nuc. acid binding	function
MSL1	21.8%	coiled-coil		interaction with MSL2
	23.6%	PEHE		interaction with MSL3, MOF
MSL2	32.5%	RING		E3 ubiquitin ligase, homeostasis
	40.5%	CXC	x	DNA binding, X targeting
MSL3	43.3%	Chromo	x	H3K36me3, H4K20me, DNA binding?
	22.4%	MRG		stimulates HAT activity
MOF	32.9%	Chromobarrel	x	modulates HAT activity
	63.0%	HAT		Histone Acetyltransferase
MLE		RB1/2	x	dsRNA binding
		ATPase		ATP hydrolysis
		Helicase		rox RNA unwinding
		G-rich	x	modulates RNA binding specificity

Figure 11. Protein domain architecture of MSL protein.

Schematic representation and summary table of the D. melanogaster and human MSL proteins. Figure is reproduced from (Keller and Akhtar, 2015) with permission of Elsevier.

The DCC combines multiple enzymatic activities and interaction surfaces for X chromosome recognition. Binding of the DCC to the X chromosome was studied in more detail by genomic approaches using chromatin immunoprecipitation followed by microarray hybridisation (ChIP-chip) or high-throughput sequencing (ChIP-seq). The DNA-binding MSL2 is the key subunit for DCC targeting to the X chromosome and H3K36me3 binding MSL3 for targeting the active genes on the X chromosome for transcription up-regulation through acetylation of H4K16 by MOF (Alekseyenko et al., 2012; Alekseyenko et al., 2006; Alekseyenko et al., 2008; Gelbart et al., 2009; Gilfillan et al., 2006; Kind et al., 2008; Larschan et al., 2007; Legube et al., 2006; Straub et al., 2008; Straub et al., 2013; Sural et al., 2008).

1.3.3 DCC targeting to the X chromosome

The DCC must distinguish the X chromosome from autosomes for faithful dosage compensation. The DCC targets the X chromosome through binding to ~300 high affinity sites (HAS), also referred to as chromosomal entry sites. The terms 'high affinity sites' or 'chromosomal entry sites' were defined already by genetic studies analysing MSL2 binding in absence of other subunits using immunofluorescence microscopy of polytene chromosome spreads (Dahlsveen et al., 2006; Demakova et al., 2003; Gilfillan et al., 2007; Gu et al., 1998; Kelley et al., 1999; Lyman et al., 1997). More recent studies using ChIP-seq showed that MSL2 is the central DNA binding subunit that recruits the DCC to HAS (Alekseyenko et al., 2008; Straub et al., 2008; Straub et al., 2013; Villa et al., 2016). Already, the early genetic studies noted that HAS are GA-rich. Indeed, the HAS contain a ~21 bp low complexity, GAGA-rich consensus sequence motif termed MSL recognition element (MRE) (Figure 12) (Alekseyenko et al., 2008; Straub et al., 2008).

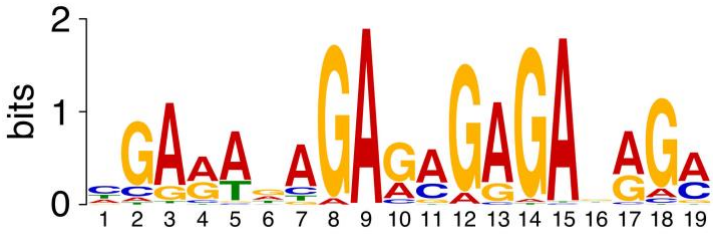


Figure 12. MSL recognition element.

Sequence motif of high affinity sites. Figure is adapted from (Villa et al., 2016) with permission of Springer Nature.

However, the MRE motif is not sufficient for X chromosome discrimination as the genome contains several thousand MRE motifs, which are only slightly enriched on the X chromosome. Furthermore, only ~2% of the MRE motifs are bound by the DCC. DCC recruitment to its genomic binding sites is modulated by epigenetic factors, as HAS are preferentially within the 3' end of active genes, in an active chromatin environment and depleted of nucleosomes (Alekseyenko et al., 2012; Alekseyenko et al., 2008; Ramirez et al., 2015; Straub et al., 2008; Straub et al., 2013). Furthermore, HAS tend to cluster as a function of chromosome domain interactions in the active X-chromosomal compartment, which is DCC-independent and invariant between males and females, as revealed by using the genome-wide chromosome conformation capture (Hi-C) and loci-specific (4C) approaches (Ramirez et al., 2015). However, earlier, more anecdotal DNA fluorescence *in situ* hybridization experiments suggested that the DCC brings some HAS in close proximity (Grimaud and Becker, 2009).

Most recently, using an *in vitro* genome-wide DNA immunoprecipitation (DIP) assay, the direct genomic binding sites of MSL2 were determined (Villa et al., 2016). Remarkably, *in vitro*, MSL2 selected sites containing the MRE motif and enriched X chromosomal sites, including ~20% of the HAS. More interestingly, binding of MSL2 to a subset of these sites required the presence of the CXC domain. These CXC-dependent sites were termed Pioneering-sites-on-the-X (PionX). The PionX harbour a consensus motif with a 5' extension to the MRE motif and a particular DNA shape with a high DNA roll between the first two base pairs. This PionX motif contributes to X chromosome discrimination as it is ~10-fold enriched on the X chromosome. However, the PionX signature, consisting of DNA motif and shape, is only present in a subset of HAS and therefore *per se* not sufficient to explain the binding of the DCC binding to all HAS. To address whether further proteins contribute to DCC targeting to HAS, Larschan and colleagues performed a gene-wide RNA interference (RNAi) screen couple to a reporter assay for DCC binding to HAS (Larschan et al., 2012). Besides general transcription regulators, they discovered some proteins with potential sequence-specific DNA binding activity contributing to DCC recruitment. One of the proteins with potential sequence-specific DNA binding activity is the gene product of CG1832. This protein was named Chromatin Linked Adaptor for MSL Proteins (CLAMP) as it is implicated in DCC recruitment to the X chromosome (Kaye et al., 2018; Larschan et al., 2012; Soruco et al., 2013). CLAMP is a seven C2H2 ZnF protein, with one N-terminal ZnF and a cluster of six ZnF at the C-terminus. The C-terminal six ZnF domains mediate sequence-specific DNA binding to the MRE motif (Kaye et al., 2018; Kuzu et al., 2016; Soruco et al., 2013). Furthermore, CLAMP is an essential gene in both sexes and binds to thousands of MRE sequences genome-wide (Soruco et al., 2013; Urban et al., 2017b). However, CLAMP binds to HAS mostly in males and in a DCC-dependent manner. There, CLAMP is suggested to interact with the DCC (Lindehell et al., 2015; Wang et al., 2013). Besides being implicated in DCC targeting, CLAMP is suggested to have further functions outside dosage compensation (Kaye et al., 2017; Rieder et al., 2017; Urban et al., 2017a; Urban et al., 2017c). CLAMP promotes long-range chromatin accessibility at its binding sites and increases global X chromosome accessibility in males. Furthermore, CLAMP is implicated in histone locus regulation and associates with boundary elements. Besides CLAMP, the small interfering RNA (siRNA) pathway, in particular siRNAs from the 1.688 g/cm$_3$ satellite repeats, may contribute to X chromosome recognition (Deshpande and Meller, 2018; Joshi and Meller, 2017; Menon et al., 2014; Menon and Meller, 2015).

Successful X chromosome discrimination and HAS targeting by the DCC is the first step in dosage compensation. In the second step, the DCC targets transcribed genes and boosts their transcription output.

1.3.4 Transcription up-regulation by the DCC

To prevent male lethality, the DCC must adjust the imbalance of X-linked gene expression between males and females. Therefore, the DCC reaches out from HAS to actively transcribed genes on the male X chromosome, making use of the chromosome interaction network (Ramirez et al., 2015). Genes actively transcribed by RNA polymerase II are marked by H3K36me3 at the gene body, a mark placed co-transcriptionally by the histone methyltransferase (HMT) Set2 (Bell et al., 2007; Carrozza et al., 2005; Larschan et al., 2007; Stabell et al., 2007). H3K36me3 is recognized by MSL3, which targets the DCC to actively transcribed genes for up-regulation (Bell et al., 2008; Larschan et al., 2007; Sural et al., 2008; Wang et al., 2013). There, MOF the histone acetyl transferase within the DCC acetylates H4K16 over gene bodies (Conrad et al., 2012; Gelbart et al., 2009; Kind et al., 2008). Outside of dosage compensation, MOF is also part of the NSL complex placing H4K16ac at promoters of housekeeping genes (Cai et al., 2010; Feller et al., 2012; Lam et al., 2012; Raja et al., 2010). However, H4K16ac at the gene body in the context of dosage compensation induces chromatin decondensation for transcription up-regulation in the two-fold range (Akhtar and Becker, 2000; Allahverdi et al., 2011; Bell et al., 2010; Corona et al., 2002; Liu et al., 2011; Prestel et al., 2010; Robinson et al., 2008; Shogren-Knaak et al., 2006; Smith et al., 2001). Transcription activation is achieved through increased transcription elongation of RNA polymerase II (Ferrari et al., 2013; Larschan et al., 2011). Further mechanisms could contribute to transcription up-regulation, for example reduced negative supercoiling mediated by topoisomerase II and supercoiling factor and the interaction of MSL1 with the elongation factor SPT5 and the general TF TFIIH via CDK7 (Chlamydas et al., 2016; Cugusi et al., 2013; Dunlap et al., 2012; Furuhashi et al., 2006; Prabhakaran and Kelley, 2012). Additional chromatin regulators are linked to dosage compensation and male X chromosome regulation, including the remodeler ISWI, the heterochromatin factors suppressor of variegation [Su(var)] 3-7 and HP1, the nuclear pore components Megator and Nup153, and the chromosomal kinase JIL-1, which is ~2-fold enriched on the male X chromosome (Badenhorst et al., 2002; Corona et al., 2002; de Wit et al., 2005; Jin et al., 2000; Mendjan et al., 2006; Regnard et al., 2011; Spierer et al., 2008; Spierer et al., 2005; Vaquerizas et al., 2010; Wang et al., 2001).

1.4 H3K36me3 chromatin

Methylation of histone lysine residues is associated with gene expression regulation. Up to three methyl-groups can be added to a lysine residue, which however retains its positive charge. Methylation of histone H3 at lysine 36 is a hallmark of active transcription and

increases gradually from mono- over di- to trimethylation from the 5’ to 3’ end of transcribed genes [reviewed in e.g. (Huang and Zhu, 2018; Li et al., 2016; McDaniel et al., 2017; Venkatesh and Workman, 2013; Wagner and Carpenter, 2012; Woo et al., 2017)]. Methylation of H3K36 is catalyzed by specific HMTs containing the common, catalytic SET domain. In *Saccharomyces cerevisiae*, a single HMT, Set2, catalyzes all three methylation states of H3K36, while higher eukaryotes have distinct enzymes for mono-/dimethylation and trimethylation. In *D. melanogaster*, Mes-4 mediates H3K36me1/me2, Ash1 mediates H3K36me2 and Set2 places H3K36me3 (Bell et al., 2007; Schmahling et al., 2018; Tanaka et al., 2007). The Set2 enzymes have a conserved domain architecture from yeast to mammals. In *S. cerevisiae*, the SET domain is located at the N-terminus while higher eukaryotes have an extended, low-complexity N-terminus preceding the SET domain. The Set2-Rpb1 interaction (SRI) domain is located at the C-terminus, mediating the interaction with RNA polymerase II. The SRI domain binds specifically to two consecutive heptapeptide repeats in the C-terminal domain (CTD) of RNA polymerase II containing serines at residue 2 and 5 in each repeat. The CTD of RNA polymerase II becomes differentially phosphorylated during the transcription cycle (Komarnitsky et al., 2000; Mayer et al., 2010). The S5 residues become phosphorylated during formation of the preinitiation complex by TFIIH, which leads to the recruitment of various elongation factors for transition of RNA polymerase II into active elongation. During the elongation phase, S5 becomes gradually dephosphorylated towards the 3’ end while S2 is gradually phosphorylated by P-TEFb and allows the binding of Set2 and transcription-coupled trimethylation of H3K36 (Carrozza et al., 2005; Gopalakrishnan et al., 2019; Kizer et al., 2005; Li et al., 2003; Stabell et al., 2007).

H3K36 trimethylation by Set2 and its biological roles are best studied in *S. cerevisiae*. There, other transcription elongation factors and histone chaperons are shown to contribute to the regulation of H3K36me3 through Set2. The H3/H4 histone chaperone Spt6, which also binds to RNA polymerase II CTD to facilitate nucleosome-reassembly in the wake of the transcribing polymerase, is required for binding of Interacts with Spt6-1 (IWS1), recruiting Set2 to the CTD (Gopalakrishnan et al., 2019; Yoh et al., 2008; Youdell et al., 2008). Furthermore, the elongation factors facilitates chromatin transcription (FACT) complex, Paf1 complex and anti-silencing factor 1 are implicated in Set2-mediated H3K36me3 (Chu et al., 2007; Chu et al., 2006; Lin et al., 2010). Heterogeneous nuclear ribonucleoprotein L associates with Set2 and stimulates H3K36me3, while the prolyl isomerase Fpr4 mediates H3P38 isomerization, which changes the conformation of H3K36 making trimethylation unfavorable (Nelson et al., 2006; Yuan et al., 2009). In addition, H3K36 methylation marks can be actively removed by Jumonji C domain containing lysine demethylases (KDM). The human KDM2A and KDM4A are shown to demethylate H3K36me2/3, in flies this is mediated

by Kdm2, Kdm4A and Kdm4B (Blackledge et al., 2010; Holowatyj et al., 2015; Whetstine et al., 2006; Zhou et al., 2012).

To translate the H3K36 methylation signal into a biological output, various readers recognize the different methylation states of H3K36 and trigger associated pathways. One function of Set2-mediated H3K36me3 in yeast is the prevention of aberrant transcription initiation within the coding region of transcribed genes (Carrozza et al., 2005; Joshi and Struhl, 2005; Keogh et al., 2005; Sun et al., 2008). The histone deacetylase complex reduced potassium dependency 3 small (Rpd3S) is recruited to H3K36me3 through the binding of the subunit ESA1-associated factor 3 (Esa3) [MORF-related gene 15 (MRG15) in humans and flies] by its chromodomain to maintain a hypoacetylated state at the gene body. The Isw1b ATP-dependent chromatin remodeling complex contributes to this pathway by properly spacing nucleosome providing a suitable substrate for deacetylation (Lee et al., 2013; Maltby et al., 2012; Smolle et al., 2012). In the Isw1b complex, Isw1 interacts with Ioc4 a PWWP domain containing protein, which recruits the complex to H3K36me3. In humans, a histone deacetylase complex is also recruited to H3K36me3 via the chromodomain-containing MRG15 subunit (Jelinic et al., 2011; Kumar et al., 2012). In addition, various PWWP domain-containing proteins are recruited to H3K36me3, including ZMYND11, which is implicated in transcription elongation and mRNA splicing, DNA (cytosine-5)-methyltransferase 3A and B to establish *de novo* DNA methylation, and GLYR1 interacting with a H3K4 demethylase and responsible for nucleosome-destabilization to facilitate transcription. (Baubec et al., 2015; Dhayalan et al., 2010; Fang et al., 2013; Fei et al., 2018; Guo et al., 2014; Morselli et al., 2015; Suzuki et al., 2006; Vermeulen et al., 2010; Wen et al., 2014). H3K36me3 readers include PSIP1, which is implicated in various processes from alternative splicing, to DNA double strand repair by homologous recombination, to DNA mismatch repair mediated by MSH6, to integration of the human immunodeficiency virus in active portions of the genome (Cherepanov et al., 2005; Ciuffi et al., 2005; Daugaard et al., 2012; Laguri et al., 2008; Li et al., 2013a; Pfister et al., 2014; Pradeepa et al., 2012; Sharma et al., 2018). Moreover, the activity of the Polycomb repressive complex 2 (PRC2) is modulated by H3K36me3, which is bound by the Tudor domain containing subunits PHF1 and PHF19 (Ballare et al., 2012; Brien et al., 2012; Cai et al., 2013; Schmitges et al., 2011; Yuan et al., 2011).

In *D. melanogaster*, MSL3 is the best studied protein shown to bind H3K36me3 via its chromodomain for dosage compensation (Larschan et al., 2007; Sural et al., 2008). Furthermore, the chromodomain-containing MRG15 is suggested to bind H3K36me3, as shown for its yeast homolog (Filion et al., 2010; Joshi and Struhl, 2005; Sun et al., 2008). MRG15 in flies is part of the Tip60 HAT complex/Domino remodeling complex and Ash1 HMT complex (Huang et al., 2017; Kusch et al., 2004; Schmahling et al., 2018). Furthermore,

the PWWP domain-containing NDF is suggested to bind H3K36me3, as shown for its human homolog GLYR1, to facilitate transcription by destabilizing nucleosomes (Fang et al., 2013; Fei et al., 2018; Vermeulen et al., 2010; Wang et al., 2013). Although the chromosomal JIL-1 kinase is not shown to directly bind H3K36me3, JIL-1 co-localizes genome-wide with the H3K36me3 mark (Regnard et al., 2011).

1.4.1 The chromosomal kinase JIL-1

In the late 1990s, the kinase JIL-1 was discovered in the lab of K. M. Johansen and J. Johansen, who most contributed to our understanding of JIL-1 function (Jin et al., 1999). JIL-1 harbors two central serine/threonine kinase domains arranged in tandem. The JIL-1 N-terminus contains an asparagine-rich and an alanine-rich stretch, and a bipartite nuclear localization signal. Furthermore, two PEST-related sequences are located in the N-terminus and one between the kinase domains, which are implicated in protein degradation (Rogers et al., 1986). JIL-1 is expressed ubiquitously at all developmental stages and associates to chromatin at all cell cycle phases (Jin et al., 1999; Zhang et al., 2003). Based on polytene chromosome squashes, JIL-1 localizes to an active chromatin environment at the decondensed interbands and is ~2-fold enriched on the dosage-compensated male X chromosome (Jin et al., 2000; Jin et al., 1999). Basal JIL-1 levels at gene bodies correlate with H3K36me3 levels and enrichment at male X-linked genes with additional H4K16ac levels (Regnard et al., 2011). Since JIL-1 is enriched on the male X chromosome in a DCC dependent way, it is linked to dosage compensation. Moreover, JIL-1 binds MSL1 and MSL3 via its kinase domains and is required for full transcription up-regulation of X-linked genes by the DCC (Jin et al., 2000; Regnard et al., 2011; Wang et al., 2013). At active chromatin, JIL-1 phosphorylates H3 at serine 10 (H3S10ph) during interphase, while H3S10ph in mitosis is catalyzed by aurora B kinase (Adams et al., 2001; Giet and Glover, 2001; Regnard et al., 2011; Wang et al., 2001). JIL-1 is an essential protein in both sexes since it is implicated in higher-order chromosome organization and ectopic recruitment causes chromatin reorganization (Bao et al., 2008; Deng et al., 2008; Deng et al., 2005; Li et al., 2013b; Wang et al., 2001). H3S10 phosphorylation by JIL-1 during interphase counteracts heterochromatin spreading by preventing H3K9 di-/trimethylation by Su(var)3-9 and subsequent binding of HP1 in euchromatic regions (Figure 13) (Cai et al., 2014; Deng et al., 2007; Wang et al., 2011a; Wang et al., 2012; Wang et al., 2014; Zhang et al., 2006). The function of JIL-1 in the maintainance of the hetero- and euchromatin boundary is best illustrated in the phenomenon of position-effect variegation (Bao et al., 2007; Deng et al., 2010; Girton et al., 2013; Lerach et al., 2006; Wang et al., 2011b). Indeed, truncations within JIL-1's C-terminus have a Su(var) phenotype, counteracting heterochromatin spreading into euchromatin (Ebert et al.,

2004). Additionally, JIL-1 is shown to phosphorylate the heterochromatin factor Su(var)3-9, the HMT specific for di- and trimethylation of H3 at lysine 9 (H3K9me2/3), at its N-terminus, which is implicated in protein-protein interaction and chromatin binding (Boeke et al., 2010; Melcher et al., 2000; Schotta et al., 2002). Besides localizing to active H3K36me3 chromatin at gene bodies, JIL-1 is also found at the arrays of retrotransposons at telomeres, consisting of the non-LTR retrotransposons HeT-A, TART and TAHRE (HTT arrays), to regulates their expression (Andreyeva et al., 2005; Silva-Sousa and Casacuberta, 2013; Silva-Sousa et al., 2012).

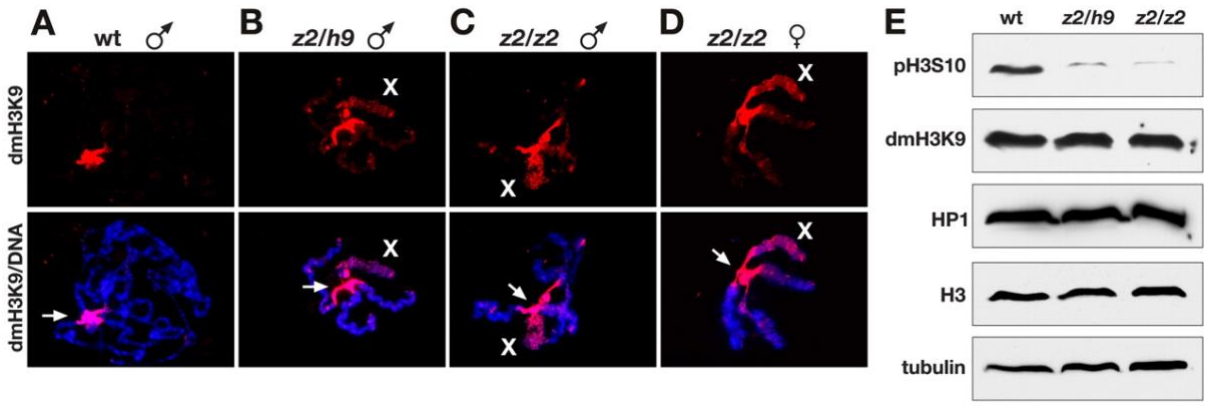


Figure 13. Heterochromatin spreading in JIL-1 mutants.

Left side, polytene squashs from wild type and JIL-1 mutant L3 larvae stained with α-H3K9me2 antibody in red and with Hoechst in blue. Right side, western blots analysis of wild type and JIL-1 mutant larval extracts for H3S10ph, H3K9me2 and HP1. Figure is reproduced from (Zhang et al., 2006) with permission of Company of Biologists.

2 Aims and objectives

The first aim was to mechanistically dissect the contribution of CLAMP to MSL2 targeting to HAS on the male X chromosome. To this end, MSL2 binding *in vivo* in absence of CLAMP was monitored by high-resolution ChIP-seq, as potentially MSL2 alone could be sufficient to bind PionX *in vivo*. To analyze cooperative effects between CLAMP and MSL2 for HAS targeting, *in vitro* genome-wide DNA immunoprecipitation with high-throughput sequencing (DIP-seq) was used (Gossett and Lieb, 2008; Liu et al., 2005). As direct protein-protein interactions are implicated in cooperative DNA binding, CLAMP interaction with MSL2 was assayed by co-IP experiments to map individual interaction domains. Further, chromatin organization could also contribute to binding site selection and cooperativity, genome-wide chromatin accessibility was studied in male and female cells after CLAMP and MSL2 depletion by assay for transposase accessibly chromatin with high-throughput sequencing (ATAC-seq) (Buenrostro et al., 2013; Buenrostro et al., 2015).

The second aim was to refine the role of chromosome architecture in DCC transfer from HAS to target genes. Therefore, chromosome architecture and compartmentalization in male and female embryos was analyzed by genome-wide chromosome conformation capture (Hi-C) (Sexton et al., 2012). To investigate potential sex-dependent differences, Hi-C data were connected to H3K36me3 and H4K16ac chromatin marks assayed by ChIP-seq as well as to gene expression measured by RNA sequencing (RNA-seq). To more specifically study the contact frequency of PionX with the active compartment, locus-specific 4C-seq after MSL2 depletion in male cells or *de novo* induction of DCC formation by *sxl* RNAi in female cells was performed and related to gene expression changes (Ghavi-Helm et al., 2014).

The third aim was to reveal the targeting mechanism of JIL-1 to H3K36me3 chromatin and its role in transcription regulation. The interaction of JIL-1 with the new PWWP domain containing protein, JIL-1 Anchoring and Stabilizing Protein (JASPer), was identified by co-IP experiments. To map the individual interaction domains, the JIL-1-JASPer complex (JJ-complex) was recombinantly expressed for subsequent co-IP assays. To analyze whether JASPer can recruit JIL-1 to H3K36me3 chromatin, *in vitro* nucleosome pull-down experiments using recombinant JASPer and JJ-complex were performed by employing a nucleosome library comprising 115 different nucleosome types (Dann et al., 2017). Furthermore, the *in vivo* localization of JASPer and JIL-1 relative to H3K36me3 was studied by high-resolution ChIP-seq in male and female cells. To gain mechanistic insight into JJ-complex enriched at the male X chromosome, JASPer, H4K16ac and MSL3 ChIP-seq after JIL-1 depletion were performed. Potential spreading of H3K9me2 into euchromatin after JIL-1 depletion was also studied by ChIP-seq. Moreover, the role of the JJ-complex in gene expression regulation and expression of transposable elements was studied by RNA-seq

after JASPer and JIL-1 depletion in male and female cells. The association of JJ-complex with other chromatin factors was investigated by co-IP coupled to mass spectrometry analysis.

3 Results

3.1 Factor cooperation for chromosome discrimination in *Drosophila*

Factor cooperation for chromosome discrimination in *Drosophila*

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Authors' contributions:

C.A., C.R. and P.B.B. conceived the study; C.A. performed all experiments except for co-immunoprecipitation and Yeast Two-Hybrid experiments; S.K. expressed proteins and performed co-immunoprecipitation experiments; E.T. and O.M. conceived, performed and analyzed Yeast Two-Hybrid experiments; C.A. performed bioinformatics analyses; All authors analyzed data; C.R. and P.B.B. provided feedback and supervision; C.A., C.R. and P.B.B. wrote the manuscript; P.B.B. secured funding.

3.2 Chromosome topology guides the *Drosophila* Dosage Compensation Complex for target gene activation

Chromosome topology guides the *Drosophila* Dosage Compensation Complex for target gene activation

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Authors' contributions:

The 4C-seq, ChIP-seq and RNA-seq experiments with Kc cells were performed by T.Sc.; 4C-seq was designed and supervised by Y.G.-H. and E.E.M.F.; Hi-C was performed by T.Se. and supervised by G.C.; ChIP-seq on H3K36me3 was carried out by C.A.; RNA-seq in S2 cells was performed by C.R.; Datasets were analyzed and visualized by T.Sc.; The study was conceptualized and supervised by P.B.B.; T.Sc. and P.B.B. wrote the manuscript with contributions from all authors.

3.3 JASPer controls interphase histone H3S10 phosphorylation by chromosomal kinase JIL-1 in *Drosophila*

JASPer controls interphase histone H3S10 phosphorylation by chromosomal kinase JIL-1 in *Drosophila*

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Authors' contributions:

C.R. conceived this study and performed experiments; C.A. performed MNase and sonication ChIP-seq experiments and all bioinformatics analysis also with support from T.S.; C.W. generated and characterized the cw2 mutant line with help from J.G., W.C. did the LacO-LacI targeting experiments with support from Y.L., and J.J. and K.M.J. supervised the work and secured funding; G.P.D. performed mononucleosome library experiments and F.W. generated the mononucleosomes and arrays for the kinase assays in T.W.M.'s lab; S.K. prepared recombinant proteins for all *in vitro* assays, RNA-seq experiments under the supervision of C.R. and spike-in ChIP-seq experiments under supervision of C.A.; All authors analyzed data; C.R. and C.A. wrote the manuscript with contributions from all authors; P.B.B. secured funding and established collaborations.

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6 Abbreviations

ADP	adenosine diphosphate
ATAC-seq	assay for transposase accessibly chromatin with high-throughput sequencing
ATP	adenosine triphosphate
bp	base pair
ChIP-chip	chromatin immunoprecipitation followed by microarray hybridisation
ChIP-seq	chromatin immunoprecipitation with high-throughput sequencing
CLAMP	Chromatin Linked Adaptor for MSL Proteins
CTD	C-terminal domain
DCC	dosage compensation complex
DIP-seq	DNA immunoprecipitation with high-throughput sequencing
DNA	deoxyribonucleic acid
E(z)	Enhancer of zeste
H3S10ph	phosphorylated histone H3 at serine 10
H3K36me3	trimethylated histone H3 at lysine 36
H4K16ac	acetylated histone H4 at lysine 16
HAS	high affinity site
HAT	histone acetyltransferase
HMT	histone methyltransferase
HP1	heterochromatin protein 1
IP	immunoprecipitation
JASPer	JIL-1 Anchoring and Stabilizing Protein
JJ-complex	JIL-1-JASPer complex
KDM	lysine demethylase
lncRNA	long non-coding RNA
MBT	malignant brain tumor
MLE	maleless
MOF	males absent on the first

MRE	MSL recognition element
MRG15	MORF-related gene 15
mRNA	messenger ribonucleic acid
MSL	male-specific-lethal
NFR	nucleosome free region
NGS	next generation sequencing
NMR	nuclear magnetic resonance
PHD	plant homeodomain
PionX	Pioneering-sites-on-the-X
PTM	post-transcriptional modification
PWM	position weight matrix
RING	really interesting new gene
RNA	ribonucleic acid
RNA-seq	RNA sequencing
RNAi	RNA interference
roX	RNA on the X
siRNA	small interfering RNA
SRI	Set2-Rpb1 interaction
SUMO	Small Ubiquitin-related Modifier
Su(var)	suppressor of variegation
SXL	Sex lethal
TAD	topologically associating domain
TF	transcription factor
ZnF	zinc finger

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