Published in final edited form as:

Curr Top Dev Biol. 2020 ; 138: 1–71. doi:10.1016/bs.ctdb.2019.11.002.

Chromatin regulation and dynamics in stem cells

David C. Klein, Sarah J. Hainer*

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, United States

Abstract

In eukaryotes, DNA is highly compacted within the nucleus into a structure known as chromatin. Modulation of chromatin structure allows for precise regulation of gene expression, and thereby controls cell fate decisions. Specific chromatin organization is established and preserved by numerous factors to generate desired cellular outcomes.

In embryonic stem (ES) cells, chromatin is precisely regulated to preserve their two defining characteristics: self-renewal and pluripotent state. This action is accomplished by a litany of nucleosome remodelers, histone variants, epigenetic marks, and other chromatin regulatory factors. These highly dynamic regulatory factors come together to precisely define a chromatin state that is conducive to ES cell maintenance and development, where dysregulation threatens the survival and fitness of the developing organism.

1. Chromatin compaction, structure, and function

To retain the genomic information required to code for a eukaryote, each cell must compact 1.7m of DNA into a nucleus of between 5 and 20 μ m (Lammerding, 2011). To accomplish this compaction, cells package genomic DNA with histone proteins into a structure known as chromatin, the basic repeating unit of which is the nucleosome. Histone proteins are extraordinarily conserved throughout eukaryotes at both the structure and sequence levels, with the shared histone fold domain being especially conserved (Postberg, Forcob, Chang, & Lipps, 2010; Sullivan & Landsman, 2003). In general, the stable association of DNA and histone proteins poses a significant obstacle to cellular processes that rely on protein-DNA interaction, including transcription, DNA replication, and DNA repair (reviewed in Bai & Morozov, 2010; Duina, 2011; Li, Carey, & Workman, 2007; Luger, 2006).

1.1 Nucleosomes are formed from DNA interacting with an octamer of four histone proteins

The nucleosome is composed of ~147 base pairs of double-stranded DNA wrapped around an octamer of histone proteins (Kornberg & Lorch, 1999). The histone octamer is comprised of an inner core tetramer of histones H3 and H4 and two outer histone H2A/H2B dimers (Luger, Mäder, Richmond, Sargent, & Richmond, 1997) (Fig. 1). Histones are small, positively charged proteins and are composed of a folded domain (termed the histone fold) that forms the nucleosome globular core and highly unstructured N- and C-terminal tails

*Corresponding author: sarah.hainer@pitt.edu.

extending from the core. The positive charge of histone proteins allows for electrostatic interactions between the histones and the negatively charged DNA. Specifically, the minor groove of DNA interacts with 14 arginine residues on the histone proteins through non-covalent hydrogen bonds. Additionally, salt bridges allow DNA and histone proteins to maintain loose association that allows nucleosome remodeling factors to reposition nucleosomes. Individual histone protein tails are often post-translationally modified (Kouzarides, 2007). These modifications have been shown to be extremely important in a number of biological processes and will be discussed; however, for reviews of histone modifications see Bannister and Kouzarides (2011), Harikumar and Meshorer (2015), Kouzarides (2007). Most of these covalent modifications are found on the N-terminal tails of histone proteins, though there are notable exceptions, such as H2BK120, a residue that is monoubiquitinated in histone crosstalk pathways (Tomson & Arndt, 2013).

Each nucleosome contains numerous structural features that can affect DNA packaging—and therefore DNA-templated activities (Fig. 2). These features include the histone fold domain, the nucleosome dyad, the DNA entry and exit sites, and the histone acidic patch (Arents & Moudrianakis, 1995). The histone fold domain is a C-terminal structural motif that is found across all core histones and is important in DNA compaction and histone dimerization (Arents & Moudrianakis, 1995; Hammond, Stromme, Huang, Patel, & Groth, 2017). The nucleosome dyad covers the H3/H3 interaction interface and is largely referred to as the center of the nucleosome (given the position “0” in the DNA superhelix). The dyad is an important site for interaction of proteins, such as FOXA/HNF3 (Cirillo et al., 1998), AMT1 (White & Luger, 2004), SOX2 (Zhu et al., 2018), and TBX2 (Zhu et al., 2018), as well as the linker histone H1 (Zhou et al., 2013). At the DNA entry/exit sites, the histone-interacting DNA participates in a process known as “nucleosome breathing” in which the DNA base pairs nearest these sites transiently release from and rewrap around the histone proteins (Anderson & Widom, 2000; Osberg, Nuebler, Korber, & Gerland, 2014); this activity can alter contacts with specific regulatory factors, such as the elongation factor and histone chaperone FACT (Hondele & Ladurner, 2013). The histone acidic patch is a region of the nucleosome composed of eight H2A amino acid residues and two H2B residues which together create a highly negatively charged surface on the nucleosome, in contrast to the rest of the nucleosome surface, which is positively charged to promote interaction with DNA (Luger et al., 1997). The acidic patch is a hotspot for interacting factors, including the SAGA coactivator (Morgan et al., 2016), the DOT1L H3K79 histone methyltransferase (Anderson et al., 2019), the Kaposi’s sarcoma herpesvirus LANA (Barbera et al., 2006), the PAF1 complex member RTF1 (Cucinotta, Hildreth, McShane, Shirra, & Arndt, 2019), the E3 ubiquitin ligase RNF168 (Mattioli, Uckelmann, Sahtoe, van Dijk, & Sixma, 2014), the Polycomb complex PRC1 (McGinty, Henrici, & Tan, 2014), the FACT histone chaperone (Hodges, Gloss, & Wyrick, 2017), and the histone H4 tail (Fan, Rangasamy, Luger, & Tremethick, 2004; Kalashnikova, Porter-Goff, Muthurajan, Luger, & Hansen, 2013). Many factors bind the acidic patch by an “arginine anchor” motif (McGinty et al., 2014). Furthermore, this surface limits the number of possible simultaneous interactions, which results in competition for binding among regulatory proteins (Dann et al., 2017; Gamarra, Johnson, Trnka, Burlingame, & Narlikar, 2018). The acidic patch is additionally intriguing

in that it is required to achieve maximal activity of the SWI/SNF, ISWI, and CHD families of nucleosome remodelers (Dann et al., 2017).

Individual nucleosomes are separated by a region of linker DNA that ranges from 20 to 90 base pairs in length, forming the basic “beads on a string” model often used to describe chromatin (Bradbury, 1989). Importantly, nucleosomes do not exist in a vacuum, and are therefore subject to the actions of neighboring nucleosomes, transcribing polymerases, nucleosome remodelers, and a host of other factors that can disrupt this periodic spacing. The sliding action of SWI/SNF family remodelers, for example, is thought to bring individual nucleosomes crashing together, forcibly evicting an H2A/H2B dimer and creating a structure known as an overlapping dinucleosome (Engelholm et al., 2009; Kato et al., 2017). Nucleosomes can also be partially dismantled, involving the loss of an H2A/H2B dimer, leaving a hexasome of histone proteins that is known as a subnucleosome (Rhee, Bataille, Zhang, & Pugh, 2014). A mammalian SWI/SNF remodeler, esBAF, regulates subnucleosome formation in murine embryonic stem (ES) cells (Hainer & Fazzio, 2015). These non-canonical nucleosome structures are depicted in Fig. 3.

1.2 Chromatin structure balances DNA compaction and accessibility

Broadly, chromatin structure can be grouped into two types: heterochromatin, which is compact and transcriptionally silent, and euchromatin, which is more accessible and therefore more permissive of DNA-templated activities. Heterochromatin can be further subdivided into constitutive heterochromatin, which remains compacted throughout the cell cycle, and facultative heterochromatin, which is preferentially, but not exclusively, heterochromatic. Constitutive heterochromatin is enriched for di- and tri-methylation of H3K9, and often marks repeat-heavy regions such as satellite DNA at centromeres (Lehnertz et al., 2003; Nishibuchi & Dejardin, 2017). Facultative heterochromatin, on the other hand, is marked by HP1-dependent H3K27 trimethylation (H3K27me3) (Jamieson et al., 2016) and is closely associated with inactive gene promoters, such as developmental genes that are repressed in ES cells but may require activation at a later time. H3K27me3 is placed largely by EZH2, a member of the PRC2 complex (Kuzmichev, Nishioka, Erdjument-Bromage, Tempst, & Reinberg, 2002), which will be discussed in greater detail later.

In contrast to heterochromatin, euchromatin is a more accessible structure (Noma, Allis, & Grewal, 2001). Euchromatic regions are the main sites of transcription by RNA Polymerase II, regulatory factor binding, and nucleosome remodeling activity. Importantly, euchromatic DNA is still compacted into an array of nucleosomes, and other factors are required to maintain gene accessibility in highly context-dependent manners. In the next section, we will discuss ways in which chromatin structure is dynamic and can be modulated to tightly regulate transcription and gene expression.

2. Chromatin dynamics regulate gene expression

Chromatin structure impacts all DNA-templated activities. The regulation of gene expression is especially important to cell identity and cell fate decisions. Transcription to RNA from a DNA template is carried out by RNA polymerases in three stages: initiation, elongation, and termination (reviewed in Liu, Bushnell, & Kornberg, 2013). In general, most

gene promoters are nucleosome-depleted regions (NDRs), which permit binding of transcription factors and successful transcription initiation (Lee, Shibata, Rao, Strahl, & Lieb, 2004; Lee et al., 2007; Varshavsky, Sundin, & Bohn, 1979). Transcription initiation can be hindered, however, when promoter DNA is wrapped into a nucleosome, making the DNA sequence more difficult for DNA-binding factors to recognize (Kornberg & Lorch, 1999). Transcription elongation can also be physically hindered by nucleosome occupancy in that transcription rates of RNA Polymerase II are slowed due to increased pausing and backtracking (Churchman & Weissman, 2011; Izban & Luse, 1991; Lee, Teyssier, Strahl, & Stallcup, 2005). Transcription termination is coordinated by numerous factors affecting polymerase function and chromatin state, including the CHD1, ISW1, and ISW2 nucleosome remodelers (Alén et al., 2002; Morillon et al., 2003; Simic et al., 2003; Xu et al., 1986). Therefore, mechanisms by which eukaryotes control chromatin dynamics are utilized during all stages of transcription to regulate gene expression.

Changes in gene transcription are tightly correlated with changes in chromatin structure (Field et al., 2008; Radman-Livaja & Rando, 2010; Schwabish & Struhl, 2004; Shivaswamy & Iyer, 2008; Weiner, Hughes, Yassour, Rando, & Friedman, 2010; Zawadzki, Morozov, & Broach, 2009). When genes are highly transcribed, the nucleosome preceding the transcription start site is evicted, generating an open and accessible promoter (Shivaswamy & Iyer, 2008; Zawadzki et al., 2009). Conversely, genes that are not expressed, or lowly expressed, have existing promoter nucleosome structure that is not altered until the gene's expression is upregulated. A highly regulated and well-positioned nucleosome, known as the +1 nucleosome, typically resides at a canonical distance downstream (in the direction of genic transcription) of each major transcriptional start site and forms the downstream border of the nucleosome-depleted region. A well-positioned -1 nucleosome forms the upstream border of the promoter. Nucleosome positioning within gene bodies is initially defined by the +1 nucleosome—the most well-positioned nucleosome in the array—with subsequent nucleosomes arranged as a result of the +1 nucleosome position (Kornberg, 1981; Kornberg & Stryer, 1988). Additionally, over the coding regions of highly transcribed genes, nucleosome occupancy decreases with high rates of transcription, whereas over lowly transcribed genes, nucleosome occupancy is not significantly disrupted (Lee et al., 2004; Shivaswamy et al., 2008). Changes to nucleosome structure, such as those mentioned above, occur through the activity of many factors, including chromatin remodeling factors, histone modifying enzymes, and histone chaperones; however, RNA Polymerase II itself is also responsible for some of the alterations which occur to nucleosome architecture. *In vitro*, RNA Polymerase II is able to transcribe DNA compacted into chromatin without evicting the nucleosome, or by creating subspecies of nucleosomes, such as hexasomes (Kulaeva, Hsieh, & Studitsky, 2010; Studitsky, Clark, & Felsenfeld, 1994; Studitsky, Kassavetis, Geiduschek, & Felsenfeld, 1997). Additionally, deactivation of RNA Polymerase II in yeast has been shown to increase -1 nucleosome occupancy, lending more support to the role of RNA Polymerase II in regulating the chromatin environment of genes (Weiner et al., 2010). Furthermore, inappropriate reassembly of nucleosomes in the wake of RNA Polymerase II transcription can lead to aberrant transcription initiation from within gene bodies (Cheung et al., 2008; Kaplan, Laprade, & Winston, 2003). Nucleosome occupancy and spacing are

tightly regulated through numerous mechanisms, the most prominent of which is direct nucleosome remodeling by ATP-dependent complexes.

3. ATP-dependent nucleosome remodeling complexes establish and maintain chromatin state

Eukaryotic cells must actively balance chromatin compaction with DNA accessibility for appropriate gene expression. To maintain a balance between compaction and accessibility, cells make use of a wide array of nucleosome remodeling factors that can alter nucleosome composition and positioning genome-wide. Nucleosome remodeling factors are protein complexes that use the energy from ATP hydrolysis to reposition (Fazzio & Tsukiyama, 2003; Lomvardas & Thanos, 2001) or remove nucleosomes (Boeger, Griesenbeck, Strattan, & Kornberg, 2004; Cairns, 2005) by altering histone-DNA contacts. Broadly, these factors use ATP to slide DNA around histone proteins, remove histone dimers or octamers, and to alter histone variant composition. Nucleosome remodeling factors are highly variable, allow other proteins to bind regulatory regions, and can permit or repress DNA-templated activities on chromatin. There are ~30 nucleosome remodeling factor ATPases in ES cells, each of which fulfills a distinct niche within the cell—often including both activating and repressing roles (Becker & Workman, 2013; Clapier & Cairns, 2009; Clapier, Iwasa, Cairns, & Peterson, 2017). By modulating histone octamer positioning, nucleosome remodelers can open or close binding sites for regulatory factors, further expanding the possible outcomes of nucleosome repositioning, even by the same ATPase. Nucleosome remodeling factors carry out their function through DNA translocation, which results in repositioning of histones along the DNA, thereby allowing them to facilitate or impede transcription and other DNA-templated processes at target loci.

ATP-dependent nucleosome remodelers are members of RNA/DNA helicase superfamily 2, also referred to as the DEAD/H superfamily (Clapier et al., 2017). There are four main families of ATP-dependent nucleosome remodelers: SWI/SNF, ISWI, INO80, and CHD (Clapier et al., 2017). Each of these families share distinct domains on their ATPase subunits that catalyze nucleosome remodeling by the complex to fulfill extraordinarily dynamic and powerful roles in regulating chromatin structure and gene expression (Fig. 4). A list of mammalian nucleosome remodeling ATPases can be found in Table 1. Broadly, SWI/SNF remodelers destabilize nucleosomes, ISWI nucleosome remodelers function to slide nucleosomes laterally, and other remodeling complexes, such as INO80, exchange H2A/H2B dimers (Yen, Vinayachandran, Batta, Koerber, & Pugh, 2012). Additionally, certain chromatin remodelers have been associated with transcription activation or repression, based on the mechanism of altering accessibility of nucleosomal DNA to other regulatory proteins, such as transcription factors. SWI/SNF family members, for example, tend to disorganize nucleosomes via sliding and ejection, and are therefore thought to promote transcription (reviewed in Rando & Winston, 2012). Alternatively, members of the ISWI family have been shown to remodel and organize nucleosomes over transcriptionally silent regions (Tirosch, Sigal, & Barkai, 2010). Across (and even within) the four families, nucleosome remodeling factors perform highly distinct activities to regulate chromatin structure and subsequent gene expression. These factors all use the shared activity of DNA

translocation around histone proteins to fulfill both activating and repressing roles with extreme precision. For comprehensive reviews of ATP-dependent nucleosome remodeling factors, see Clapier et al. (2017) and Becker and Workman (2013).

3.1 SWI/SNF family nucleosome remodeling factors

The first characterized nucleosome remodelers were identified from two convergent screens in *Saccharomyces cerevisiae* (Neigeborn & Carlson, 1984; Stern, Jensen, & Herskowitz, 1984). Over the next decade, it became clear that these genes belonged to the same complex, and this complex became known as SWI/SNF in yeast. Although the SWI/SNF complex is known as BAF (Brahma-associated factors) in higher eukaryotes, the family of nucleosome remodeling factors retains the SWI/SNF name. The SWI/SNF family is defined by the presence of a bromodomain and a helicase/SANT ATPase (HSA) domain that facilitates binding of actin and/or actin-related proteins (Szerlong et al., 2008). SWI/SNF family remodelers are typically associated with increased chromatin accessibility via nucleosome sliding or eviction, which can have downstream activating or repressive effects (Kasten, Clapier, & Cairns, 2011). The SWI/SNF-defining bromodomain binds acetylated histone lysines to promote nucleosome remodeling at these sites (Lee, Park, Kim, Kwon, & Kwon, 2010). In addition to complex targeting, the SWI/SNF family member Sth1's bromodomain is necessary for enhanced remodeling activity, suggesting a regulatory role (Chatterjee et al., 2011).

In part because of their extensive variety in mammalian cells, nucleosome remodelers can have many interchangeable subunits that are often differently utilized by cell type. At least six cell type-specific BAF complexes have been identified in mammalian cells, including those specific to cardiac cells (cBAF, containing BAF45C and BAF60C) (Lickert et al., 2004; Takeuchi & Bruneau, 2009), neural progenitors (npBAF, containing BAF45A and BAF53A) (Lamba, Hayes, Karl, & Reh, 2008; Lessard et al., 2007; Wu et al., 2007), neural cells (nBAF, containing BAF45B and BAF53B) (Olave, Wang, Xue, Kuo, & Crabtree, 2002; Wu et al., 2007), hematopoietic stem cells (hscBAF) (Buscariet et al., 2014), and ES cells (esBAF, containing BRG1 and BAF155 but not BAF170) (Ho, Rohan, et al., 2009). Other non-cell type-specific BAF complexes have been defined by subunit composition, including GLTSCR1-containing BAF (GBAF or non-canonical/ncBAF, containing BRD9 and GLTSCR1/1L instead of an ARID subunit) (Alpsy & Dykhuizen, 2018; Middeljans et al., 2012) and polybromo-containing BAF (PBAF, containing ARID2, PBRM1, and PHF10) (Wang et al., 1996). Ectopic expression of specific BAF subunits can hijack embryonic development; for example, expression of BAF60C in a developing embryo, rather than BAF60A, leads to beating cardiomyocytes outside of the heart precursor region (Takeuchi & Bruneau, 2009). BAF complexes are combinatorially assembled in a stepwise manner: first, a dimer of BAF155 and/or BAF170 subunits is formed; followed by loading of BAF60 subunits to form the initial core complex; then BAF47 and BAF57 to form the BAF core complex (Fig. 5) (Mashtalir et al., 2018). The immense variety of BAF complexes allows for specialization and target specificity, including roles in development that are too extensive to outline in this chapter; for a targeted review of BAF complexes in development, see Alfert, Moreno, and Kerl (2019) and Ho and Crabtree (2010).

3.2 INO80 family nucleosome remodeling factors

The Inositol-requiring 80 (INO80) family's namesake was first identified in *S. cerevisiae* as a mutant causing inositol auxotrophy (Ebbert, Birkmann, & Schuller, 1999); since then, roles for INO80 have been uncovered in DNA repair, replication, and transcription (Poli, Gasser, & Papamichos-Chronakis, 2017; Poli et al., 2016; Xue et al., 2015). The INO80 family is defined by the presence of an HSA domain like that of SWI/SNF family, but without an ATPase C-terminal bromodomain. There are two general groups of INO80 members, those belonging to the INO80 class and the SWR1 class. Mammals have two SWR1-class members—SRCAP and p400—as well as an INO80-class member (INO80). Although INO80 is the lone member of the INO80 class, the complex may exist in different compositions to act at both euchromatic and heterochromatic regions of chromatin (Runge, Raab, & Magnuson, 2018).

INO80 mediates nucleosome spacing, histone eviction, and replication fork progression, including a role for eviction of RNA Polymerase II and the PAF1 complex at replication-transcription fork collisions (Lafon et al., 2015; Poli et al., 2017, 2016). Specifically, INO80 translocates DNA proximal to H2A/H2B dimers to destabilize nucleosomes and promote both nucleosome sliding and histone variant exchange (Brahma et al., 2017). INO80 can also be found at upward of 90% of nucleosome-depleted regions in *S. cerevisiae* (Yen, Vinayachandran, & Pugh, 2013) and its presence at promoters correlates positively with gene expression (Klopf, Schmidt, Clauder-Munster, Steinmetz, & Schuller, 2017).

The 17-subunit Tat-Interactive Protein 60kDa (Tip60)/p400 complex is one of two members of the SWR1 class of INO80 remodelers, the primary functions of which are to perform histone acetylation and histone H2A.Z/H2B dimer exchange (Doyon, Selleck, Lane, Tan, & Cote, 2004; Kobor et al., 2004). Tip60 is the mammalian homolog of the histone acetyltransferase NuA4, while p400 is the homolog of the nucleosome remodeler SWR1 (Doyon & Cote, 2004; Doyon et al., 2004; Latrick et al., 2016). While the acetylation and incorporation of H2A.Z are generally associated with transcriptional activation, Tip60-p400 instead acts as a repressor of differentiation-associated genes in murine ES cells (Fazzio, Huff, & Panning, 2008a). The complex does so by localizing to gene promoters marked by H3K4me3 and acetylating histones in that region (Fazzio et al., 2008a). The presence of active transcription marks combined with the repressive role for Tip60-p400 in ES cells suggests a heightened importance for the state of chromatin decorated by canonical active and repressive marks, rather than a simple on/off program of gene expression.

Along with p400, the SNF2-related CREBBP activating protein (SRCAP) is a homolog of SWR1 that also promotes exchange of H2A. Z, including deposition at promoters (Wong, Cox, & Chrivia, 2007). SRCAP shares the YL1 subunit with Tip60-p400 complex (Cai et al., 2005) and makes use of YL1 to facilitate transfer of H2A.Z from the remodeler to the nucleosome (Liang et al., 2016). SRCAP interacts with INO80 and has roles in chromosome double-strand break recognition and repair, as well as in regulating transcription (Gerhold & Gasser, 2014).

3.3 ISWI family nucleosome remodeling factors

The Imitation Switch (ISWI) family of nucleosome remodelers was first identified in *D. melanogaster* as a homolog of the yeast SWI/SNF complex (Elfring, Deuring, Mccallum, Peterson, & Tamkun, 1994). Members of the ISWI family of nucleosome remodelers are defined by the presence of HAND, SANT, and SLIDE domains that mediate nucleosome interaction (Clapier & Cairns, 2012). Broadly, ISWI family remodelers are important for nucleosome assembly and spacing, as well as higher-order chromatin compaction (Clapier & Cairns, 2009). ISWI remodeling complexes are substantially smaller than SWI/SNF complexes—composed of 2–4 subunits—and are canonically associated with repression of transcription (Clapier & Cairns, 2009). Mammalian ISWI complexes include WICH, NORC, WCRF/ACF, CHRAC, and RSF, all of which exclusively use the ATPase SNF2H, while NURF and CERF use the SNF2L ATPase (Längst & Becker, 2001).

ISWI remodelers are dependent on the nucleosome acidic patch for maximum enzymatic activity (Dann et al., 2017). Furthermore, ISWI remodelers display enhanced remodeling activity at H2A.Z-containing nucleosomes *in vitro* (Goldman, Garlick, & Kingston, 2010). Beyond repression of transcription, ISWI family remodelers function in three DNA damage repair pathways: homologous recombination, non-homologous end-joining, and nucleotide excision repair (Aydin, Vermeulen, & Lans, 2014; Lan et al., 2010; Nakamura et al., 2011; Yadon & Tsukiyama, 2011). In ES cells, ISWI family remodelers have been implicated in the control of cell fate decisions, neural tube formation, and neurite outgrowth (Andersen, Lu, & Horvitz, 2006; Yadon & Tsukiyama, 2011).

3.4 CHD family nucleosome remodeling factors

The Chromodomain helicase DNA-binding (CHD) superfamily of nucleosome remodeling factors contains nine proteins grouped into three subfamilies: those containing PHD domains (CHD3–5), SANT domains and Brahma and Kismet (BRK) domains (CHD6–9), and neither (CHD1 and CHD2) (Micucci, Sperry, & Martin, 2015). The CHD family is defined by the presence of two N-terminal tandem chromodomains that mediate chromatin interaction by binding to methylated lysine residues in the histone tails, and two SNF2-like ATP-dependent helicase domains (Clapier et al., 2017; Micucci et al., 2015). The first CHD family member to be identified was CHD1 (Delmas, Stokes, & Perry, 1993); however, the chromodomain itself had been discovered two years earlier as a shared region between the repressive proteins HP1 and Polycomb in *D. melanogaster* (Paro & Hogness, 1991). Functionally, CHD1 maintains open chromatin by binding to H3K4 trimethylated regions of chromatin and excluding H3K27me3, a repressive mark associated with heterochromatin (Gaspar-Maia et al., 2009). Although CHD family nucleosome remodelers are grouped into subfamilies based on shared domains, CHD family members can exhibit specialized nucleosome binding preferences and activities even within the same subfamily. CHD7 and CHD8, for example, slide nucleosomes along DNA, while CHD6 does not slide nucleosomes to remodel chromatin (Manning & Yusufzai, 2017).

The NuRD complex (originally known as the Mi-2 complex) was isolated and characterized by four separate groups in the same year (Tong, Hassig, Schnitzler, Kingston, & Schreiber, 1998; Wade, Jones, Vermaak, & Wolffe, 1998; Xue et al., 1998; Zhang, LeRoy, Seelig, Lane,

& Reinberg, 1998). Each of these studies described a complex containing parallel ATP-dependent nucleosome remodeling activity and histone deacetylase activity, a combination unique to NuRD. The enzymatic subunits of the canonical NuRD complex are an ATP-dependent remodeler (CHD3 or CHD4, though CHD3 is not expressed in ES cells), and two histone deacetylases (HDAC1 and HDAC2). The MBD3 and MBD2 subunits are mutually exclusive within the NuRD complex and confer complex naming (MBD2/NuRD or MBD3/NuRD) (Le Guezennec et al., 2006). There are three MBD3 isoforms, termed MBD3A, MBD3B, and MBD3C. MBD3A and MBD3B are found in all cell types examined, but MBD3C is unique to stem cells (Ee et al., 2017). MBD2 contains a domain (MBD) that binds methylated CpG dinucleotides on DNA (Hendrich & Bird, 1998) and a C-terminal transcription repression domain. MBD3 is the only MBD protein that does not bind methylated DNA, although it does bind hydroxymethylated cytosines (Yildirim et al., 2011). Despite mutually exclusive association within NuRD complexes, MBD2 and MBD3 localize to many of the same genomic regions (Gunther et al., 2013; Hainer et al., 2016). MBD3/NuRD is a largely repressive complex and functions in opposition to gene activating complexes like esBAF and STAT3 (Yildirim et al., 2011). In addition to transcriptional control, NuRD activity is also associated with higher-order chromatin assembly, maintenance of genome stability, hematopoietic stem cell differentiation, various human cancers, and aging (Lai & Wade, 2011; Pegoraro et al., 2009; Yoshida et al., 2008).

3.5 Summary

Nucleosome remodeling enzymes harness the power of a single biochemical activity—ATP hydrolysis—to alter DNA-histone interactions and regulate chromatin structure with a seemingly endless number of potential outcomes. Making use of unique domains and complex assemblies, nucleosome remodeling factors establish and preserve a diverse suite of chromatin states as a means by which to control DNA accessibility, and therefore all DNA-templated processes. In ES cells, nucleosome remodeling factors are generally important for maintenance of self-renewal and pluripotency, as well as specification into developmental lineages. The dynamic local chromatin states maintained by ATP-dependent nucleosome remodeling factors provide important context for the various modifications associated with active and repressed gene expression; indeed, local chromatin dynamics can even lead to opposite gene expression profiles from those canonically associated with the modifications decorating these regions, as is the case with Tip60-p400-mediated regions (Fazzio, Huff, & Panning, 2008b). In the next section, we will discuss these histone modifications in greater detail, examining some of the best-characterized modifications, their roles, and the techniques used to examine them.

4. Histone modifications provide an additional layer of gene regulation

Histone proteins are composed of globular alpha-helix domains and N- and C-terminal tail extensions, which are intrinsically disordered (Luger et al., 1997). Both the globular domains and tails of histone proteins are subject to a vast array of post-translational modifications. Histone modifying enzymes attach covalent moieties, including methyl groups, acetyl groups, phosphate groups, ubiquitin, and Small Ubiquitin-like Modifiers (SUMOs) to specific amino acid residues on histone proteins (reviewed in Fuchs, Larabee, &

Strahl, 2009; Smith & Shilatifard, 2010). These modifications regulate gene expression and other DNA-templated activities by affecting chromatin structure through altering DNA-histone interactions and providing a platform for recruitment of additional regulatory proteins that can further influence chromatin function and dynamics. Histone post-translational modifications (both acetylation and methylation of histone tails) were first identified by Alfred Mirsky's group (Allfrey, Faulkner, & Mirsky, 1964). Lysine residues are the most commonly modified amino acid in histone proteins, and modified residues are most often found on the N-terminal histone tails, though some modifications have been identified within the globular domains (Kouzarides, 2007). All four core histone proteins can be modified, and a summary of the best-characterized modifications can be found in Fig. 6.

Among the most prominent technical advancements regarding investigating the role and localization of histone modifications is the development of chromatin immunoprecipitation (ChIP) (Gilmour & Lis, 1984; Solomon, Larsen, & Varshavsky, 1988). This technology was coupled with deep sequencing, or ChIP-seq (Albert et al., 2007), to provide previously unparalleled resolution with which to examine protein localization on chromatin. ChIP-seq and its various modifications allow one to profile the localization of a protein on chromatin, including histone modification marks. Recently, an analogous technique to ChIP-seq, CUT&RUN (Skene & Henikoff, 2017), has successfully been used to profile localization of pluripotency factors in individual ES cells (Hainer, Boskovic, McCannell, Rando, & Fazzio, 2019). With the refinement of genomic profiling techniques, it is likely that future experiments will bring additional clarity to the state and roles of histone modifications and the enzymes that deposit and remove them.

4.1 Histone acetylation and deacetylation

The addition of an acetyl group to residues on histone proteins is a modification typically associated with active transcription. Acetyl groups are covalently attached to histone residues post-translationally by histone acetyltransferases (HATs), while histone deacetylases (HDACs) remove acetyl marks (Carrozza et al., 2005; Close et al., 2006; Gilbert, Gore, Herman, & Carducci, 2004; Govind, Zhang, Qiu, Hofmeyer, & Hinnebusch, 2007; Keogh et al., 2005). There are six HAT families, including GCN5/PCAF, MYST, TAF_{II}250, CBP/p300, SRC1, HAT1, and ATF-2 (Marmorstein & Zhou, 2014). Individual HAT families are well conserved at the sequence level within families, but not between families (Kuo & Allis, 1998) and include a histone acetyltransferase domain (Marmorstein, 2001).

Histone acetylation facilitates activation of transcription by neutralizing the basic charge of the lysine residue, thereby loosening DNA-histone contacts (Pokholok et al., 2005). In addition, acetylation can serve as a recruitment mark for chromatin modifying factors. For example, HATs enhance SWI/SNF binding to promoter nucleosomes through recognition of acetylated histone residues by the ATPase bromodomain (Hassan, Neely, & Workman, 2001; Lee et al., 2010). Both histone acetylation and ubiquitylation have been shown to interfere with formation of higher-order chromatin structure by disrupting chromatin interactions (Fierz et al., 2011). Commonly acetylated histone residues can be found in Fig. 6. For a more complete review on HATs, see Marmorstein and Zhou (2014).

Histone deacetylases, commonly referred to as HDACs, remove acetyl groups from modified proteins. The 18 human HDACs are sorted into four classes, deemed Class I, Class II, Class III, and Class IV (Seto & Yoshida, 2014). Broadly, Class I, II, and IV HDACs are grouped into the histone deacetylase or classical HDAC family, and are dependent on metals (specifically zinc) to hydrolyze acetylated substrates via a set of shared active site residues, while Class III HDACs use NAD⁺ to deacetylate modified lysines (Seto & Yoshida, 2014). For a comprehensive review on HDAC structure, function, and classification, see Seto and Yoshida (2014).

4.2 Histone methylation and demethylation

Histone methylation comes in varying states, as residues can be mono-, di-, or tri-methylated in a stepwise manner (Zee et al., 2010), and these marks are associated with different functions depending on the residue modified and the extent of methylation. At H3K4, for example, monomethylation (H3K4me1) is a mark of open chromatin and enhancers, while H3K4 trimethylation (H3K4me3) tends to decorate promoters and actively transcribed regions (Bannister & Kouzarides, 2011). These methyl groups are covalently attached to histone amino acids by a family of enzymes known as histone methyltransferases. Histone methyltransferases can be general or highly specialized, and can include somewhat redundant complexes, like the human H3K4 methyltransferases MLL1–6. Most histone methyltransferases are marked by a SET (Su-39, Enhancer of zeste, Trithorax) domain, with the exception of the DOT1L H3K79 methyltransferase (Dillon, Zhang, Trievel, & Cheng, 2005). Histone methyltransferases catalyze the transfer of methyl groups from the donor molecule S-Adenosyl Methionine (SAM) to target proteins, many—though not all—of which are histones (Dillon et al., 2005). Histone methylation is associated with either transcriptional activation (e.g., H3K4) or, more often, transcriptional repression (e.g., H3K9, H3K27, H4K20, and H1K26), but the function of these marks is often highly context- and location-specific. Commonly methylated histone residues can be found in Fig. 6.

Histone methyl groups are also regulated by enzymes that remove these modifications, known as histone demethylases. There are two main families of histone demethylases: the Lysine-Specific Demethylase (LSD) family, and the Jumonji (JMJ) family. Of the two, JMJ is the larger family, comprised of over 30 demethylases, while LSD is comprised of only two demethylases, LSD1 and LSD2 (Kooistra & Helin, 2012). Both LSDs share a SWI3P, RSC8P, and MOIRA (SWIRM) domain and an amine oxidase domain, along with either a spacer region (LSD1) or a CW-type zinc finger domain (LSD2) (Kooistra & Helin, 2012). LSD1 utilizes the substrates H3K4 (mono- or di-methylated), H3K9 (mono- or di-methylated), plus three non-histone substrates in p53, E2F1, and DNMT1. LSD2, meanwhile, is specific to mono- and di-methylated H3K4 alone (Ciccone et al., 2009; Karytinis et al., 2009). JMJ demethylases exhibit extraordinary diversity of targets. Importantly, only JMJ-family demethylases are known to act upon trimethylated residues (Shi, 2007; Whetstine et al., 2006).

Histone modifications have extensive downstream effects depending on the residue and modification. Marks associated with active transcription include acetylation of H3K27 (H3K27ac), H3K56me3, H3K4me3, and H3K36me, while repression-associated

modifications include deacetylation and some methylation marks (H3K9me3, H3K27me3, H3K56me3, and H4K20me). H3K4 methylation is currently the most extensively studied histone modification, likely due to its role in decorating actively transcribed promoters (H3K4me3) and enhancers (H3K4me1). H3K4 methylation is placed by the SET1/COMPASS complex in yeast and by the MLL1–6, SET1D1A, and SET1D1B complexes in humans (Dou et al., 2006; Takahashi et al., 2011). Similar to H3K4me1, H3K27ac decorates regions of active transcription, and is used as the canonical mark by which to identify an active enhancer. H3K27 can also be trimethylated, a repressive mark placed by the PcG proteins in a coordinated positive feedback loop with H2AK119 ubiquitylation, by the PRC2 and PRC1 complexes, respectively (Cao, Tsukada, & Zhang, 2005). Although H3K27me3 is a canonical repressive mark, it also marks “poised” genes in stem cells, along with H3K4me3, leading to what is known as a bivalent gene promoter (Fig. 7) (Azuara et al., 2006; Bernstein et al., 2006). H3K36 methylation is placed by the SET2 methyltransferase and marks genes that are in the process of being transcribed; as RNA Polymerase II passes along the gene, SET2 is recruited by the phosphorylated C-terminal domain of RNA Polymerase II subunit RPB1 and the methyl mark is left on the gene in its wake (Kizer et al., 2005; Youdell et al., 2008). This mark prevents cryptic initiation of transcripts from within the transcribed gene (Carrozza et al., 2005). Finally, H3K56ac is catalyzed by the RTT109 histone acetyltransferase and ASF1 chaperone in yeast (Driscoll, Hudson, & Jackson, 2007; Schneider, Bajwa, Johnson, Bhaumik, & Shilatifard, 2006; Tsubota et al., 2007) and by p300/CBP and GCN5 in *Drosophila* and mammals (Das, Lucia, Hansen, & Tyler, 2009; Tjeertes, Miller, & Jackson, 2009). Acetylation of H3K56 enhances nucleosome unwrapping and “breathing,” allowing for increased DNA accessibility and remodeling by nucleosome remodeling complexes (Neumann et al., 2009). H3K56ac and H2A.Z both mark promoter-proximal nucleosomes with high turnover rate (Kaplan et al., 2008; Raisner et al., 2005; Rufiange, Jacques, Bhat, Robert, & Nourani, 2007) and are thus associated with transcriptional activation.

Importantly, the majority of these marks themselves do not appear to directly activate or silence genes, with acetylation being a notable exception; instead, these marks are correlated with genes activated and silenced through other mechanisms (Bannister & Kouzarides, 2011). Even canonical “activating” marks—like histone acetylation and trimethylation of H3K4— can be present at repressed genes, as is the case with Tip60-p400-repressed genes during stem cell differentiation (Fazzio et al., 2008a). In addition to correlation with activity state, histone modifications can regulate higher-order chromatin structure; H4K16ac, for example, is known to inhibit higher-order chromatin folding and compaction through decreased nucleosome-nucleosome stacking, perhaps due to a decreased interaction of the H4 tail with the histone acidic patch (Shogren-Knaak, Ishii, Pazin, Davie, & Peterson, 2006; Zhang, Erler, & Langowski, 2017).

Histone modifications can also create or eliminate protein binding sites. Upon methylation of H3K9 by a SUV39H methyltransferase, a binding site for HP1 proteins is created that is dependent on a functional HP1 chromodomain and a dimethylated H3K9 (Lachner, O’Carroll, Rea, Mechtler, & Jenuwein, 2001). Similarly, H3K36me3 placed by the SET2 methyltransferase serves as a recruitment cue for the RPD3S deacetylase to coding regions to repress cryptic intragenic transcription (Carrozza et al., 2005). Conversely, complex

binding to chromatin can be disrupted by histone modifications, as is the case with H3K4 methylation and NuRD complex binding (Kouzarides, 2007). Chromatin modifications are highly dependent on the chromatin context in which they are found. For example, the traditionally repression-associated mark H3K27me3 is associated with genes that are inactive, but poised for activation in ES cells. These so-called bivalent genes will be examined in greater detail later in this chapter.

While the above sections have detailed some of the better characterized roles associated with various histone modifications, there are numerous other roles that are less well-studied. These roles include gene bookmarking as a means through which epigenetic information is passed between parent and daughter cells through mitosis (H4K5ac and H4K8ac) (Zhao, Nakamura, Fu, Lazar, & Spector, 2011) and histone “crosstalk” to regulate downstream epigenetic marks (such as H2BK120ub and proline isomerization at H3P38 to regulate H3K36me) (Kouzarides, 2007; Tomson & Arndt, 2013). With new approaches to examine epigenetic marks, powerful single-cell genomic techniques, and decreasing costs of next-generation sequencing, it is likely that new roles for established marks and new epigenetic marks and modifications themselves will be discovered in the near future.

4.3 Polycomb group proteins mediate H3K27me3 and silencing of developmental genes

Polycomb Group (PcG) proteins have been associated with developmental processes since their identification (Lewis, 1947, 1978). PcG includes two complexes, Polycomb-Related Complex 1 (PRC1) and PRC2. PRC1 was purified in 1999 from *D. melanogaster* and found to contain four proteins: Polycomb, Polyhomeotic, dRING, and Posterior Sex Combs (PSC) (Shao et al., 1999). The Sex Combs on Midleg (SCM) protein was later found to copurify with the complex and to be incorporated *in vitro* (Kang et al., 2015; Peterson et al., 2004), and plays a major recruitment role in the PRC1 complex (Kassis & Kennison, 2010), as is the case for the chromobox (CBX) family of proteins that can recognize and bind H3K27me3 (Vincenz & Kerppola, 2008). When added to arrayed nucleosomes, PRC1 was able to block remodeling by BAF, but not to disrupt the arrays by cutting (Shao et al., 1999). PRC1's RING1 and RING2 subunits catalyze the placement of ubiquitin on H2AK119, a mark that serves as a recruitment cue for the PRC2 complex (Cao et al., 2005). In addition to placing the H2AK119Ub mark, PRC1 reads the PRC2-catalyzed H3K27me3 mark as a recruitment cue (Blackledge et al., 2014).

In addition to facilitating gene repression through H3K27me3, Enhancer of Zeste Homolog 2 (EZH2) serves as a scaffold for DNA methyltransferases that methylate cytosines to repress gene promoters (Vire et al., 2006). Functionally, PRC2 binds to chromatin and carries out H3K27me3 via EZH2, an action that works to silence target genes. The exact mechanism by which PRC2 is recruited to target sites is unknown; however, the JARID2 (Pasini et al., 2010) and PCL (Hunkapiller et al., 2012) proteins appear to be linked to this targeting.

5. Histone chaperones and histone variants regulate chromatin structure

Individual histone proteins within the nucleosome octamer can be substituted by a number of variants that provide an additional level of complexity. The canonical histone proteins

H2A, H2B, H3, and H4 are regulated in a cell-cycle-dependent manner (Marzluff, Gongidi, Woods, Jin, & Maltais, 2002). In contrast to the canonical histones, genes encoding histone variants are expressed and the variants deposited onto chromatin in a replication-independent manner (Ahmad & Henikoff, 2002). Replacement of canonical histones with histone variants, such as the replacement of H2A with H2A.Z over promoters (Santisteban, Hang, & Smith, 2011; Wan et al., 2009; Zhang, Roberts, & Cairns, 2005), plays an important role in chromatin dynamics during transcription and other DNA-templated activities. Many different variant forms of each histone exist throughout various organisms (Tables 2 and 3) and reviewed in Buschbeck and Hake (2017). Sequence differences between histone variants and canonical histones are found in many regions of the histone proteins: either in the terminal tails, the globular fold domains, or in specific amino acid residues (Doyen, An, et al., 2006; Doyen, Montel, et al., 2006; Henikoff & Ahmad, 2005). While incorporation of histone variants in place of canonical histones impacts chromatin structure in various ways, many post-translational modification sites are conserved between variants and canonical histones (McKittrick, Gafken, Ahmad, & Henikoff, 2004). Therefore, exchanging the canonical histones with these variants may not alter nucleosome recognition by some chromatin regulatory proteins.

Histone exchange is carried out by an extensive class of proteins called histone chaperones. Histone chaperones typically do not require the use of ATP to carry out this exchange; rather, histone chaperones use spontaneous DNA movement around the dyad axis to destabilize the nucleosome and promote histone exchange (Hondele et al., 2013). Histone exchange can be targeted to swap a core histone protein with a histone variant (Venkatesh & Workman, 2015) to transiently promote transcription elongation through the nucleosome (Hsieh et al., 2013; Kulaeva, Hsieh, Chang, Luse, & Studitsky, 2013; Kulaeva et al., 2010; Li et al., 2007) and to replace histone proteins that have been dissociated in the wake of the transcribing polymerase (Hsieh et al., 2013; Li et al., 2007), among other roles. Histone chaperones can be specific to individual histone variants (e.g., Holliday Junction Recognition Protein (HJURP) for CENP-A) or more general (α -thalassemia X-linked mental retardation protein (ATR-X) is associated with deposition of both H3.3 and macroH2A) (Skene & Henikoff, 2013).

Among the best-characterized histone variants are those found at *cis*-regulatory regions, H2A.Z and H3.3; however, there are numerous histone variants for H2A and H3, and their incorporation can be regulated by genomic location, depositing histone chaperone, higher-order chromatin state, and numerous other factors (Tables 2 and 3). Although mammalian variants of H2B exist, they are often highly cell type-specific and commonly involved in gametogenesis (Draizen et al., 2016). A variant of histone H4, H4G, has very recently been discovered (Long et al., 2019), but as little is known about it at present, this section will focus on variants of H2A and H3, as they are highly conserved general regulators with numerous well-defined roles.

6. Histone H2A variants

6.1 H2A.Z is a histone variant associated with active transcription

H2A.Z is arguably the best-characterized histone variant to date. Originally identified with H2A.X in *S. cerevisiae* (West & Bonner, 1980), H2A.Z is highly conserved across species and is expressed in every eukaryote examined to date. H2A.Z is highly incorporated at regions of active transcription, especially at promoters and enhancers. Studies suggest that H2A.Z can be deposited into a nucleosome either through ATP-dependent histone exchange (Mizuguchi et al., 2004) or with the help of replication-independent histone chaperones (Park, Chodaparambil, Bao, McBryant, & Luger, 2005). When an H2A.Z/H2B dimer is incorporated into a nucleosome, steric hinderances are induced between the newly incorporated dimer and the H3/H4 tetramer, effectively destabilizing the nucleosome (Giaimo, Ferrante, Herchenrother, Hake, & Borggreffe, 2019). This change may promote active transcription, DNA repair processes, and chromatin domain segregation (Suto, Clarkson, Tremethick, & Luger, 2000). H2A.Z can localize to the 5' ends of active and inactive genes (the +1 and -1 nucleosomes), though it is most often associated with the +1 and -1 nucleosomes flanking the NDR region of active genes (Guillemette et al., 2005). H2A.Z also localizes to the borders of euchromatic domains, and it is thought to prevent heterochromatic invasion into euchromatic space, with INO80 complexes helping to accomplish this role (Papamichos-Chronakis, Watanabe, Rando, & Peterson, 2011; Raisner et al., 2005). In addition to INO80, ISWI complexes display enhanced activity at H2A.Z-containing nucleosomes; H2A.Z may therefore regulate ISWI complexes, serving as a stimulatory cue for nucleosome remodeling activity (Goldman et al., 2010). In eukaryotes, SWI/SNF family remodelers (RSC and esBAF) act on H2A.Z-containing nucleosomes (Cakiroglu et al., 2019; Hainer & Fazzio, 2015).

6.2 H2A.X is a marker of DNA double-strand breaks

First characterized in 1980 (West & Bonner, 1980), H2A.X is now best known as a marker of DNA damage. Upon recognition of a DNA double-strand break, S139 on H2A.X becomes phosphorylated, then referenced as γ H2A.X (Rogakou, Pilch, Orr, Ivanova, & Bonner, 1998). This phosphorylation, primarily carried out by the ATM, ATR, and DNA-PK kinases (Sharma, Singh, & Almasan, 2012), recruits the INO80 nucleosome remodeling complex (Van Attikum, Fritsch, Hohn, & Gasser, 2004). γ H2A.X is found in high levels in ES cells relative to differentiated cells (Turinetto et al., 2012); furthermore, γ H2A.X is an important regulator of ES cell proliferative rate, and its deposition at nucleolar ribosomal DNA promoters assembles the nucleolar remodeling complex, thereby repressing rRNA transcription and controlling ES cells' self-renewal rate (Eleuteri, Aranda, & Ernfors, 2018; Turinetto et al., 2012).

6.3 macroH2A is associated with repression and heterochromatin

macroH2A is defined by its C-terminal "macro" domain, which is approximately twice the size of the corresponding histone fold region in core H2A and may allow the variant to resist nucleosome remodeling activity (Buschbeck & Hake, 2017; Chakravarthy et al., 2005). macroH2A associates with heterochromatin but is not preferentially deposited in repressed regions of chromatin; rather, macroH2A is deposited throughout the genome (by the action

of multiple histone chaperones) and subsequently pruned away from regions of active transcription by the FACT histone chaperone (Sun et al., 2018). macroH2A variants inhibit transcription initiation by interfering with p300-dependent histone acetylation (Doyen, An, et al., 2006).

Additionally, macroH2A variants interfere with nucleosome remodeling and sliding by SWI/SNF and ACF remodelers through the variant's non-histone domain (Angelov et al., 2003; Doyen, An, et al., 2006; Sun et al., 2018; Timinszky, 2009). Importantly, macroH2A also impedes reactivation of pluripotent genes during reprogramming to iPSCs (Gaspar-Maia et al., 2013).

7. Histone H3 variants

7.1 H3.3 marks regulatory, repetitive, and actively transcribed regions

H3.3 is deposited at regions of active transcription, a process highly regulated by multiple factors including Histone Cell Cycle Regulation Defective Homolog A (HIRA), ATRX, Death domain associated protein (DAXX) and p400 (Ahmad & Henikoff, 2002; Chen, Zhao, et al., 2013; Elsaesser, Goldberg, & Allis, 2010; Hake et al., 2006; Jin & Felsenfeld, 2007; Jin et al., 2009; Lewis, Elsaesser, Noh, Stadler, & Allis, 2010; McKittrick et al., 2004; Pradhan et al., 2016; Wirbelauer, Bell, & Schubeler, 2005). The structure of H3.3 is highly similar to that of H3.1 and H3.2, with the exception of five amino acids found on the surface of the H3/H4 tetramer (Tachiwana, Osakabe, et al., 2011), which suggests that factor binding to H3.3-containing nucleosomes could be different from core H3-containing nucleosomes. Additionally, murine ES cell H3.3 S31 can be phosphorylated, a mark that promotes activity of the p300 HAT and acetylation of enhancers (Martire et al., 2019).

H3.3 can be deposited in both replication-dependent and replication-independent manners (Ahmad & Henikoff, 2002; Tagami, Ray-Gallet, Almouzni, & Nakatani, 2004). Interestingly, H3.3 exchange occurs at both active and, with a lower turnover rate, inactive promoters (Kraushaar et al., 2013). Venkatesh and Workman have proposed that H3.3 exchange at inactive promoters may maintain a poised state, wherein the promoter undergoes rapid histone turnover in preparation for gene activation and subsequent transcription (Venkatesh & Workman, 2015). At many promoters and enhancers, H3.3 co-localizes with H2A.Z; these nucleosomes are believed to be less stable than nucleosomes containing core histones and thus more conducive to open chromatin structure and transcriptional activation (Chen, Zhao, et al., 2013; Jin & Felsenfeld, 2007; Jin et al., 2009).

In addition to actively transcribed and regulatory regions of the genome, H3.3 is specifically deposited at telomeres and other repeat regions. While HIRA deposits H3.3 at actively transcribed regions, ATRX and DAXX regulate H3.3 deposition at telomeres, pericentric heterochromatin, and other regions of repetitive chromatin (Dyer, Qadeer, Valle-Garcia, & Bernstein, 2017; Lewis et al., 2010). HIRA-mediated H3.3 deposition facilitates PRC2 binding at developmental loci in ES cells and subsequent H3K27me3 of these promoters (Banaszynski et al., 2013), underscoring a specialized role for H3.3 in ES cells that will be expanded upon later in the chapter.

7.2 CENP-A is a centromere-specific variant of histone H3

CENP-A is an H3 variant that is found at centromeres and is conserved from *S. cerevisiae* (in which it is called CENH3) through humans. It bears relatively low sequence identity to canonical H3 (approximately 60% in the histone fold domain and less in the N-terminus) (Chittori et al., 2018; Tachiwana, Kagawa, et al., 2011; Yoda et al., 2000). Regulation of CENP-A synthesis and deposition are key to proper progression through the cell cycle and mitosis (Earnshaw & Rothfield, 1985; Hori et al., 2014; Nardi, Zasadzinska, Stellfox, Knippler, & Foltz, 2016; Niikura et al., 2015; Okada, Okawa, Isobe, & Fukagawa, 2009; Palmer, O'Day, Wener, Andrews, & Margolis, 1987; Shuaib, Ouararhni, Dimitrov, & Hamiche, 2010).

8. Histone chaperones

8.1 FACT facilitates H2A/H2B dimer exchange to promote nucleosome-templated activities

Facilitates Chromatin Transactions (FACT) is a highly conserved complex that plays important roles in several nuclear processes including DNA replication, DNA repair, and transcription initiation and elongation (reviewed in Duina, 2011; Formosa, 2008, 2012; Gurova, Chang, Valieva, Sandleh, & Studitsky, 2018). FACT is a heterodimer composed of suppressor of Ty 16 (SPT16) and Structure-Specific Recognition Protein 1 (SSRP1) (Orphanides, LeRoy, Chang, Luse, & Reinberg, 1998; Orphanides, Wu, Lane, Hampsey, & Reinberg, 1999). In human cells, FACT was initially identified by its ability to allow RNA Polymerase II to transcribe through nucleosomal DNA (Orphanides et al., 1998). Additionally, human FACT binds histone H2A/H2B dimers while human SSRP1 and *S. pombe* SPT16 can both bind H3/H4 dimers (Belotserkovskaya et al., 2003; Ransom, Dennehey, & Tyler, 2010; Stuwe et al., 2008). Therefore, FACT can act as both an H2A/H2B and H3/H4 histone chaperone. FACT activity is also required for proper regulation of transcription initiation. SPT16 nucleosome reassembly occurs over certain gene promoters and this activity is required for proper transcriptional repression of these genes (Adkins & Tyler, 2006). In addition to contributing to transcription initiation and elongation, FACT participates in mRNA nuclear export (Hautbergue et al., 2009; Herold, Teixeira, & Izaurralde, 2003). These studies demonstrate a multifaceted role for the FACT complex in regulating multiple stages of transcription and mRNA processing.

Recently, FACT has been implicated in maintenance of pluripotency in stem cells. While FACT had long been thought of as a marker of actively proliferating cells (Hertel et al., 1999), FACT has more recently been associated with pluripotent cells than with actively proliferating cells (marked by Ki-67 and SPT16 intestinal immunohistochemistry) (Garcia et al., 2011). Recently, it was demonstrated that FACT is required for maintenance of pluripotency (Mylonas & Tessarz, 2018), a role consistent with the previously mentioned work as well as FACT's known interactions with the master regulator of pluripotency OCT4 (Ding, Xu, Faiola, Ma'ayan, & Wang, 2012; Pardo et al., 2010). Subsequent studies have suggested that FACT inhibition can both facilitate and impede establishment of pluripotency but that the complex may not be necessary for maintenance of pluripotency (Kolundzic et

al., 2018; Shen, Formosa, & Tantin, 2018). Further studies will be necessary to determine the true extent of FACT's role(s) in stem cells and pluripotency.

8.2 CAF1 and ASF1 promote incorporation of H3 and H4 onto newly synthesized DNA

Chromatin Assembly Factor 1 (CAF1) is a histone chaperone responsible for loading histones H3 and H4 onto newly synthesized DNA (Smith & Stillman, 1989; Stillman, 1986). CAF1 binds multiple H3/H4 dimers at once, thereby promoting tetramerization and nucleosome formation on DNA (Liu, Roemer, Port, & Churchill, 2012). CAF1 does so in parallel with Anti-Silencing Factor 1 (ASF1). Like CAF1, ASF1 binds H3/H4 dimers to promote loading onto newly synthesized DNA; unlike CAF1, however, monomeric ASF1 binds only one H3/H4 dimer at a time, and ASF1 is excluded from a CAF1-H3/H4 complex via a tight interaction between CAF1 and the histone dimers (Liu et al., 2012). CAF1 and ASF1 are most active during S-phase as loading of H3/H4 occurs on newly synthesized DNA. Beyond post-replication H3/H4 incorporation, CAF1 and ASF1 are also active in passing through the DNA damage checkpoint upon successful completion of DNA repair (Kim & Haber, 2009). In adult stem cells, CAF1 prevents expression of differentiation-associated genes (Clemot, Molla-Herman, Mathieu, Huynh, & Dostatni, 2018).

ASF1 has key epigenetic roles via its interaction with the RTT109 histone acetyltransferase; without the ASF1-H3/H4 interaction, RTT109 cannot perform H3K56 acetylation (Recht et al., 2006). ASF1 can also recruit the HAT1 acetyltransferase to modify H4K5 and H4K12 (Fillingham et al., 2008), two modifications that have been implicated in memory and learning (Park, Rehrauer, & Mansuy, 2013; Peleg et al., 2010). H4K5ac also prevents mitotic compaction of some cell type-specific genes, allowing for quick resumption of transcription following cytokinesis (Zhao et al., 2011), a role that may be particularly important in stem cells for a poised differentiation state.

8.3 HIRA deposits H3.3 at actively transcribed and regulatory regions of chromatin

Histone Cell Cycle Regulation Defective Homolog A (HIRA) performs replication-independent incorporation of histone variants, including H3.3 that is associated with active transcription and regulatory regions (Lamour et al., 1995; Lorain et al., 1996). ASF1 seems to assist HIRA in H3.3 deposition, as sites that are HIRA-bound, but not ASF1-bound, are not enriched for H3.3 (Adam, Polo, & Almouzni, 2013; Pchelintsev et al., 2013; Tang et al., 2006). Like CAF1, HIRA has a role in overcoming DNA damage to prepare chromatin for resumption of transcription after genotoxic events (Adam et al., 2013). While HIRA is essential for H3.3 enrichment at active and repressed genes, it is not essential for H3.3 enrichment at telomeres (Goldberg et al., 2010); rather, the histone chaperones ATRX and DAXX that fill this role (Lewis et al., 2010). Interestingly, upon depletion of HIRA (and/or H3.3 itself), ES cells exhibit diminished PRC2 occupancy and decreased H3K27me3 at bivalent genes, though this H3K27me3 loss does not correlate with a global increase in gene expression (Banaszynski et al., 2013). Furthermore, ES cells depleted of H3.3 display a low nucleosome turnover rate, improper lineage specification despite functional pluripotency, increased expression of trophectoderm markers, and a failure of HIRA to localize to chromatin (Banaszynski et al., 2013).

8.4 ATRX/DAXX deposit H3.3 at telomeres and pericentric heterochromatin

ATRX and DAXX form an H3.3-depositing complex, wherein DAXX acts as the primary H3.3 chaperone (Drane, Ouarrhni, Depaux, Shuaib, & Hamiche, 2010; Lewis et al., 2010) and ATRX appears to recognize H3K9me3 (Dyer et al., 2017). ATRX is a transcriptional repressor (Gibbons et al., 2000; Gibbons, Picketts, Villard, & Higgs, 1995) that also directly interacts with PRC2; this interaction may be important to recruit PRC2 to genomic loci (Dyer et al., 2017; Sarma et al., 2014). DAXX is essential for H3.3 deposition at telomeres in murine ES cells (Lewis et al., 2010) and at pericentric heterochromatin regions in murine embryonic fibroblasts (Drane et al., 2010).

8.5 The NAP1 family import newly translated histones from the cytoplasm to the nucleus

The Nucleosome Assembly Protein 1 (NAP1) chaperone was first identified based on its ability to facilitate nucleosome assembly *in vitro* (Ishimi, Yasuda, Hirosumi, Hanaoka, & Yamada, 1983). NAP1 is primarily involved in nuclear histone import and regulation of transcription (Avvakumov, Nourani, & Cote, 2011). NAP1 binds newly translated H2A and H2B proteins in the cytosol and shuttles them into the nucleus via a direct interaction with Importin 9 (Mosammaparast, Ewart, & Pemberton, 2002). NAP1 also has H3/H4 tetramer binding activity, including the ability to load an entire H3/H4 tetramer onto DNA *in vitro* (Bowman et al., 2011; McBryant et al., 2003).

8.6 INO80 family members possess both nucleosome remodeling and histone chaperone activities

INO80 family members, such as Tip60-p400, act as histone chaperones as well as ATP-dependent nucleosome remodeling complexes. p400 facilitates incorporation of H3.3 at promoters and enhancers *in vitro* (Pradhan et al., 2016). Additionally, *Drosophila* Tip60 acetylates γ H2Av—the *Drosophila* homolog of γ H2A.X—and exchanges it with an unphosphorylated H2Av (Kusch et al., 2004). Along with p400, the SRCAP complex is able to incorporate H2A.Z/H2B dimers into mononucleosomes (Ruhl et al., 2006; Wong et al., 2007). Finally, INO80 globally regulates H2A.Z incorporation via direction of H2A.Z/H2B localization and through a histone exchange function that allows the complex to switch H2A.Z/H2B and H2A/H2B dimers (Papamichos-Chronakis et al., 2011). Specifically, INO80 performs this exchange by translocating along DNA at the H2A/H2B dimer and displacing DNA from the nucleosome surface, thereby creating torsional strain and permitting exchange of H2A.Z/H2B dimers without additional chaperones (Brahma et al., 2017).

9. Chromatin structure is dynamic and highly regulated

Chromatin structure is the result of dynamic interplay between histone positioning, variants, modifications, and localization, with the product being DNA compaction and regulated gene expression. Each of these processes must be individually regulated and function synergistically to maintain the integrity of the genome. In spite of often contradictory roles and complex regulatory pathways that require fidelity from each member, cells are able to maintain a balance between gene expression and repression through precise regulation by chromatin modifiers. In the remainder of this chapter, we will discuss in more detail how

stem cells make use of unique nucleosome remodeling factors, histone methylation patterns, trends in histone variant incorporation, and long-range chromatin interactions. In doing so, stem cells carefully regulate their chromatin state to preserve stem cell state, as well as to facilitate proper differentiation when required by the organism.

10. Stem cell chromatin is dynamic and tuned to regulate cell fate

ES cells carefully regulate their two defining properties—self-renewal and pluripotency—through a suite of coordinated changes in gene expression modulated by chromatin structure. In ES cells, careful regulation of chromatin state is crucial to establish and preserve the qualities that both prevent and drive differentiation of ES cells into adult cell types. While there are many factors that drive an ES cell's decision to differentiate into a progenitor cell among one of the three germ layers—including cell signaling (Reya & Clevers, 2005), physical strain (Miller & Davidson, 2013), and metabolic changes (Romito & Cobellis, 2016)—the key to ES cell pluripotency lies within their regulated gene expression; chromatin regulation therefore plays an enormous role in ES cell fate and function. Between a suite of transcription factors called master regulators of pluripotency, a largely euchromatic state of compaction, and carefully curated epigenetic marks, ES cells establish and preserve this pluripotent state by diverse mechanisms that harness chromatin's dynamic nature. As one may expect based on the diverse range of specialized eukaryotic cells, this regulation can be carried out through multiple mechanisms. Depending on differentiation signals received, pluripotent ES cells are able to upregulate specific subsets of genes corresponding to any lineage. Thus, the structure and features of ES cell chromatin largely differ from that of most somatic cell types. In this section, we will discuss the ways in which ES cells regulate their chromatin state to maintain proper cellular function and fate.

ES cell chromatin is largely euchromatic (Young, 2011), and thereby permissive of gene transcription. This heightened accessibility does not always directly correspond with increased gene transcription, however. ES cell chromatin structure is preserved by a diverse suite of nucleosome remodeling factors, including the ES cell-specific SWI/SNF family complex esBAF. While the remainder of this chapter will focus on nucleosome remodeling factors, histone-based chromatin organization, and higher-order chromatin structures, it is important to note that pluripotency is affected and regulated by numerous processes that are not direct results of chromatin state changes.

11. ES cells carefully regulate their chromatin via specialized transcription factors

11.1 Master regulators of pluripotency

Among ES cell transcription factors, OCT3/4 (henceforth referred to as OCT4), SOX2, and NANOG are commonly known as core factors that maintain ES cell pluripotency in conjunction with a large network of proteins that includes both transcription factors and chromatin modifiers (Morey, Santanach, & Di Croce, 2015; Orkin & Hochedlinger, 2011). OCT4, SOX2, and NANOG cooperate in a positive feedback loop to maintain their own expression in pluripotent cells as well as the expression of known ES cell regulators such as

the LIF signaling pathway or microRNA genes (Chen et al., 2008; Marson et al., 2008; Okumura-Nakanishi, Saito, Niwa, & Ishikawa, 2005; Tomioka et al., 2002; Young, 2011). Chromatin regulatory complexes in both ATP-dependent nucleosome remodeler and histone modifying families have been shown to interact with OCT4, SOX2, or NANOG (Ang et al., 2011; Liang et al., 2008), and the pluripotency network also includes long non-coding RNAs (Guttman et al., 2011).

Differentiated or somatic cells can be reprogrammed to the pluripotent state—termed induced pluripotent stem cells—through ectopic expression of the core pluripotency factors OCT4, SOX2, and KLF4 (Takahashi & Yamanaka, 2006). These cells reactivate their endogenous pluripotency factors, deactivate somatic genes, and re-establish activating histone marks and other hallmarks of ES cell chromatin structure (Apostolou & Hochedlinger, 2013).

11.2 Pioneer transcription factors

A subset of transcription factors are able to bind to compacted chromatin and modulate local nucleosome architecture to facilitate downstream events; these transcription factors are known as pioneer transcription factors (reviewed in Iwafuchi-Doi, 2019; Mayran & Drouin, 2018; Zaret & Carroll, 2011). Recently, many traditionally described transcription factors have been identified as pioneer transcription factors, but the mechanism of function for many of these factors remains incompletely understood. Transcription factors with pioneering activity have been shown to move through the nucleus more slowly than other transcription factors in fluorescence recovery after photobleaching experiments, suggesting that these factors may bind to closed chromatin nonspecifically and scan for a binding site at which to remain (Iwafuchi-Doi, 2019; Zaret, Lerner, & Iwafuchi-Doi, 2016). With respect to stem cells, it has been proposed that OCT4 can act as a pioneer transcription factor (Zaret & Carroll, 2011) through recruitment of the esBAF complex to inaccessible regulatory sites. OCT4 interacts with major nucleosome remodeling factors, including esBAF, lending credence to this hypothesis (Ding et al., 2012; Pardo et al., 2010; van den Berg et al., 2010). Nucleosome remodeling by esBAF at these sites subsequently allows for binding and regulation by the remaining core transcription factors (Hainer & Fazzio, 2015; King & Klose, 2017). In addition to OCT4, SOX2 has pioneering activity, and both pluripotency factors (as well as the pioneer factor FOXA) bind to cell-type-restricted enhancers to premark them for activation in differentiated cells (Iwafuchi-Doi et al., 2016; Kim et al., 2018). This premarking function is required for future enhancer activation in differentiated cells, and premarked enhancers are in an open configuration but are not decorated with canonical enhancer histone modifications (H3K27ac, H3K4me1, and H3K4me2) (Kim et al., 2018). Along with OCT4 and SOX2, additional pioneer factors, such as p63, GATA, and AP-1 have been reported to recruit nucleosome remodeling factors to their target sites (Bao et al., 2015; Engelen et al., 2011; Hu et al., 2011; Takaku et al., 2016; Vierbuchen et al., 2017). Recent studies have shown that esBAF is likely required to remodel nucleosome structure prior to factor binding during embryogenesis (Hainer et al., 2019). Pioneer transcription factors are clearly key regulators of pluripotency and cell fate; however, their regulation of, by, and/or with nucleosome remodeling factors (such as OCT4 and esBAF) remains incompletely understood.

12. Embryonic stem cell chromatin is poised for action

ES cell chromatin is uniquely packaged to promote expression of genes required for self-renewal and to prevent differentiation into progenitor cell populations unless acted upon by the proper regulatory pathway. Therefore, ES cells maintain themselves in a poised state, whereby the cells can exit this self-renewal cycle to differentiate into the progenitors for any of the 200+ cell types found in the adult organism. ES cell chromatin is packaged and regulated to accomplish this activity using numerous methods, including a high euchromatin-to-heterochromatin ratio, bivalent gene promoters, and specialized nucleosome remodelers and transcription factors (Azuara et al., 2006; de Dieuleveult et al., 2016; Harikumar & Meshorer, 2015; Orkin & Hochedlinger, 2011). In the following sections, we will discuss the features that define chromatin state in ES cells and the ways in which these features are established and maintained.

13. Histone modifications are specifically regulated in stem cells to maintain pluripotency and facilitate differentiation

13.1 H3K56ac regulates pluripotency factors and developmental regulators

In ES cells, H3K56ac is thought to be important for both pluripotency and differentiation through its interactions with OCT4, SOX2, and NANOG at pluripotency genes, and through its enrichment at developmental regulators such as the *HOX* genes during differentiation (Tan, Xue, Song, & Grunstein, 2013; Xie et al., 2009). Of the core pluripotency factors, only OCT4 directly interacts with H3K56ac (Tan et al., 2013); however, H3K56ac can be found at many of the OCT4/SOX2/NANOG binding sites (Xie et al., 2009). Deletion of *Sirt6*, a NAD-dependent deacetylase that targets H3K56, leads to upregulation of *OCT4*, *SOX2*, and *NANOG* during differentiation, and mis-expression of markers from all three germ layers (Etchegaray & Mostoslavsky, 2015). H3K56 deacetylation by SIRT6 functions to maintain genome integrity, while hyperacetylation of H3K56 is associated with genome instability and sensitivity to genotoxins, such as MMS (Yang, Zwaans, Eckersdorff, & Lombard, 2009).

13.2 Bivalent promoters mark lowly expressed but poised genes in ES cells

Overall, ES cell chromatin is enriched for active histone modifications such as H3K4me3 and H3K9ac (reviewed in Meshorer & Misteli, 2006). Pluripotent chromatin is, however, distinguishable by a subset of gene promoters (between 3000 and 4000) (Li, Lian, Dai, Xiang, & Dai, 2013) marked with both active (H3K4me3) and repressive (H3K27me3) histone modifications, placed, respectively, by complexes in the Trithorax and Polycomb (PRC2) groups (Fig. 7). The first evidence of bivalent chromatin marks in murine ES cells was identified using ChIP and tiling oligonucleotide arrays where a large region of H3K27me3 was accompanied by smaller patches of H3K4me3 (Azuara et al., 2006; Bernstein et al., 2006). These bivalent promoters mark lowly expressed genes in undifferentiated cells, and are therefore poised for activation in response to developmental signaling (Azuara et al., 2006; Bernstein et al., 2006; Voigt, Tee, & Reinberg, 2013). Transcription of these genes is repressed, but not blocked: bivalent genes remain open to transcription factors and are therefore poised for upregulation upon differentiation as required by the organism (Voigt et al., 2013). During differentiation, one mark is typically

lost from the promoter while the other becomes enriched depending on whether the gene is expressed or silenced. In ES cells, bivalent chromatin is resolved by SWI/SNF-mediated eviction of PcG proteins (Stanton et al., 2017) and by ASF1A-driven clearing of H3K27me3 (Gao, Gan, Lou, & Zhang, 2018). Eviction of the repressive H3K27me3 mark and the PcG proteins responsible for its placement allows derepression of bivalent lineage specification genes (Stanton et al., 2017). Consistent with their poised state, bivalent genes tend to display low levels of DNA methylation, another repressive epigenetic mark, with the CpG islands of germline and Polycomb genes becoming increasingly methylated as ES cells differentiate and commit to specific lineages (Mohn et al., 2008; Vastenhouw & Schier, 2012). Bivalent genes are largely lineage specification genes (such as members of the SOX, FOX, PAX, IRX, and POU/OCT families; Bernstein et al., 2006) and remain accessible but not expressed in ES cells. Bivalent chromatin marks are largely specific to stem cells (including ES cells, iPSCs, and hematopoietic stem cells Cui et al., 2009; Harikumar & Meshorer, 2015). Bivalent genes have, however, been identified in non-stem cells, including differentiated T cells and some cancer cell types (Bapat et al., 2010; Barski et al., 2007; Lin et al., 2015; McGarvey et al., 2008; Rodriguez et al., 2008; Roh, Cuddapah, Cui, & Zhao, 2006). While bivalent promoters were initially thought to be restricted to developmentally-regulated genes to enable fast transition between active and repressed expression states, it is clear that bivalency is more complex, as bivalent promoters have been identified in different gene families in numerous different cell types. Furthermore, regulation of the bivalent state is carried out by a wide variety of different proteins and regulators that extend well beyond the Trithorax and Polycomb group members that place the epigenetic marks of bivalency. Some important regulators include nucleosome remodeling complexes such as Tip60-p400, esBAF, CHD7, and NuRD (Alajem et al., 2015; Fazzio et al., 2008b; Ho, Jothi, et al., 2009; Lei et al., 2015; Reynolds, Salmon-Divon, et al., 2012; Schnetz et al., 2010). Tip60-p400 significantly co-localizes with H3K4me3, particularly near the TSS, and the complex mostly acts to repress gene expression in ES cells (Fazzio et al., 2008b). Knockdown of Tip60-p400 results in deregulation of 4% of genes, most of which are upregulated; interestingly, many of those found to be upregulated were classical bivalent early-differentiation genes, which are normally silenced in ES cells (Fazzio et al., 2008b). The esBAF subunit BAF60A was mapped by ChIP-seq to reveal a distribution similar to that of H3K27me3 around TSSs, with significant enrichment at promoters of bivalent genes; after BAF60A depletion, these marks were found to be significantly redistributed genome-wide (Alajem et al., 2015). While esBAF tends to function as an activator of gene expression and PRC2 as a repressor, the two function synergistically to properly regulate expression of differentiation-associated genes (Ho et al., 2011). CHD7 associates with a PcG cluster, containing SUZ12, RING1B, and EZH2, suggesting a connection between CHD7 and H3K27me3 (Schnetz et al., 2010). Finally, the NuRD ATPase CHD4 removes acetyl groups from H3K27 in ES cells, thereby allowing the subsequent recruitment of PcG proteins and H3K27me3 (Reynolds, Salmon-Divon, et al., 2012). CHD4 also interacts with the H3K4 demethylase LSD1, which occupies a majority of active genes and approximately two thirds of bivalent genes (Whyte et al., 2012). Nucleosome remodeling factors play critical roles in cell fate decisions and maintenance of stem cell characteristics; while their relationships with bivalency have been discussed, the next section will substantially expand upon these roles as well as other functions of nucleosome remodeling factors in ES cells.

14. Chromatin state is precisely regulated by nucleosome remodeling factors in ES cells

14.1 esBAF maintains stem cell pluripotency by preserving chromatin state

One of the best described ES cell-specific nucleosome remodeling complex is esBAF. esBAF is characterized by the exclusive use of BRG1 as its ATPase, whereas BAF complexes in differentiated cells use a combination of BRG1 and BRM (Ho, Jothi, et al., 2009; Ho, Ronan, et al., 2009). Murine esBAF is also assembled around a BAF155 homodimer, rather than a BAF155/BAF170 heterodimer, and exclusively uses the ARID1A and BAF53A subunits (Mashtalir et al., 2018), rather than other possible subunits (ARID1B, ARID2, BAF53B). Interestingly, human ES cells are reliant on BAF170 for maintenance of pluripotency, as well as BAF155 (Zhang et al., 2014). In addition to the canonical esBAF complex, a non-canonical BRD9-containing esBAF complex has recently been described, as has a BRD7-containing PBAF complex in ES cells (Gatchalian et al., 2018; Kaeser, Aslanian, Dong, Yates, & Emerson, 2008). BAF complexes are crucial for differentiation of ES cell state. The Panning group confirmed the role of esBAF subunits in ES cell pluripotency via an RNAi screen (Fazzio et al., 2008b). The next year, the Paddison group elucidated a mechanism by which BAF is required for ES cell development (Schaniel et al., 2009). During differentiation and upon RNAi-mediated depletion of the core esBAF components BAF57 and BAF155, NANOG was upregulated and heterochromatin formation was stifled, demonstrating the requirement for esBAF in NANOG silencing and appropriate chromatin compaction during differentiation (Schaniel et al., 2009). Functionally, esBAF preserves pluripotency in ES cells largely by facilitating LIF/STAT3 signaling and by coordinating function (both stimulatory and antagonistic) with PcG proteins (Ho et al., 2011). Interestingly, BAF subunits BAF155 and BRG1 are also major players in regulation of X-chromosome inactivation, creating a nucleosome-depleted region at X-located gene promoters that is necessary for gene silencing (Keniry et al., 2019). Upon depletion of esBAF, H2A.Z occupancy is greatly reduced, and subnucleosome formation appears to be more prevalent at sites of H2A.Z occupancy (Hainer & Fazzio, 2015) suggesting that esBAF regulates H2A.Z localization and incorporation in stem cells, though it is unclear precisely how esBAF does so.

Underscoring the importance of esBAF in development, maternal BRG1 is essential for murine zygotic genome activation (in which the maternally contributed RNA and proteins are silenced and the zygote begins transcription of its own genes) by the 2-cell stage (Bouniol, Nguyen, & Debey, 1995); furthermore, zygotic BRG1 is essential for proliferation of both the inner cell mass and the trophectoderm of blastocysts (Bultman et al., 2000). Deletion of the murine homolog of BAF155, SRG3, is lethal peri-implantation, showing severe deficiencies in vascular formation and circulation when inactivated via a transgenic construct (Han et al., 2008). Haploinsufficiency of SNF5/BAF47 predisposes developing mice to malignant rhabdoid tumors, a cancer brought on by biallelic inactivation of BAF47 (Nakayama et al., 2017; Roberts, Galusha, McMenamin, Fletcher, & Orkin, 2000; Wang et al., 2019). Interestingly, *Brm*^{-/-} mice develop normally, highlighting the divergent roles of the two BAF ATPases (Reyes et al., 1998). Similarly to its role with PcG targets, esBAF functions in opposition to MBD3/NuRD complex at shared targets (Yildirim et al., 2011),

suggesting that esBAF is a key regulator of balance between activating and silencing pathways in ES cells. Further highlighting its importance, BRG1 depletion in blastocysts results in reduced expression of OCT4 and NANOG—among other pluripotency-associated genes—while differentiation-associated gene expression rises (Kidder, Palmer, & Knott, 2009). Although we have highlighted specific roles for esBAF as an activator of transcription, it is important to note that this is not an exclusive function; indeed, esBAF suppresses non-coding transcription from over 57,000 nucleosome-depleted regions in ES cells (Hainer et al., 2015), demonstrating a widespread role in transcriptional repression as well.

14.2 CHD proteins are regulators of ES cell pluripotency

Among the most studied nucleosome remodeling factors in ES cells is CHD1. CHD1 uses two N-terminal tandem chromodomains to bind methylated lysine residues in the histone tails and promote nucleosome sliding. CHD1 is essential for maintaining pluripotency in naive stem cells via interaction with the Mediator complex (Gaspar-Maia et al., 2009). Mediator is a large (~30 subunit) coactivator complex that is necessary for RNA Polymerase II transcription and has both permissive and repressive roles in differentiation via interactions with various lineage specification regulators (Yin & Wang, 2014), such as NANOG (Tutter et al., 2009) and SOX9 (Zhou et al., 2002). At gene promoters, Mediator facilitates assembly of the pre-initiation complex and subsequently recruits CHD1 to transcription start sites (Lin et al., 2011). CHD1 features an N-terminal serine-rich region that is not essential for ES cell viability, but is required for pluripotency (Piatti et al., 2015). CHD1 is also essential for establishment of pluripotency during reprogramming from fibroblasts to induced pluripotent stem cells (Gaspar-Maia et al., 2009). Specifically, *Chd1*^{-/-} ES cells cannot give rise to primitive endoderm, but rather differentiate along neural lineages (Gaspar-Maia et al., 2009). CHD1 is enriched at active genes, but specifically depleted at bivalent genes, suggesting a role as an activator of transcription (Gaspar-Maia et al., 2009). Beyond roles that specifically promote pluripotency in ES cells, CHD1 is also important for ES cells to prevent spurious heterochromatin formation over accessible regions of chromatin (Lin et al., 2011). *Chd1*^{-/-} mouse embryos display reduced mRNA and intergenic RNA transcription (Guzman-Ayala et al., 2015), suggesting that the remodeler is a key factor in maintaining ES cells' distinct rapid transcription rate, a property known as "hypertranscription" (Efroni et al., 2008). In addition to facilitating transcription genome-wide, CHD1 has recently been shown to facilitate repair of promoter-proximal DNA double-strand breaks by recruitment of DNA repair proteins (Bulut-Karslioglu et al., 2019). CHD7 has also been implicated in maintenance of pluripotency in ES cells, with a role that may to be similar to that of CHD1 in murine ES cells (Zentner, Tesar, & Scacheri, 2011). CHD7, however, associates with three distinct classes of protein clusters: one associated with enhancers (including CHD7, p400, OCT4, SIX2, NANOG, SMAD1, and STAT3), another with c-MYC and n-MYC-regulated genes, and a third associated with PcG (Schnetz et al., 2010). In addition, CHD7 regulates neural crest formation from multipotent cells through association with the PBAF complex (Bajpai et al., 2010). Therefore, CHD7 regulates the expression of some ES cell-specific genes, possibly through interaction with other regulators of ES cell gene expression.

14.3 MBD3/NuRD generally represses expression of differentiation genes

In an RNAi screen to identify chromatin regulators important for murine ES cell self-renewal, the Panning group identified MBD3 as important for maintenance of the ES cell state (Fazzio et al., 2008b). ES cells derived from *Mbd3*^{-/-} mouse embryos are capable of self-renewal in culture in the absence of LIF and are unable to differentiate properly, with differentiation skewed toward the trophectoderm lineage (Kaji et al., 2006; Kaji, Nichols, & Hendrich, 2007; Zhu, Fang, Li, & Zhang, 2009). MBD3 is essential for MBD3/NuRD complex assembly (Kaji et al., 2006). MBD3/NuRD target genes exhibit increased H3K27ac and decreased H3K27me3 in *Mbd3*^{-/-} ES cells (Reynolds, Latos, et al., 2012; Reynolds, Salmon-Divon, et al., 2012), suggesting that MBD3 regulates pluripotency genes through deacetylation and recruitment of PRC2. MBD3/NuRD also regulates nucleosome positioning across enhancer and promoter regions to control factor access to enhancers during the lineage commitment process, as well as prevent coactivators (e.g., Mediator) and RNA Polymerase II from functioning at these regions (Bornelov et al., 2018). Furthermore, depletion of the MBD3/NuRD member MTA proteins leads to improper expression of differentiation-associated genes, resulting in inability to contribute to embryogenesis (Burgold et al., 2019). MBD3/NuRD is necessary for deacetylation of H3K27, although MBD3/NuRD does not appear to act at bivalent genes, but rather is repelled from chromatin binding by H3K27me3 (Harikumar & Meshorer, 2015; Kaji et al., 2006). MBD3 is also essential for pluripotency—though not viability—of ES cells; specifically, MBD3-deficient cells fail to properly silence lineage specification genes (Kaji et al., 2006). In ES cells, depletion of the MBD3/NuRD ATPase CHD4 leads to loss of self-renewal, decreased proliferation, and increased embryoid body differentiation (Zhao, Han, et al., 2017). In addition, the MBD3/NuRD histone deacetylase HDAC1 binds promoters of pluripotency genes; cells treated with an HDAC inhibitor result in a differentiation phenotype (Kidder & Palmer, 2012). Furthermore, MBD3C—a stem cell-specific isoform—interacts with the MLL1 H3K4 methyltransferase complex component WDR5, an interaction that is unique to stem cells (Ee et al., 2017). It remains to be determined whether this interaction or an alternative function for MBD3C contributes to ES cell pluripotency or self-renewal.

14.4 The ISWI remodeler ATPase SNF2H is essential during development

Deletion of the ISWI ATPase SNF2H is embryonic lethal in mice, suggesting high functional importance in ES cells (Saladi & de la Serna, 2010). Specifically, SNF2H deletion is lethal pre-implantation due to cell growth defects of blastocyst embryos (Stopka & Skoultschi, 2003). It is possible, however, to generate ES cells with a SNF2H functional knockout caused by a frameshift in exon 6 (Barisic, Stadler, Iurlaro, & Schübeler, 2019). Although yeast ISW1 has been known to regulate nucleosome spacing, this functional knockout enabled *in vivo* confirmation of this function in mammalian cells (Barisic et al., 2019). Complicating the issue of SNF2H requirement is the extreme variability of SNF2H-containing complexes. Because SNF2H is incorporated into the RSF, WICH, NoRC, CHRAC, and ACF complexes, it is difficult to identify the precise roles for which ISWI complexes are essential in ES cells. While the alternative ISWI ATPase, SNF2L, is not embryonic lethal in mice, depletion of the ISWI family NURF complex member BPTF disrupts expression of lineage specification genes across all three germ layers, particularly those regulated by SMAD, suggesting a link between BPTF and SMAD signaling in

regulation of lineage-specific genes (Landry et al., 2008). Interestingly, depletion of H3K4me3 leads to eviction of BPTF from chromatin, defects in recruitment of SNF2L, and compromised spatial regulation of *HOX* gene expression (Wysocka et al., 2006). One function of SNF2H in cells that contributes to its essential nature is recruitment to DNA double-strand break sites. The deacetylase SIRT6 is rapidly recruited to double-strand breaks and subsequently recruits SNF2H, which then facilitates open chromatin at the break sites (Kokavec et al., 2017). Both SIRT6 and SNF2H are required for recruitment of other repair factors, including BRCA1, 53BP1, and RPA, suggesting a role for ISWI complexes in preventing genome instability (Kokavec et al., 2017). In hematopoietic stem and progenitor cells, SNF2H is necessary to promote maturation into erythroid and myeloid lineages, as well as to allow proliferation of committed erythroid cell populations (Kokavec et al., 2017). SNF2H and SNF2L have been shown to position nucleosomes adjacent to CTCF and other transcription factor binding sites (Wiechens et al., 2016); indeed, SNF2H/L seem to regulate a specific group of transcription factors including CTCF, whereas BAF regulates distinct factors such as OCT4, SOX2, NANOG, and REST (Barisic et al., 2019). Selective reliance of CTCF on SNF2H suggests that higher-order chromatin domains and topologically-associated domains (TADs) may depend upon ISWI activity but not on BRG1.

14.5 INO80 remodelers repress transcription of differentiation-associated genes

Despite roles that are often associated with active transcription—like the histone acetyltransferase activity of Tip60-p400 and deposition of H2A.Z and H3.3—INO80-family complexes fulfill important transcriptional repression roles in ES cells (Cai et al., 2005; Doyon et al., 2004; Pradhan et al., 2016; Ruhl et al., 2006; Squatrito, Gorrini, & Amati, 2006). As previously noted, the INO80 superfamily member Tip60-p400 is essential for normal self-renewal in ES cells, and *Tip60* knockout is lethal at the blastocyst stage (Fazzio et al., 2008a; Hu et al., 2009). Tip60-p400 maintains pluripotency in ES cells by repressing differentiation-associated genes, despite its role as a HAT, which has traditionally been associated with activation of transcription (Fazzio et al., 2008a). This may be due to a unique interaction of Tip60-p400 with the traditionally cytosolic HDAC6, which shows nuclear localization in ES cells and promotes recruitment of Tip60-p400 to target genes, especially differentiation-associated genes that are normally repressed by Tip60-p400 (Chen, Hung, et al., 2013). While HDAC6 requires its deacetylase domains to silence differentiation genes, it does not regulate gene expression by deacetylating histones near Tip60-p400 target promoters; rather, the catalytic domains of HDAC6 are required for its interaction with Tip60-p400 (Chen, Hung, et al., 2013). Interestingly, Tip60 shows acetyltransferase-dependent activity in differentiation but acetyltransferase-independent activity in promoting self-renewal of ES cells (Acharya et al., 2017). Unlike *Tip60*^{-/-} mice, acetyltransferase-deficient mice do not display compromised self-renewal and are able to proceed past the blastocyst stage (Acharya et al., 2017; Hu et al., 2009).

Comparatively little is known about SRCAP, including its role(s) in pluripotent cells. SRCAP remodels nucleosomes primarily through incorporation of the variant H2A.Z (Ruhl et al., 2006; Wong et al., 2007). SRCAP is recruited to sites of H2A.Z deposition along with Tip60-p400 via their shared subunit, GAS41, a reader of acetylated lysines (Hsu et al., 2018). Interestingly, PCI domain-containing protein 2 (PCID2), a highly expressed protein

in multipotent hematopoietic progenitor cells, impedes SRCAP remodeling activity by blocking deposition of H2A.Z and recruitment of transcription factor PU.1 to lymphoid fate regulator genes via an interaction with the zinc finger HIT-type containing 1 (ZNHIT1) protein (Ye et al., 2017). Additionally, SRCAP assembly and ATPase activity is promoted by the transcription factor ZBTB3; depletion of ZBTB3 disrupts ES cell self-renewal and viability. Activity of ZBTB3 is itself activated by the lncRNA LncKdm2b by promoting the assembly of the SRCAP complex in *trans* (Ye et al., 2018). SRCAP therefore fulfills the INO80 paradigm of seemingly disparate roles in stem cells through its role in specifying lymphoid fate as well as its role in preserving stem cell pluripotency.

The INO80 complex itself largely fulfills an activating role with respect to pluripotency genes in stem cells—including *Oct4*, *Nanog*, *Sox2*, *Klf4*, and *Esrrb* (Wang et al., 2014)—by promoting recruitment of Mediator and RNA Polymerase II to these genes.

14.6 Polycomb group proteins silence developmental genes in ES cells

Polycomb group (PcG) complexes mediate H3K27 methylation and are typically associated with gene repression (Di Croce & Helin, 2013). There are two broad classes of PcG complexes, PRC1 and PRC2, each of which includes numerous subcomplexes. PRC1 includes six major complexes, each defined by a Polycomb Group Ring Finger (PCGF) subunit, PCGF1–6. In addition to the PCGF subunit, PRC1 complexes include the RING1/2 E3 ubiquitin ligase, RVBP/YAF2 or a chromobox (CBX) protein, and a unique set of associated proteins (Gao et al., 2012). The canonical PRC1 complexes are PRC1.2 (containing PCGF2) and PRC1.4 (containing PCGF4/BMI1); these complexes are recruited to chromatin by the H3K27me3 mark deposited by PRC2 (Gao et al., 2012). In ES cells, canonical PRC1 functions to maintain pluripotency in a CBX7-dependent manner, whereas CBX2 and CBX4 become more abundant in PRC1 complexes upon ES cell differentiation into embryoid bodies (Morey et al., 2012). This switch from CBX7 to CBX2 and CBX4 is regulated by the miR-125 and miR-181 microRNAs (O’Loghlen et al., 2012). The non-canonical PRC1 complexes (PRC1.1, PRC1.3, PRC1.5, and PRC1.6) are recruited to chromatin through H3K27me3-independent mechanisms and catalyze ubiquitylation of H2A at lysine 119 (H2AK119), a mark that is not necessary for PRC1 target binding but is necessary for efficient repression of genes (specifically drivers of cellular differentiation) in ES cells (Endoh et al., 2012; Tavares et al., 2012). PCGF1 is somewhat distinct in that it functions in gene activation during ES cell lineage specification through positive regulation of endoderm- and mesoderm-associated transcription factor expression (Yan et al., 2017). PRC1.3 and PRC1.5 mainly function to activate transcription in ES cells via an interaction with the TEX10 pluripotency factor (Zhao, Huang, et al., 2017). PCGF6 is necessary for proper self-renewal and differentiation, likely through PRC1.6’s role in recruiting RING1B and mediating H2AK119ub (Endoh et al., 2017; Zhao, Tong, et al., 2017).

PRC2, meanwhile, includes the PRC2.1 and PRC2.2 complexes, which are recruited by distinct mechanisms and have divergent gene silencing functions (Hauri et al., 2016; Jones & Wang, 2010; van Mierlo, Veenstra, Vermeulen, & Marks, 2019). The accepted model for PcG activity is that PRC2 methylates H3K27—a process that then creates PRC1 binding sites and enables subsequent H2AK119ub, a mark recognized by the CBX proteins of the

PRC1 complex (Endoh et al., 2012; Eskeland et al., 2010). PRC1 can also compact chromatin independently of its ubiquitylation activity (Buchwald et al., 2006; Eskeland et al., 2010). Specific to ES cells, PcG proteins are known to directly repress hundreds of development-associated genes (Aloia, Di Stefano, & Di Croce, 2013; Boyer et al., 2006; Lee et al., 2006). H3K27me3 can be disrupted at various cell stages using direct inhibition of PRC2 function via disruption of the EZH2/EED interaction, a novel technique that was used to show that PRC2 is required for self-renewal at all but the earliest stage of human ES cell development (Moody et al., 2017). Upon differentiation, promoters that are marked by H3K27me3 in pluripotent cells often become DNA methylated, and the H3K27-methylated gene landscape changes dramatically—including loss of bivalency (Mohn et al., 2008). In human ES cells, PcG knockouts cause pluripotency loss and subsequent mesoendoderm fate specification, as well as a failure to differentiate into ectoderm lineages upon EZH1 and EZH2 loss (Shan et al., 2017). In murine ES cells, PcG deficiency causes loss of BMP4 but not pluripotency loss; however, when these cells are converted to a primed state, they undergo similar spontaneous differentiation to PcG-deficient human ES cells (Shan et al., 2017). Interestingly, human ES cells appear more dependent on EZH2 than murine ES cells, as EZH2 deficiency confers a more severe self-renewal and proliferation defect in human ES cells than mouse (Collinson et al., 2016). In ES cells, H3K27me3 deposition and subsequent gene silencing is also regulated by H2A.Z and H3.3 deposition; however, the mechanism by which this silencing occurs has yet to be elucidated (Wang et al., 2018).

Increased DNA methylation at H3K27me3 sites suggests some level of crosstalk between PcG-mediated silencing and DNA methylation. PcG binding and subsequent gene silencing also appears to be antagonistic to esBAF-mediated gene activation, particularly at LIF signaling targets that are dependent on BRG1 to facilitate establishment of STAT3 binding sites (Ho, Jothi, et al., 2009; Ho et al., 2011). It is likely that this STAT3 binding site establishment (and perhaps that of other pluripotency factors) is enabled by binding of BRG1 at the two nucleosomes flanking relatively long nucleosome-depleted promoter regions in ES cells (de Dieuleveult et al., 2016). Although STAT3 binding appears more reliant on BRG1 than vice versa, STAT3 and BRG1 recruitment are mutually dependent, and both LIF and BRG1 must be expressed at sufficient levels to enable proper expression and function of transcription factors that regulate self-renewal, such as TBX3, TFCP2L1, ESRRB, SOCS3, and TCL1 (Ho et al., 2011). Importantly, however, PcG and esBAF do not exist in a simple antagonistic relationship; esBAF also promotes PcG-mediated silencing at *Hox* loci, working with PcG to facilitate their repression in ES cells (Ho et al., 2011). In summary, PcG proteins coordinate gene silencing for hundreds of differentiation-associated genes, working with and against other chromatin modifying factors to facilitate maintenance of pluripotency in ES cells and proper lineage differentiation as is required by the maturing organism.

14.7 Variants of H2A and H3 have specialized roles in pluripotent cells

Along with nucleosome remodeling factors like esBAF, the histone variant H2A.Z also functions at PcG target genes; H2A.Z is enriched in ES cells at PcG targets and is necessary for the lineage commitment process (Creyghton et al., 2008). Furthermore, H2A cannot compensate for H2A.Z loss during early development (Creyghton et al., 2008; Faast et al.,

2001; Hu et al., 2013). PRC1 and PRC2 are not, however, required for targeting of H2A.Z to developmental genes in ES cells (Illingworth, Botting, Grimes, Bickmore, & Eskeland, 2012). Like the H3 variant H3.3, H2A.Z can be found at regulatory regions of the ES cell genome, such as enhancers (Hu et al., 2013). H2A.Z regulates chromatin to facilitate access of both active and repressive factors, including OCT4, MLL complexes, and PRC2 (Hu et al., 2013). H2A.Z also promotes ES cell differentiation by regulation of nucleosomes themselves (Li et al., 2012) and of epigenetic histone marks, including the bivalent chromatin mark H3K27me3 (Surface et al., 2016; Wang et al., 2018). H2A.Z can be ubiquitylated, and without this mark, murine ES cells undergo faulty lineage commitment (Surface et al., 2016). Prior work has suggested that PRC1-mediated ubiquitylation of H2A is important for silencing bivalent genes in murine ES cells (de Napoles et al., 2004; Endoh et al., 2012). H2A.Z contains an acidic patch domain distinct from that of core H2A that is also necessary for regulation of lineage commitment by interplay between transcription and chromatin dynamics (Subramanian et al., 2013).

The histone H3 variant H3.3 marks active promoter regions and other regulatory regions genome-wide in ES cells, including super-enhancers (Deaton et al., 2016; Goldberg et al., 2010). In addition to H3.3's presence at enhancer loci, H3.3 can be phosphorylated at S31 to promote enhancer acetylation (Martire et al., 2019). H3.3-containing nucleosomes are interaction hotspots for pluripotency factors, nucleosome remodelers, and other factors that may help to establish and preserve pluripotency in stem cells. Binding sites for ES cell-specific pluripotency factors have been identified at regions of rapid H3.3-containing nucleosome turnover, suggesting that H3.3 may facilitate binding of pluripotency factors in ES cells (Ha, Kraushaar, & Zhao, 2014). Furthermore, the -1 nucleosome of expressed genes in ES cells contains the H3.3 variant; upon differentiation, an H3.3-containing nucleosome can be found shifted into the +1 position, possibly to regulate gene expression (Schlesinger et al., 2017). In addition to interactions with pluripotency factors, H3.3 facilitates silencing of developmental promoters via PRC2 recruitment and subsequent H3K27me3 deposition (Banaszynski et al., 2013). While HIRA and H3.3 are required for H3K27me3 establishment at developmental gene promoters in ES cells, H3K4me3 was largely maintained upon H3.3 depletion (Banaszynski et al., 2013). By forcing H3.1 expression and knocking down H3.3 expression in ES cells, however, myogenic differentiation is impaired, and both H3K4me3 and H3K27me3 are diminished (Harada et al., 2015). It therefore appears that H3.3 deposition and/or placement is essential for the presence of both bivalent promoter marks, and that H3.3 may play a greater role in regulation of bivalent promoters than has previously been known. Further, H3.3 is incorporated by ATRX/DAXX to facilitate silencing of repetitive elements via the KAP1 co-repressor, and this incorporation appears to function upstream of H3K9me3 and heterochromatin formation at these regions (Elsässer, Noh, Diaz, Allis, & Banaszynski, 2015).

Several developmental roles for H3.3 in ES cells have been suggested. Without H3.3, mice show near-complete embryonic lethality by E6.5, and those mice that survived displayed early (E8.5) expression of *Brachyury*, a mesoderm marker, and more closely resembled E7.5 embryos (Jang, Shibata, Starmer, Yee, & Magnuson, 2015). Similarly, the Jiao group demonstrated a requirement for H3.3 in neural stem cells to properly proliferate and

differentiate—a requirement that could be overcome by overexpression of H3.3, MOF, and GLI1, but not H3.1 or an H3.3 mutant with K36 mutated to arginine (Xia & Jiao, 2017).

15. Long-range chromatin interactions are critical for regulation of pluripotency

While ES cells precisely regulate chromatin structure, accessibility, and transcription at individual gene loci, long-range chromatin interactions include physical contacts made between enhancers, promoters, insulators, gene clusters, and other features that regulate gene expression beyond the sequence, transcription factor, and individual nucleosome levels. These interactions are mappable genome-wide by Hi-C, ChIA-PET, GAM, and OligoPAINT, among more techniques (Beagrie et al., 2017; Beliveau et al., 2012; Dekker, 2016; Li et al., 2010; Lieberman-Aiden et al., 2009). CCCTC-binding factor (CTCF) facilitates looping interactions within chromosomes, and it is canonically referred to as an insulator protein; however, CTCF has also been implicated in transcriptional activation (Vostrov & Quitschke, 1997; Vostrov, Taheny, & Quitschke, 2002) and splicing (Shukla et al., 2011). CTCF works with cohesin and Mediator complexes to mediate loop formation and establish the 3D genomic landscape, consisting of domains of decreasing size known as chromosomal territories, compartments, topologically-associating domains (TADs), and subTADs (Dekker & Misteli, 2015). TADs feature high interaction frequencies within domains, but low interaction frequencies across domains (Cavalli & Misteli, 2013; Dekker & Misteli, 2015; Dixon et al., 2012; Nora et al., 2012) (Fig. 8). The expression of genes within a single TAD are correlated during differentiation (Nora et al., 2012), but TAD formation, regulation, and function are largely unknown processes at present.

Long-range chromatin interactions are key regulatory features of ES cell chromatin (Dixon et al., 2015, 2012). At the *Nanog* gene locus, for example, there are numerous DNase I hypersensitivity sites (DHSs) spread across a 160kb region between *Nanog* and the next gene, *Dppa3* (Levasseur, Wang, Dorschner, Stamatoyannopoulos, & Orkin, 2008). When the pluripotency factors OCT4, NANOG, ZFP281, and NAC1 bind at these DHSs, they alter higher-order chromatin structure to loop out the extragenic region and bring the *Nanog*, *Dppa3*, and *Gdf3* promoters into close proximity; furthermore, upon depletion of OCT4, this looping event collapses, and ES cells fail to maintain pluripotency (Keenen & de la Serna, 2009; Levasseur et al., 2008). This long-range interaction highlights another key aspect of pluripotency factors: they are often involved in complex autoregulatory circuits that can interfere with attempts to understand the actions of individual pluripotency factors.

Among the most prominent of long-range chromatin interactions in ES cells are those of clusters of enhancers that function together, known as super-enhancers (also referred to as stretch enhancers) (Parker et al., 2013; Peng & Zhang, 2018). Super-enhancers are prevalent in ES cells, and are reorganized upon exit from a pluripotent state to facilitate cellular differentiation and specification, as shown through promoter capture experiments (Novo et al., 2018). Super-enhancers tend to regulate sets of genes that control cell identity and fate, and they are occupied by between 12% and 36% of enhancer-associated RNA Polymerase II and cofactors, despite making up less than 3% of total identified enhancers in ES cells; this

heightened association likely explains super-enhancers high RNA abundance (Hnisz et al., 2013; Whyte et al., 2013). More specifically, super-enhancers in ES cells are highly occupied by the master regulators of pluripotency, OCT4, SOX2, and NANOG, as well as Mediator and ASH2L, an MLL complex subunit that recruits the master regulators to super-enhancer loci and forms a complex with OCT4 to regulate pluripotency genes (Tsai et al., 2019; Whyte et al., 2013). In addition, TEX10 is a pluripotency factor that is enriched at super-enhancers, localizing to them in a SOX2-dependent manner to regulate histone acetylation and DNA demethylation at super-enhancers (Ding et al., 2015). Interestingly, the master regulators of pluripotency are themselves regulated by super-enhancers, highlighting the complex autoregulatory circuits involved in maintaining pluripotency in stem cells (Blinka, Reimer, Pulakanti, & Rao, 2016; Li et al., 2014; Whyte et al., 2013; Zhou et al., 2014). Indeed, deletion of a super-enhancer can alter transcription of genes that are not currently assigned to that super-enhancer (Moorthy et al., 2017), suggesting that super-enhancers may regulate multiple promoters and function as operators within larger regulatory pathways than have currently been identified.

Long-range chromatin interactions are not unique to ES cells; however, they are specific, critical, and highly regulated. Over the course of cellular differentiation, over one third of long-range chromatin interactions change, including changes to both active and inactive compartments throughout the genome (Dixon et al., 2015). These altered long-range chromatin interactions serve to modify expression of developmental genes (both positively and negatively), to silence pluripotency factors, and to generally facilitate differentiation into correct lineages. Although the 3D interactome remains incompletely characterized, it is clear that ES cells undergo drastic changes to long-range chromatin interactions over the course of their differentiation; as such, 3D chromatin interactions represent a diverse and understudied source of gene regulation.

16. ES cells regulate chromatin by common processes to preserve pluripotency

In this chapter, we have discussed the importance of genetic and epigenetic regulators that govern lineage fidelity and permit lineage specification. Specifically, ES cells carefully regulate the expression of developmental and pluripotency genes through unique mechanisms, including bivalent histone modifications over the promoters of developmental genes, highly accessible chromatin structure that is permissive of enhanced transcription, prevalent use of enhancers and super-enhancers largely regulated by pluripotency factors OCT4, SOX2, and NANOG, and unique application of chromatin machinery used in somatic cells (including nucleosome remodelers esBAF, Tip60-p400, and CHD1) and histone variants (including H3.3 and H2A.Z). Despite the vast array of unique and specialized tools utilized to regulate ES cell chromatin, at their core these processes reflect the mechanisms used to regulate chromatin in cells in the adult organism. The effectors and pathways are unique; however, as ES cells must precisely regulate their chromatin architecture to reflect the dynamic nature of this cell type. While chromatin regulatory processes are as critical within all cell types, their function within ES cells is precisely guided to ensure proper development. In recent years, our understanding of mechanisms that govern proliferation of

ES cells, and those leading to lineage commitment, has increased. Further characterization of the mechanisms discussed throughout this chapter will continue to help identify effective methods for reprogramming differentiated cells, thereby facilitating the development of stem cell based therapies. Additionally, cells in various disease states take on chromatin characteristics reminiscent of those in ES cells, including hijacking of chromatin machinery, as in MYC-driven “transcription addiction,” acquisition of novel super-enhancers to regulate oncogenes, and transition to an un- or less-differentiated state (Hnisz et al., 2013; Jordan, 2007; Loven et al., 2013). In sum, stem cells represent a unique application of fundamental biological processes to maintain a delicate balance and facilitate a rapid shift between gene repression and activation in the pluripotent state and along the pathway of lineage specification.

Acknowledgments

We thank members of the Hainer lab for critical reading of this chapter. This work was supported by a Charles E. Kaufman Foundation New Investigator Award and National Institutes of Health grant 1R35GM133732-01 to S.J.H.

References

- Acharya D, Hainer SJ, Yoon Y, Wang F, Bach I, Rivera-Perez JA, et al. (2017). *Cell Reports*, 19, 671–679. [PubMed: 28445719]
- Adam S, Polo SE, & Almouzni G (2013). *Cell*, 155, 94–106. [PubMed: 24074863]
- Adkins MW, & Tyler JK (2006). *Molecular Cell*, 21, 405–416. [PubMed: 16455495]
- Ahmad K, & Henikoff S (2002). *Molecular Cell*, 9, 1191–1200. [PubMed: 12086617]
- Alajem A, Biran A, Harikumar A, Sailaja BS, Aaronson Y, Livyatan I, et al. (2015). *Cell Reports*, 10, 2019–2031. [PubMed: 25818293]
- Albert I, Mavrich TN, Tomsho LP, Qi J, Zanton SJ, Schuster SC, et al. (2007). *Nature*, 446, 572–576. [PubMed: 17392789]
- Alén C, Kent NA, Jones HS, O’Sullivan J, Aranda A, & Proudfoot NJ (2002). *Molecular Cell*, 10, 1441–1452. [PubMed: 12504018]
- Alfert A, Moreno N, & Kerl K (2019). *Epigenetics & Chromatin*, 12, 19. [PubMed: 30898143]
- Allfrey V, Faulkner R, & Mirsky A (1964). *Proceedings of the National Academy of Sciences of the United States of America*, 51, 8.
- Aloia L, Di Stefano B, & Di Croce L (2013). *Development*, 140, 2525–2534. [PubMed: 23715546]
- Alps A, & Dykhuizen EC (2018). *The Journal of Biological Chemistry*, 293, 3892–3903. [PubMed: 29374058]
- Andersen EC, Lu X, & Horvitz HR (2006). *Development*, 133, 2695–2704. [PubMed: 16774993]
- Anderson CJ, Baird MR, Hsu A, Barbour EH, Koyama Y, Borgnia MJ, et al. (2019). *Cell Reports*, 26, 1681–1690.e5. [PubMed: 30759380]
- Anderson JD, & Widom J (2000). *Journal of Molecular Biology*, 296, 979–987. [PubMed: 10686097]
- Ang YS, Tsai SY, Lee DF, Monk J, Su J, Ratnakumar K, et al. (2011). *Cell*, 145, 183–197. [PubMed: 21477851]
- Angelov D, Molla A, Perche P-Y, Hans F, Côté J, Khochbin S, et al. (2003). *Molecular Cell*, 11, 1033–1041. [PubMed: 12718888]
- Apostolou E, & Hochedlinger K (2013). *Nature*, 502, 462–471. [PubMed: 24153299]
- Arents G, & Moudrianakis EN (1995). *Proceedings of the National Academy of Sciences of the United States of America*, 92, 11170–11174.
- Arimura Y, Kimura H, Oda T, Sato K, Osakabe A, Tachiwana H, et al. (2015). *Scientific Reports*, 5, 9628. [PubMed: 26166249]
- Aul RB, & Oko RJ (2001). *Developmental Biology*, 239, 376–387. [PubMed: 11784042]

- Avvakumov N, Nourani A, & Cote J (2011). *Molecular Cell*, 41, 502–514. [PubMed: 21362547]
- Aydin ÖZ, Vermeulen W, & Lans H (2014). *Cell Cycle* (Georgetown, Texas), 13, 3016–3025.
- Azuara V, Perry P, Sauer S, Spivakov M, Jorgensen HF, John RM, et al. (2006). *Nature Cell Biology*, 8, 532–538. [PubMed: 16570078]
- Bai L, & Morozov AV (2010). *Trends in Genetics*, 26, 476–483. [PubMed: 20832136]
- Bajpai R, Chen DA, Rada-Iglesias A, Zhang J, Xiong Y, Helms J, et al. (2010). *Nature*, 463, 958–962. [PubMed: 20130577]
- Banaszynski LA, Wen DC, Dewell S, Whitcomb SJ, Lin MY, Diaz N, et al. (2013). *Cell*, 155, 107–120. [PubMed: 24074864]
- Bannister AJ, & Kouzarides T (2011). *Cell Research*, 21, 381–395. [PubMed: 21321607]
- Bao X, Rubin AJ, Qu K, Zhang J, Giresi PG, Chang HY, et al. (2015). *Genome Biology*, 16, 284. [PubMed: 26683334]
- Bapat SA, Jin V, Berry N, Balch C, Sharma N, Kurrey N, et al. (2010). *Epigenetics*, 5, 716–729. [PubMed: 20676026]
- Barbera AJ, Chodaparambil JV, Kelley-Clarke B, Joukov V, Walter JC, Luger K, et al. (2006). *Science*, 311, 856–861. [PubMed: 16469929]
- Barisic D, Stadler MB, Iurlaro M, & Schübeler D (2019). *Nature*, 569, 136–140. [PubMed: 30996347]
- Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, et al. (2007). *Cell*, 129, 823–837. [PubMed: 17512414]
- Beagrie RA, Scialdone A, Schueler M, Kraemer DC, Chotalia M, Xie SQ, et al. (2017). *Nature*, 543, 519–524. [PubMed: 28273065]
- Becker PB, & Workman JL (2013). *Cold Spring Harbor Perspectives in Biology*, 5, a017905. [PubMed: 24003213]
- Beliveau BJ, Joyce EF, Apostolopoulos N, Yilmaz F, Fonseka CY, McCole RB, et al. (2012). *Proceedings of the National Academy of Sciences of the United States of America*, 109, 21301–21306.
- Belotserkovskaya R, Oh S, Bondarenko VA, Orphanides G, Studitsky VM, & Reinberg D (2003). *Science*, 301, 1090–1093. [PubMed: 12934006]
- Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, et al. (2006). *Cell*, 125, 315–326. [PubMed: 16630819]
- Blackledge NP, Farcas AM, Kondo T, King HW, McGouran JF, Hanssen LL, et al. (2014). *Cell*, 157, 1445–1459. [PubMed: 24856970]
- Blinka S, Reimer MH Jr., Pulakanti K, & Rao S (2016). *Cell Reports*, 17, 19–28. [PubMed: 27681417]
- Boeger H, Griesenbeck J, Strattan JS, & Kornberg RD (2004). *Molecular Cell*, 14, 667–673. [PubMed: 15175161]
- Bornelov S, Reynolds N, Xenophontos M, Gharbi S, Johnstone E, Floyd R, et al. (2018). *Molecular Cell*, 71, 56–72.e4. [PubMed: 30008319]
- Bouniol C, Nguyen E, & Debey P (1995). *Experimental Cell Research*, 218, 57–62. [PubMed: 7537698]
- Bowman A, Ward R, Wiechens N, Singh V, El-Mkami H, Norman DG, et al. (2011). *Molecular Cell*, 41, 398–408. [PubMed: 21329878]
- Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, et al. (2006). *Nature*, 441, 349–353. [PubMed: 16625203]
- Bradbury EM (1989). *Journal of Molecular Recognition*, 2 i–i.
- Brahma S, Udugama MI, Kim J, Hada A, Bhardwaj SK, Hailu SG, et al. (2017). *Nature Communications*, 8, 15616.
- Buchwald G, van der Stoop P, Weichenrieder O, Perrakis A, van Lohuizen M, & Sixma TK (2006). *The EMBO Journal*, 25, 2465–2474. [PubMed: 16710298]
- Bultman S, Gebuhr T, Yee D, La Mantia C, Nicholson J, Gilliam A, et al. (2000). *Molecular Cell*, 6, 1287–1295. [PubMed: 11163203]
- Bulut-Karslioglu A, Jin H, Guzman-Ayala M, Williamson AJK, Hejna M, Whetton AD, et al. (2019). *BioRxiv*.

- Burgold T, Barber M, Kloet S, Cramard J, Gharbi S, Floyd R, et al. (2019). *The EMBO Journal*, 38, e100788. [PubMed: 31036553]
- Buscarlet M, Krasteva V, Ho L, Simon C, Hébert J, Wilhelm B, et al. (2014). *Blood*, 123, 1720–1728. [PubMed: 24478402]
- Buschbeck M, & Hake SB (2017). *Nature Reviews Molecular Cell Biology*, 18, 299–314. [PubMed: 28144029]
- Cai Y, Jin JJ, Florens L, Swanson SK, Kusch T, Li B, et al. (2005). *Journal of Biological Chemistry*, 280, 13665–13670. [PubMed: 15647280]
- Cairns BR (2005). *Current Opinion in Genetics & Development*, 15, 185–190. [PubMed: 15797201]
- Cakiroglu A, Clapier CR, Ehrensberger AH, Darbo E, Cairns BR, Luscombe NM, et al. (2019). *Genome Research*, 29, 988–998. [PubMed: 31097474]
- Cao R, Tsukada Y, & Zhang Y (2005). *Molecular Cell*, 20, 845–854. [PubMed: 16359901]
- Carrozza MJ, Li B, Florens L, Suganuma T, Swanson SK, Lee KK, et al. (2005). *Cell*, 123, 581–592. [PubMed: 16286007]
- Cavalli G, & Misteli T (2013). *Nature Structural & Molecular Biology*, 20, 290–299.
- Chakravarthy S, Gundimella SK, Caron C, Perche PY, Pehrson JR, Khochbin S, et al. (2005). *Molecular and Cellular Biology*, 25, 7616–7624. [PubMed: 16107708]
- Chatterjee N, Sinha D, Lemma-Dechassa M, Tan S, Shogren-Knaak MA, & Bartholomew B (2011). *Nucleic Acids Research*, 39, 8378–8391. [PubMed: 21749977]
- Chen PB, Hung JH, Hickman TL, Coles AH, Carey JF, Weng Z, et al. (2013). *eLife*, 2, e01557. [PubMed: 24302573]
- Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, et al. (2008). *Cell*, 133, 1106–1117. [PubMed: 18555785]
- Chen P, Zhao J, Wang Y, Wang M, Long H, Liang D, et al. (2013). *Genes & Development*, 27, 2109–2124. [PubMed: 24065740]
- Cheung V, Chua G, Batada NN, Landry CR, Michnick SW, Hughes TR, et al. (2008). *PLoS Biology*, 6, e277. [PubMed: 18998772]
- Chittori S, Hong J, Saunders H, Feng H, Ghirlando R, Kelly AE, et al. (2018). *Science*, 359, 339–343. [PubMed: 29269420]
- Churchman L, & Weissman J (2011). *Nature*, 469, 368–373. [PubMed: 21248844]
- Ciccone DN, Su H, Hevi S, Gay F, Lei H, Bajko J, et al. (2009). *Nature*, 461, 415–418. [PubMed: 19727073]
- Cirillo LA, McPherson CE, Bossard P, Stevens K, Cherian S, Shim EY, et al. (1998). *The EMBO Journal*, 17, 244–254. [PubMed: 9427758]
- Clapier CR, & Cairns BR (2009). *Annual Review of Biochemistry*, 78, 273–304.
- Clapier CR, & Cairns BR (2012). *Nature*, 492, 280–284. [PubMed: 23143334]
- Clapier CR, Iwasa J, Cairns BR, & Peterson CL (2017). *Nature Reviews. Molecular Cell Biology*, 18, 407–422. [PubMed: 28512350]
- Clemot M, Molla-Herman A, Mathieu J, Huynh JR, & Dostatni N (2018). *Development*, 145, 1–13.
- Close P, Hawkes N, Cornez I, Creppe C, Lambert CA, Rogister B, et al. (2006). *Molecular Cell*, 22, 521–531. [PubMed: 16713582]
- Collinson A, Collier AJ, Morgan NP, Sienerth AR, Chandra T, Andrews S, et al. (2016). *Cell Reports*, 17, 2700–2714. [PubMed: 27926872]
- Creyghton MP, Markoulaki S, Levine SS, Hanna J, Lodato MA, Sha K, et al. (2008). *Cell*, 135, 649–661. [PubMed: 18992931]
- Cucinotta CE, Hildreth AE, McShane BM, Shirra MK, & Arndt KM (2019). *Nucleic Acids Research*, 47, 8410–8423. [PubMed: 31226204]
- Cui K, Zang C, Roh T-Y, Schones DE, Childs RW, Peng W, et al. (2009). *Cell Stem Cell*, 4, 80–93. [PubMed: 19128795]
- Dann GP, Liszczak GP, Bagert JD, Muller MM, Nguyen UTT, Wojcik F, et al. (2017). *Nature*, 548, 607–611. [PubMed: 28767641]
- Das C, Lucia MS, Hansen KC, & Tyler JK (2009). *Nature*, 459, 113–117. [PubMed: 19270680]

- de Dieuleveult M, Yen K, Hmitou I, Depaux A, Boussouar F, Bou Dargham D, et al. (2016). *Nature*, 530, 113–116. [PubMed: 26814966]
- de Napoles M, Mermoud JE, Wakao R, Tang YA, Endoh M, Appanah R, et al. (2004). *Developmental Cell*, 7, 663–676. [PubMed: 15525528]
- Deaton AM, Gomez-Rodriguez M, Mieczkowski J, Tolstorukov MY, Kundu S, Sadreyev RI, et al. (2016). *eLife*, 5, 1–24.
- Dekker J (2016). *Nature Reviews. Molecular Cell Biology*, 17, 741–742.
- Dekker J, & Misteli T (2015). *Cold Spring Harbor Perspectives in Biology*, 7, a019356. [PubMed: 26430217]
- Delmas V, Stokes DG, & Perry RP (1993). *Proceedings of the National Academy of Sciences of the United States of America*, 90, 2414–2418.
- Di Croce L, & Helin K (2013). *Nature Structural & Molecular Biology*, 20, 1147.
- Dillon SC, Zhang X, Trievel RC, & Cheng X (2005). *Genome Biology*, 6, 227. [PubMed: 16086857]
- Ding J, Huang X, Shao N, Zhou H, Lee DF, Faiola F, et al. (2015). *Cell Stem Cell*, 16, 653–668. [PubMed: 25936917]
- Ding J, Xu H, Faiola F, Ma'ayan A, & Wang J (2012). *Cell Research*, 22, 155–167. [PubMed: 22083510]
- Dixon JR, Jung I, Selvaraj S, Shen Y, Antosiewicz-Bourget JE, Lee AY, et al. (2015). *Nature*, 518, 331–336. [PubMed: 25693564]
- Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, et al. (2012). *Nature*, 485, 376–380. [PubMed: 22495300]
- Dou Y, Milne TA, Ruthenburg AJ, Lee S, Lee JW, Verdine GL, et al. (2006). *Nature Structural & Molecular Biology*, 13, 713–719.
- Doyen CM, An W, Angelov D, Bondarenko V, Miettinen F, Studitsky VM, et al. (2006). *Molecular Cell Biology*, 26, 1156–1164.
- Doyen CM, Montel F, Gautier T, Menoni H, Claudet C, Delacour-Larose M, et al. (2006). *The EMBO Journal*, 25, 4234–4244. [PubMed: 16957777]
- Doyon Y, & Cote J (2004). *Current Opinion in Genetics & Development*, 14, 147–154. [PubMed: 15196461]
- Doyon Y, Selleck W, Lane WS, Tan S, & Cote J (2004). *Molecular Cell Biology*, 24, 1884–1896.
- Draizen EJ, Shaytan AK, Marino-Ramirez L, Talbert PB, Landsman D, & Panchenko AR (2016). *Database-the Journal of Biological Databases and Curation*, 2016, 1–10.
- Drane P, Ouarrhni K, Depaux A, Shuaib M, & Hamiche A (2010). *Genes & Development*, 24, 1253–1265. [PubMed: 20504901]
- Driscoll R, Hudson A, & Jackson SP (2007). *Science (New York, N.Y.)*, 315, 649–652.
- Duina AA (2011). *Genetics Research International*, 2011, 625210. [PubMed: 22567361]
- Dyer MA, Qadeer ZA, Valle-Garcia D, & Bernstein E (2017). *Cold Spring Harbor Perspectives in Medicine*, 7, a026567. [PubMed: 28062559]
- Earnshaw WC, & Rothfield N (1985). *Chromosoma*, 91, 313–321. [PubMed: 2579778]
- Ebbert R, Birkmann A, & Schuller HJ (1999). *Molecular Microbiology*, 32, 741–751. [PubMed: 10361278]
- Ee LS, McCannell KN, Tang Y, Fernandes N, Hardy WR, Green MR, et al. (2017). *Stem Cell Reports*, 8, 1488–1496. [PubMed: 28528697]
- Efroni S, Duttagupta R, Cheng J, Dehghani H, Hoepfner DJ, Dash C, et al. (2008). *Cell Stem Cell*, 2, 437–447. [PubMed: 18462694]
- Eleuteri B, Aranda S, & Ernfors P (2018). *Cell Reports*, 23, 1853–1866. [PubMed: 29742439]
- Elfring L, Deuring R, McCallum C, Peterson CL, & Tamkun J (1994). *Molecular Cell Biology*, 14, 2225–2234.
- Elsaesser SJ, Goldberg AD, & Allis CD (2010). *Current Opinion in Genetics & Development*, 20, 110–117.
- Elsässer SJ, Noh K-M, Diaz N, Allis CD, & Banaszynski LA (2015). *Nature*, 522, 240. [PubMed: 25938714]

- Endoh M, Endo TA, Endoh T, Isono K, Sharif J, Ohara O, et al. (2012). *PLoS Genetics*, 8, 1–10, e1002774.
- Endoh M, Endo TA, Shinga J, Hayashi K, Farcas A, Ma KW, et al. (2017). *Elife*, 6, 1–26.
- Engelholm M, de Jager M, Flaus A, Brenk R, van Noort J, & Owen-Hughes T (2009). *Nature Structural & Molecular Biology*, 16, 151–158.
- Engelen E, Akinci U, Bryne JC, Hou J, Gontan C, Moen M, et al. (2011). *Nature Genetics*, 43, 607–611. [PubMed: 21532573]
- Eskeland R, Leeb M, Grimes GR, Kress C, Boyle S, Sproul D, et al. (2010). *Molecular Cell*, 38, 452–464. [PubMed: 20471950]
- Etchegaray J-P, & Mostoslavsky R (2015). *Cell Cycle* (Georgetown, Texas), 14, 2187–2188.
- Faast R, Thonglairoam V, Schulz TC, Beall J, Wells JRE, Taylor H, et al. (2001). *Current Biology*, 11, 1183–1187. [PubMed: 11516949]
- Fan JY, Rangasamy D, Luger K, & Tremethick DJ (2004). *Molecular Cell*, 16, 655–661. [PubMed: 15546624]
- Fazio TG, Huff JT, & Panning B (2008a). *Cell Cycle*, 7, 3302–3306. [PubMed: 18948739]
- Fazio TG, Huff JT, & Panning B (2008b). *Cell*, 134, 162–174. [PubMed: 18614019]
- Fazio TG, & Tsukiyama T (2003). *Molecular Cell*, 12, 1333–1340. [PubMed: 14636590]
- Field Y, Kaplan N, Fondufe-Mittendorf Y, Moore IK, Sharon E, Lubling Y, et al. (2008). *PLoS Computational Biology*, 4, e1000216. [PubMed: 18989395]
- Fierz B, Chatterjee C, McGinty RK, Bar-Dagan M, Raleigh DP, & Muir TW (2011). *Nature Chemical Biology*, 7, 113–119. [PubMed: 21196936]
- Fillingham J, Recht J, Silva AC, Suter B, Emili A, Stagljar I, et al. (2008). *Molecular Cell Biology*, 28, 4342–4353.
- Formosa T (2008). *Molecular BioSystems*, 4, 1085–1093. [PubMed: 18931784]
- Formosa T (2012). *Biochimica et Biophysica Acta*, 1819, 247–255. [PubMed: 21807128]
- Fuchs S, Larabee R, & Strahl B (2009). *Biochimica et biophysica acta*, 1789, 26–36.
- Furuya M, Tanaka M, Teranishi T, Matsumoto K, Hosoi Y, Saeki K, et al. (2007). *The Journal of Reproduction and Development*, 53, 895–902. [PubMed: 17519519]
- Gamarra N, Johnson SL, Trnka MJ, Burlingame AL, & Narlikar GJ (2018). *eLife*, 7, 1–25.
- Gao Y, Gan H, Lou Z, & Zhang Z (2018). *Proceedings of the National Academy of Sciences of the United States of America*, 115, E6162–E6171.
- Gao Z, Zhang J, Bonasio R, Strino F, Sawai A, Parisi F, et al. (2012). *Molecular Cell*, 45, 344–356. [PubMed: 22325352]
- Garcia H, Fleishman D, Kolesnikova K, Safina A, Commane M, Paszkiewicz G, et al. (2011). *Oncotarget*, 2, 783–796. [PubMed: 21998152]
- Gaspar-Maia A, Alajem A, Polesso F, Sridharan R, Mason MJ, Heidersbach A, et al. (2009). *Nature*, 460, 863–868. [PubMed: 19587682]
- Gaspar-Maia A, Qadeer ZA, Hasson D, Ratnakumar K, Adrian Leu N, Leroy G, et al. (2013). *Nature Communications*, 4, 1565.
- Gatchalian J, Malik S, Ho J, Lee DS, Kelso TWR, Shokhirev MN, et al. (2018). *Nature Communications*, 9, 5139.
- Gerhold C, & Gasser S (2014). *Trends in Cell Biology*, 24, 12.
- Giaimo BD, Ferrante F, Herchenrother H, Hake SB, & Borggreffe T (2019). *Epigenetics & Chromatin*, 12, 37. [PubMed: 31200754]
- Gibbons RJ, McDowell TL, Raman S, O'Rourke DM, Garrick D, Ayyub H, et al. (2000). *Nature Genetics*, 24, 368–371. [PubMed: 10742099]
- Gibbons RJ, Picketts DJ, Villard L, & Higgs DR (1995). *Cell*, 80, 837–845. [PubMed: 7697714]
- Gilbert J, Gore S, Herman J, & Carducci M (2004). *Clinical Cancer Research*, 10, 4589–4596. [PubMed: 15269129]
- Gilmour D, & Lis J (1984). *Proceedings of the National Academy of Sciences of the United States of America*, 81, 4.

- Goldberg AD, Banaszynski LA, Noh KM, Lewis PW, Elsaesser SJ, Stadler S, et al. (2010). *Cell*, 140, 678–691. [PubMed: 20211137]
- Goldman JA, Garlick JD, & Kingston RE (2010). *The Journal of Biological Chemistry*, 285, 4645–4651. [PubMed: 19940112]
- Govind CK, Zhang F, Qiu H, Hofmeyer K, & Hinnebusch AG (2007). *Molecular Cell*, 25, 31–42. [PubMed: 17218269]
- Guillemette B, Bataille AR, Gevry N, Adam M, Blanchette M, Robert F, et al. (2005). *PLoS Biology*, 3, e384. [PubMed: 16248679]
- Gunther K, Rust M, Leers J, Boettger T, Scharfe M, Jarek M, et al. (2013). *Nucleic Acids Research*, 41, 3010–3021. [PubMed: 23361464]
- Gurova K, Chang HW, Valieva ME, Sandlesh P, & Studitsky VM (2018). *Biochimica et biophysica acta. Gene regulatory mechanisms*, 1861, 892–904.
- Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G, et al. (2011). *Nature*, 477, 295–300. [PubMed: 21874018]
- Guzman-Ayala M, Sachs M, Koh FM, Onodera C, Bulut-Karslioglu A, Lin C-J, et al. (2015). *Development (Cambridge, England)*, 142, 118–127.
- Ha M, Kraushaar DC, & Zhao K (2014). *Epigenetics & Chromatin*, 7, 38. [PubMed: 25598842]
- Hainer SJ, Boskovic A, McCannell KN, Rando OJ, & Fazzio TG (2019). *Cell*, 177, 1319–1329.e11. [PubMed: 30955888]
- Hainer SJ, & Fazzio TG (2015). *Cell Reports*, 13, 61–69. [PubMed: 26411677]
- Hainer SJ, Gu W, Carone BR, Landry BD, Rando OJ, Mello CC, et al. (2015). *Genes & Development*, 29, 362–378. [PubMed: 25691467]
- Hainer SJ, McCannell KN, Yu J, Ee LS, Zhu LJ, Rando OJ, et al. (2016). *Elife*, 5, 1–24.
- Hake SB, Garcia BA, Duncan EM, Kauer M, Dellaire G, Shabanowitz J, et al. (2006). *The Journal of Biological Chemistry*, 281, 559–568. [PubMed: 16267050]
- Hammond CM, Stromme CB, Huang H, Patel DJ, & Groth A (2017). *Nature Reviews. Molecular Cell Biology*, 18, 141–158. [PubMed: 28053344]
- Han D, Jeon S, Sohn DH, Lee C, Ahn S, Kim WK, et al. (2008). *Developmental Biology*, 315, 136–146. [PubMed: 18206867]
- Happel N, Schulze E, & Doenecke D (2005). *Biological Chemistry*, 386, 541–551. [PubMed: 16006241]
- Harada A, Maehara K, Sato Y, Konno D, Tachibana T, Kimura H, et al. (2015). *Nucleic Acids Research*, 43, 775–786. [PubMed: 25539924]
- Harikumar A, & Meshorer E (2015). *EMBO Reports*, 16, 1609–1619. [PubMed: 26553936]
- Hassan AH, Neely KE, & Workman JL (2001). *Cell*, 104, 817–827. [PubMed: 11290320]
- Hauri S, Comoglio F, Seimiya M, Gerstung M, Glatter T, Hansen K, et al. (2016). *Cell Reports*, 17, 583–595. [PubMed: 27705803]
- Hautbergue GM, Hung ML, Walsh MJ, Snijders AP, Chang CT, Jones R, et al. (2009). *Current Biology*, 19, 1918–1924. [PubMed: 19836239]
- Hayakawa K, Ohgane J, Tanaka S, Yagi S, & Shiota K (2012). *Epigenetics*, 7, 1029–1036. [PubMed: 22868987]
- Hendrich B, & Bird A (1998). *Molecular Cell Biology*, 18, 6538–6547.
- Henikoff S, & Ahmad K (2005). *Annual Review of Cell and Developmental Biology*, 21, 133–153.
- Herold A, Teixeira L, & Izaurralde E (2003). *The EMBO Journal*, 22, 2472–2483. [PubMed: 12743041]
- Hertel L, De Andrea M, Bellomo G, Santoro P, Landolfo S, & Gariglio M (1999). *Experimental Cell Research*, 250, 313–328. [PubMed: 10413586]
- Hnisz D, Abraham BJ, Lee TI, Lau A, Saint-Andre V, Sigova AA, et al. (2013). *Cell*, 155, 934–947. [PubMed: 24119843]
- Ho L, & Crabtree GR (2010). *Nature*, 463, 474–484. [PubMed: 20110991]
- Ho L, Jothi R, Ronan JL, Cui K, Zhao K, & Crabtree GR (2009). *Proceedings of the National Academy of Sciences of the United States of America*, 106, 5187–5191.

- Ho L, Miller EL, Ronan JL, Ho WQ, Jothi R, & Crabtree GR (2011). *Nature Cell Biology*, 13, 903. [PubMed: 21785422]
- Ho L, Ronan JL, Wu J, Staahl BT, Chen L, Kuo A, et al. (2009). *Proceedings of the National Academy of Sciences of the United States of America*, 106, 5181–5186.
- Hodges AJ, Gloss LM, & Wyrick JJ (2017). *Genetics*, 206, 1339–1348. [PubMed: 28468903]
- Hoeijmakers WA, Salcedo-Amaya AM, Smits AH, Francoijs KJ, Treeck M, Gilberger TW, et al. (2013). *Molecular Microbiology*, 87, 1061–1073. [PubMed: 23320541]
- Hondele M, & Ladurner AG (2013). *Nucleus*, 4, 443–449. [PubMed: 24413069]
- Hondele M, Stuwe T, Hassler M, Halbach F, Bowman A, Zhang ET, et al. (2013). *Nature*, 499, 111. [PubMed: 23698368]
- Hori T, Shang WH, Toyoda A, Misu S, Monma N, Ikeo K, et al. (2014). *Developmental Cell*, 29, 740–749. [PubMed: 24960696]
- Hsieh FK, Kulaeva OI, Patel SS, Dyer PN, Luger K, Reinberg D, et al. (2013). *Proceedings of the National Academy of Sciences of the United States of America*, 110, 7654–7659.
- Hsu C-C, Zhao D, Shi J, Peng D, Guan H, Li Y, et al. (2018). *Cell Discovery*, 4, 28. [PubMed: 29900004]
- Hu G, Cui K, Northrup D, Liu C, Wang C, Tang Q, et al. (2013). *Cell Stem Cell*, 12, 180–192. [PubMed: 23260488]
- Hu Y, Fisher JB, Koprowski S, McAllister D, Kim MS, & Lough J (2009). *Developmental Dynamics*, 238, 2912–2921. [PubMed: 19842187]
- Hu G, Schones DE, Cui K, Ybarra R, Northrup D, Tang Q, et al. (2011). *Genome Research*, 21, 1650–1658. [PubMed: 21795385]
- Hunkapiller J, Shen Y, Diaz A, Cagney G, McCleary D, Ramalho-Santos M, et al. (2012). *PLoS Genetics*, 8, e1002576. [PubMed: 22438827]
- Illingworth RS, Botting CH, Grimes GR, Bickmore WA, & Eskeland R (2012). *PLoS One*, 7, e34848. [PubMed: 22496869]
- Ishimi Y, Yasuda H, Hirosumi J, Hanaoka F, & Yamada M (1983). *Journal of Biochemistry*, 94, 735–744. [PubMed: 6643419]
- Iwafuchi-Doi M (2019). *Wiley Interdisciplinary Reviews Systems Biology and Medicine*, 11 e1427. [PubMed: 29949240]
- Iwafuchi-Doi M, Donahue G, Kakumanu A, Watts JA, Mahony S, Pugh BF, et al. (2016). *Molecular Cell*, 62, 79–91. [PubMed: 27058788]
- Izban M, & Luse D (1991). *Genes & Development*, 5, 683–696. [PubMed: 2010092]
- Izzo A, Ziegler-Birling C, Hill PWS, Brondani L, Hajkova P, Torres-Padilla ME, et al. (2017). *The Journal of Cell Biology*, 216, 3017–3028. [PubMed: 28794128]
- Jamieson K, Wiles ET, McNaught KJ, Sidoli S, Leggett N, Shao Y, et al. (2016). *Genome Research*, 26, 97–107. [PubMed: 26537359]
- Jang CW, Shibata Y, Starmer J, Yee D, & Magnuson T (2015). *Genes & Development*, 29, 1377–1392. [PubMed: 26159997]
- Jin C, & Felsenfeld G (2007). *Genes & Development*, 21, 1519–1529. [PubMed: 17575053]
- Jin C, Zang C, Wei G, Cui K, Peng W, Zhao K, et al. (2009). *Nature Genetics*, 41, 941–945. [PubMed: 19633671]
- Jones A, & Wang H (2010). *Protein & Cell*, 1, 1056–1062. [PubMed: 21213100]
- Jordan CT (2007). *Best Practice & Research. Clinical Haematology*, 20, 13–18. [PubMed: 17336250]
- Kaaser MD, Aslanian A, Dong M-Q, Yates JR 3rd, & Emerson BM (2008). *The Journal of Biological Chemistry*, 283, 32254–32263. [PubMed: 18809673]
- Kaji K, Caballero IM, MacLeod R, Nichols J, Wilson VA, & Hendrich B (2006). *Nature Cell Biology*, 8, 285–292. [PubMed: 16462733]
- Kaji K, Nichols J, & Hendrich B (2007). *Development*, 134, 1123–1132. [PubMed: 17287250]
- Kalashnikova AA, Porter-Goff ME, Muthurajan UM, Luger K, & Hansen JC (2013). *Journal of the Royal Society, Interface*, 10, 20121022.

- Kang H, McElroy KA, Jung YL, Alekseyenko AA, Zee BM, Park PJ, et al. (2015). *Genes & Development*, 29, 1136–1150. [PubMed: 26063573]
- Kaplan CD, Laprade L, & Winston F (2003). *Science*, 301, 1096–1099. [PubMed: 12934008]
- Kaplan T, Liu CL, Erkmann JA, Holik J, Grunstein M, Kaufman PD, et al. (2008). *PLoS Genetics*, 4, e1000270. [PubMed: 19023413]
- Karytinos A, Forneris F, Profumo A, Ciossani G, Battaglioli E, Binda C, et al. (2009). *The Journal of Biological Chemistry*, 284, 17775–17782. [PubMed: 19407342]
- Kassis JA, & Kennison JA (2010). *Molecular Cell Biology*, 30, 2581–2583.
- Kasten MM, Clapier CR, & Cairns BR (2011). *Cell*, 144 310.e1. [PubMed: 21241897]
- Kato D, Osakabe A, Arimura Y, Mizukami Y, Horikoshi N, Saikusa K, et al. (2017). *Science*, 356, 205–208. [PubMed: 28408607]
- Keenen B, & de la Serna IL (2009). *Journal of Cellular Physiology*, 219, 1–7. [PubMed: 19097034]
- Keniry A, Jansz N, Gearing LJ, Wanigasuriya I, Chen J, Nefzger CM, et al. (2019). *BioRxiv*.
- Keogh MC, Kurdistani SK, Morris SA, Ahn SH, Podolny V, Collins SR, et al. (2005). *Cell*, 123, 593–605. [PubMed: 16286008]
- Kidder BL, & Palmer S (2012). *Nucleic Acids Research*, 40, 2925–2939. [PubMed: 22156375]
- Kidder BL, Palmer S, & Knott JG (2009). *Stem Cells*, 27, 317–328. [PubMed: 19056910]
- Kim JA, & Haber JE (2009). *Proceedings of the National Academy of Sciences of the United States of America*, 106, 1151–1156.
- Kim HS, Tan Y, Ma W, Merkurjev D, Destici E, Ma Q, et al. (2018). *Nature*, 556, 510–514. [PubMed: 29670286]
- King HW, & Klose RJ (2017). *eLife*, 6, 1–24.
- Kizer KO, Phatnani HP, Shibata Y, Hall H, Greenleaf AL, & Strahl BD (2005). *Molecular Cell Biology*, 25, 3305–3316.
- Klopf E, Schmidt HA, Clauder-Munster S, Steinmetz LM, & Schuller C (2017). *Nucleic Acids Research*, 45, 3752–3766. [PubMed: 28025392]
- Kobor MS, Venkatasubrahmanyam S, Meneghini MD, Gin JW, Jennings JL, Link AJ, et al. (2004). *PLoS Biology*, 2, E131. [PubMed: 15045029]
- Kokavec J, Zikmund T, Savvulidi F, Kulvait V, Edelman W, Skoultchi AI, et al. (2017). *Stem Cells*, 35, 1614–1623. [PubMed: 28276606]
- Kolundzic E, Ofenbauer A, Bulut SI, Uyar B, Baytek G, Sommermeier A, et al. (2018). *Development Cell*, 46, 611–626.
- Kooistra SM, & Helin K (2012). *Nature Reviews. Molecular Cell Biology*, 13, 297–311. [PubMed: 22473470]
- Kornberg R (1981). *Nature*, 292, 579–580. [PubMed: 7254354]
- Kornberg R, & Lorch Y (1999). *Cell*, 98, 285–294. [PubMed: 10458604]
- Kornberg RD, & Stryer L (1988). *Nucleic Acids Research*, 16, 6677–6690. [PubMed: 3399412]
- Kouzarides T (2007). *Cell*, 128, 693–705. [PubMed: 17320507]
- Kraushaar DC, Jin W, Maunakea A, Abraham B, Ha M, & Zhao K (2013). *Genome Biology*, 14, R121. [PubMed: 24176123]
- Kujirai T, Horikoshi N, Sato K, Maehara K, Machida S, Osakabe A, et al. (2017). *Nucleic Acids Research*, 45, 3612. [PubMed: 27913734]
- Kulaeva OI, Hsieh FK, Chang HW, Luse DS, & Studitsky VM (2013). *Biochimica et Biophysica Acta*, 1829, 76–83. [PubMed: 22982194]
- Kulaeva OI, Hsieh F, & Studitsky V (2010). *Proceedings of the National Academy of Sciences of the United States of America*, 107, 11325–11330.
- Kuo M-H, & Allis CD (1998). *BioEssays*, 20, 615–626. [PubMed: 9780836]
- Kusch T, Florens L, Macdonald WH, Swanson SK, Glaser RL, Yates JR 3rd, et al. (2004). *Science*, 306, 2084–2087. [PubMed: 15528408]
- Kuzmichev A, Nishioka K, Erdjument-Bromage H, Tempst P, & Reinberg D (2002). *Genes & Development*, 16, 2893–2905. [PubMed: 12435631]

- Lachner M, O'Carroll D, Rea S, Mechtler K, & Jenuwein T (2001). *Nature*, 410, 116–120. [PubMed: 11242053]
- Lafon A, Taranum S, Pietrocola F, Dingli F, Loew D, Brahma S, et al. (2015). *Molecular Cell*, 60, 784–796. [PubMed: 26656161]
- Lai AY, & Wade PA (2011). *Nature Reviews. Cancer*, 11, 588–596. [PubMed: 21734722]
- Lamba DA, Hayes S, Karl MO, & Reh T (2008). *Developmental Dynamics*, 237, 3016–3023. [PubMed: 18816825]
- Lammerding J (2011). *Comprehensive Physiology*, 1, 783–807. [PubMed: 23737203]
- Lamour V, Lecluse Y, Desmaze C, Spector M, Bodescot M, Aurias A, et al. (1995). *Human Molecular Genetics*, 4, 791–799. [PubMed: 7633437]
- Lan L, Ui A, Nakajima S, Hatakeyama K, Hoshi M, Watanabe R, et al. (2010). *Molecular Cell*, 40, 976–987. [PubMed: 21172662]
- Landry J, Sharov AA, Piao Y, Sharova LV, Xiao H, Southon E, et al. (2008). *PLoS Genetics*, 4, e1000241. [PubMed: 18974875]
- Längst G, & Becker P (2001). *Journal of Cell Science*, 114, 2561–2568. [PubMed: 11683384]
- Lattrick CM, Marek M, Ouararhni K, Papin C, Stoll I, Ignatyeva M, et al. (2016). *Nature Structural & Molecular Biology*, 23, 309–316.
- Le Guezennec X, Vermeulen M, Brinkman AB, Hoeijmakers WA, Cohen A, Lasonder E, et al. (2006). *Molecular Cell Biology*, 26, 843–851.
- Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, et al. (2006). *Cell*, 125, 301–313. [PubMed: 16630818]
- Lee HS, Park JH, Kim SJ, Kwon SJ, & Kwon J (2010). *The EMBO Journal*, 29, 1434–1445. [PubMed: 20224553]
- Lee C-K, Shibata Y, Rao B, Strahl BD, & Lieb JD (2004). *Nature Genetics*, 36, 900–905. [PubMed: 15247917]
- Lee DY, Teyssier C, Strahl BD, & Stallcup MR (2005). *Endocrine Reviews*, 26, 147–170. [PubMed: 15479858]
- Lee W, Tillo D, Bray N, Morse R, Davis R, Hughes T, et al. (2007). *Nature Genetics*, 39, 1235–1244. [PubMed: 17873876]
- Lehnertz B, Ueda Y, Derijck AAHA, Braunschweig U, Perez-Burgos L, Kubicek S, et al. (2003). *Current Biology*, 13, 1192–1200. [PubMed: 12867029]
- Lei I, West J, Yan Z, Gao X, Fang P, Dennis JH, et al. (2015). *The Journal of Biological Chemistry*, 290, 19343–19352. [PubMed: 26070559]
- Lessard J, Wu JI, Ranish JA, Wan M, Winslow MM, Staahl BT, et al. (2007). *Neuron*, 55, 201–215. [PubMed: 17640523]
- Levasseur DN, Wang J, Dorschner MO, Stamatoiyannopoulos JA, & Orkin SH (2008). *Genes & Development*, 22, 575–580. [PubMed: 18283123]
- Lewis PH (1947). *Drosophila Information Service*, 21, 69.
- Lewis EB (1978). *Nature*, 276, 565–570. [PubMed: 103000]
- Lewis PW, Elsaesser SJ, Noh KM, Stadler SC, & Allis CD (2010). *Proceedings of the National Academy of Sciences of the United States of America*, 107, 14075–14080.
- Li B, Carey M, & Workman JL (2007). *Cell*, 128, 707–719. [PubMed: 17320508]
- Li G, Fullwood MJ, Xu H, Mulawadi FH, Velkov S, Vega V, et al. (2010). *Genome Biology*, 11, R22. [PubMed: 20181287]
- Li Z, Gadue P, Chen K, Jiao Y, Tuteja G, Schug J, et al. (2012). *Cell*, 151, 1608–1616. [PubMed: 23260146]
- Li Q, Lian S, Dai Z, Xiang Q, & Dai X (2013). *Database: The Journal of Biological Databases and Curation*, 2013, bat057.
- Li Y, Rivera CM, Ishii H, Jin F, Selvaraj S, Lee AY, et al. (2014). *PLoS One*, 9, e114485. [PubMed: 25486255]
- Liang X, Shan S, Pan L, Zhao J, Ranjan A, Wang F, et al. (2016). *Nature Structural & Molecular Biology*, 23, 317–323.

- Liang J, Wan M, Zhang Y, Gu P, Xin H, Jung SY, et al. (2008). *Nature Cell Biology*, 10, 731–739. [PubMed: 18454139]
- Lickert H, Takeuchi JK, von Both I, Walls JR, McAuliffe F, Lee Adamson S, et al. (2004). *Nature*, 432, 107–112. [PubMed: 15525990]
- Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, et al. (2009). *Science*, 326, 289–293. [PubMed: 19815776]
- Lin B, Lee H, Yoon JG, Madan A, Wayner E, Tonning S, et al. (2015). *Oncotarget*, 6, 5369–5381. [PubMed: 25749033]
- Lin JJ, Lehmann LW, Bonora G, Sridharan R, Vashisht AA, Tran N, et al. (2011). *Genes & Development*, 25, 2198–2209. [PubMed: 21979373]
- Liu X, Bushnell DA, & Kornberg RD (2013). *Biochimica et Biophysica Acta*, 1829, 2–8. [PubMed: 23000482]
- Liu WH, Roemer SC, Port AM, & Churchill ME (2012). *Nucleic Acids Research*, 40, 11229–11239. [PubMed: 23034810]
- Lomvardas S, & Thanos D (2001). *Cell*, 106, 685–696. [PubMed: 11572775]
- Long M, Sun X, Shi W, Yanru A, Leung STC, Ding D, et al. (2019). *Nucleic Acids Research*, 47, 8399–8409. [PubMed: 31219579]
- Lorain S, Demczuk S, Lamour V, Toth S, Aurias A, Roe BA, et al. (1996). *Genome Research*, 6, 43–50. [PubMed: 8681138]
- Loven J, Hoke HA, Lin CY, Lau A, Orlando DA, Vakoc CR, et al. (2013). *Cell*, 153, 320–334. [PubMed: 23582323]
- Luger K (2006). *Chromosome Research*, 14, 5–16. [PubMed: 16506092]
- Luger K, Mäder AW, Richmond RK, Sargent DF, & Richmond TJ (1997). *Nature*, 389.
- Manning BJ, & Yusufzai T (2017). *The Journal of Biological Chemistry*, 292, 11927–11936. [PubMed: 28533432]
- Marmorstein R (2001). *Journal of Molecular Biology*, 311, 433–444. [PubMed: 11492997]
- Marmorstein R, & Zhou MM (2014). *Cold Spring Harbor Perspectives in Biology*, 6, a018762. [PubMed: 24984779]
- Marson A, Levine SS, Cole MF, Frampton GM, Brambrink T, Johnstone S, et al. (2008). *Cell*, 134, 521–533. [PubMed: 18692474]
- Martianov I, Brancorsini S, Catena R, Gansmuller A, Kotaja N, Parvinen M, et al. (2005). *Proceedings of the National Academy of Sciences of the United States of America*, 102, 2808–2813.
- Martire S, Gogate AA, Whitmill A, Tafessu A, Nguyen J, Teng YC, et al. (2019). *Nature Genetics*, 51, 941–946. [PubMed: 31152160]
- Marzluff WF, Gongidi P, Woods KR, Jin J, & Maltais LJ (2002). *Genomics*, 80, 487–498. [PubMed: 12408966]
- Mashtalir N, D'Avino AR, Michel BC, Luo J, Pan J, Otto JE, et al. (2018). *Cell*, 175, 1272–1288.e20. [PubMed: 30343899]
- Mattiroli F, Uckelmann M, Sahtoe DD, van Dijk WJ, & Sixma TK (2014). *Nature Communications*, 5, 3291.
- Mayran A, & Drouin J (2018). *The Journal of Biological Chemistry*, 293, 13795–13804. [PubMed: 29507097]
- McBryant SJ, Park YJ, Abernathy SM, Laybourn PJ, Nyborg JK, & Luger K (2003). *The Journal of Biological Chemistry*, 278, 44574–44583. [PubMed: 12928440]
- McGarvey KM, Van Neste L, Cope L, Ohm JE, Herman JG, Van Criekinge W, et al. (2008). *Cancer Research*, 68, 5753–5759. [PubMed: 18632628]
- McGinty RK, Henrici RC, & Tan S (2014). *Nature*, 514, 591. [PubMed: 25355358]
- McKittrick E, Gaffken P, Ahmad K, & Henikoff S (2004). *Proceedings of the National Academy of Sciences of the United States of America*, 101, 1525–1530.
- Meshorer E, & Misteli T (2006). *Nature Reviews. Molecular Cell Biology*, 7, 540–546. [PubMed: 16723974]
- Micucci J, Sperry E, & Martin D (2015). *Stem Cells and Development*, 24, 9.

- Middeljans E, Wan X, Jansen PW, Sharma V, Stunnenberg HG, & Logie C (2012). PLoS One, 7, e33834. [PubMed: 22442726]
- Miller CJ, & Davidson LA (2013). Nature Reviews Genetics, 14, 733–744.
- Mizuguchi G, Shen X, Landry J, Wu W, Sen S, & Wu C (2004). Science, 303, 343–348. [PubMed: 14645854]
- Mohn F, Weber M, Rebhan M, Roloff TC, Richter J, Stadler MB, et al. (2008). Molecular Cell, 30, 755–766. [PubMed: 18514006]
- Molaro A, Young JM, & Malik HS (2018). Genome Research, 28, 460–473. [PubMed: 29549088]
- Moody JD, Levy S, Mathieu J, Xing Y, Kim W, Dong C, et al. (2017). Proceedings of the National Academy of Sciences of the United States of America, 114, 10125–10130.
- Moorthy SD, Davidson S, Shchuka VM, Singh G, Malek-Gilani N, Langroudi L, et al. (2017). Genome Research, 27, 246–258. [PubMed: 27895109]
- Morey L, Pascual G, Cozzuto L, Roma G, Wutz A, Benitah SA, et al. (2012). Cell Stem Cell, 10, 47–62. [PubMed: 22226355]
- Morey L, Santanach A, & Di Croce L (2015). Molecular Cell Biology, 35, 2716–2728.
- Morgan MT, Haj-Yahya M, Ringel AE, Bandi P, Brik A, & Wolberger C (2016). Science, 351, 725–728. [PubMed: 26912860]
- Morillon A, Karabetsou N, O'Sullivan J, Kent N, Proudfoot N, & Mellor J (2003). Cell, 115, 425–435. [PubMed: 14622597]
- Mosammaparast N, Ewart CS, & Pemberton LF (2002). The EMBO Journal, 21, 6527–6538. [PubMed: 12456659]
- Mylonas C, & Tessarz P (2018). Life Science Alliance, 1, e201800085. [PubMed: 30456357]
- Nakamura K, Kato A, Kobayashi J, Yanagihara H, Sakamoto S, Oliveira DVNP, et al. (2011). Molecular Cell, 41, 515–528. [PubMed: 21362548]
- Nakayama RT, Pulice JL, Valencia AM, McBride MJ, McKenzie ZM, Gillespie MA, et al. (2017). Nature Genetics, 49, 1613–1623. [PubMed: 28945250]
- Nardi IK, Zasadzinska E, Stellfox ME, Knippler CM, & Foltz DR (2016). Molecular Cell, 61, 774–787. [PubMed: 26942680]
- Neigeborn L, & Carlson M (1984). Genetics, 108, 845–858. [PubMed: 6392017]
- Neumann H, Hancock SM, Buning R, Routh A, Chapman L, Somers J, et al. (2009). Molecular Cell, 36, 153–163. [PubMed: 19818718]
- Niikura Y, Kitagawa R, Ogi H, Abdulle R, Pagala V, & Kitagawa K (2015). Developmental Cell, 32, 589–603. [PubMed: 25727006]
- Nishibuchi G, & Dejardin J (2017). Chromosome Research, 25, 77–87. [PubMed: 28078514]
- Noma K, Allis CD, & Grewal SI (2001). Science, 293, 1150–1155. [PubMed: 11498594]
- Nora EP, Lajoie BR, Schulz EG, Giorgetti L, Okamoto I, Servant N, et al. (2012). Nature, 485, 381–385. [PubMed: 22495304]
- Novo CL, Javierre BM, Cairns J, Segonds-Pichon A, Wingett SW, Freire-Pritchett P, et al. (2018). Cell Reports, 22, 2615–2627. [PubMed: 29514091]
- Okada M, Okawa K, Isobe T, & Fukagawa T (2009). Molecular Biology of the Cell, 20, 3986–3995. [PubMed: 19625449]
- Okumura-Nakanishi S, Saito M, Niwa H, & Ishikawa F (2005). The Journal of Biological Chemistry, 280, 5307–5317. [PubMed: 15557334]
- Olave I, Wang W, Xue Y, Kuo A, & Crabtree GR (2002). Genes & Development, 16, 2509–2517. [PubMed: 12368262]
- Oliver MI, Concha C, Gutierrez S, Bustos A, Montecino M, Puchi M, et al. (2002). Journal of Cellular Biochemistry, 85, 851–859. [PubMed: 11968024]
- O'Loughlin A, Munoz-Cabello AM, Gaspar-Maia A, Wu HA, Banito A, Kunowska N, et al. (2012). Cell Stem Cell, 10, 33–46. [PubMed: 22226354]
- Orkin SH, & Hochedlinger K (2011). Cell, 145, 835–850. [PubMed: 21663790]
- Orphanides G, LeRoy G, Chang CH, Luse DS, & Reinberg D (1998). Cell, 92, 105–116. [PubMed: 9489704]

- Orphanides G, Wu W, Lane W, Hampsey M, & Reinberg D (1999). *Nature*, 400, 284–288. [PubMed: 10421373]
- Osberg B, Nuebler J, Korber P, & Gerland U (2014). *Nucleic Acids Research*, 42, 13633–13645. [PubMed: 25428353]
- Palmer D, O'Day K, Wener M, Andrews B, & Margolis R (1987). *Journal of Cell Biology*, 104, 10.
- Papamichos-Chronakis M, Watanabe S, Rando OJ, & Peterson CL (2011). *Cell*, 144, 200–213. [PubMed: 21241891]
- Pardo M, Lang B, Yu L, Prosser H, Bradley A, Babu MM, et al. (2010). *Cell Stem Cell*, 6, 382–395. [PubMed: 20362542]
- Park YJ, Chodaparambil JV, Bao Y, McBryant SJ, & Luger K (2005). *The Journal of Biological Chemistry*, 280, 1817–1825. [PubMed: 15516689]
- Park CS, Rehrauer H, & Mansuy IM (2013). *BMC Genomics*, 14, 539. [PubMed: 23927422]
- Parker SC, Stitzel ML, Taylor DL, Orozco JM, Erdos MR, Akiyama JA, et al. (2013). *Proceedings of the National Academy of Sciences of the United States of America*, 110, 17921–17926.
- Paro R, & Hogness DS (1991). *Proceedings of the National Academy of Sciences of the United States of America*, 88, 263–267.
- Pasini D, Cloos PA, Walfridsson J, Olsson L, Bukowski JP, Johansen JV, et al. (2010). *Nature*, 464, 306–310. [PubMed: 20075857]
- Pchelintsev NA, McBryan T, Rai TS, van Tuyn J, Ray-Gallet D, Almouzni G, et al. (2013). *Cell Reports*, 3, 1012–1019. [PubMed: 23602572]
- Pegoraro G, Kubben N, Wickert U, Gohler H, Hoffmann K, & Misteli T (2009). *Nature Cell Biology*, 11, 1261–1267. [PubMed: 19734887]
- Peleg S, Sananbenesi F, Zovoilis A, Burkhardt S, Bahari-Javan S, Agis-Balboa RC, et al. (2010). *Science*, 328, 753–756. [PubMed: 20448184]
- Peng Y, & Zhang Y (2018). *Animal Models and Experimental Medicine*, 1, 169–179. [PubMed: 30891562]
- Peterson AJ, Mallin DR, Francis NJ, Ketel CS, Stamm J, Voeller RK, et al. (2004). *Genetics*, 167, 1225–1239. [PubMed: 15280237]
- Petter M, Selvarajah SA, Lee CC, Chin WH, Gupta AP, Bozdech Z, et al. (2013). *Molecular Microbiology*, 87, 1167–1182. [PubMed: 23373537]
- Piatti P, Lim CY, Nat R, Villunger A, Geley S, Shue YT, et al. (2015). *Scientific Reports*, 5, 8007. [PubMed: 25620209]
- Pokholok DK, Harbison CT, Levine S, Cole M, Hannett NM, Lee TI, et al. (2005). *Cell*, 122, 517–527. [PubMed: 16122420]
- Poli J, Gasser SM, & Papamichos-Chronakis M (2017). *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 372, 20160289. [PubMed: 28847826]
- Poli J, Gerhold CB, Tosi A, Hustedt N, Seeber A, Sack R, et al. (2016). *Genes & Development*, 30, 337–354. [PubMed: 26798134]
- Postberg J, Forcob S, Chang WJ, & Lipps HJ (2010). *BMC Evolutionary Biology*, 10, 259. [PubMed: 20738881]
- Pradhan SK, Su T, Yen L, Jacquet K, Huang C, Cote J, et al. (2016). *Molecular Cell*, 61, 27–38. [PubMed: 26669263]
- Radman-Livaja M, & Rando OJ (2010). *Developmental Biology*, 339, 258–266. [PubMed: 19527704]
- Raisner RM, Hartley PD, Meneghini MD, Bao MZ, Liu CL, Schreiber SL, et al. (2005). *Cell*, 123, 233–248. [PubMed: 16239142]
- Rando OJ, & Winston F (2012). *Genetics*, 190, 351–387. [PubMed: 22345607]
- Ransom M, Dennehey BK, & Tyler JK (2010). *Cell*, 140, 183–195. [PubMed: 20141833]
- Recht J, Tsubota T, Tanny JC, Diaz RL, Berger JM, Zhang X, et al. (2006). *Proceedings of the National Academy of Sciences of the United States of America*, 103, 6988–6993.
- Reya T, & Clevers H (2005). *Nature*, 434, 843–850. [PubMed: 15829953]
- Reyes JC, Barra J, Muchardt C, Camus A, Babinet C, & Yaniv M (1998). *The EMBO Journal*, 17, 6979–6991. [PubMed: 9843504]

- Reynolds N, Latos P, Hynes-Allen A, Loos R, Leaford D, O'Shaughnessy A, et al. (2012). *Cell Stem Cell*, 10, 583–594. [PubMed: 22560079]
- Reynolds N, Salmon-Divon M, Dvinge H, Hynes-Allen A, Balasooriya G, Leaford D, et al. (2012). *The EMBO Journal*, 31, 593–605. [PubMed: 22139358]
- Rhee HS, Bataille AR, Zhang L, & Pugh BF (2014). *Cell*, 159, 1377–1388. [PubMed: 25480300]
- Roberts CW, Galusha SA, McMenamin ME, Fletcher CD, & Orkin SH (2000). *Proceedings of the National Academy of Sciences of the United States of America*, 97, 13796–13800.
- Rodriguez J, Munoz M, Vives L, Frangou CG, Groudine M, & Peinado MA (2008). *Proceedings of the National Academy of Sciences of the United States of America*, 105, 19809–19814.
- Rogakou EP, Pilch DR, Orr AH, Ivanova VS, & Bonner WM (1998). *The Journal of Biological Chemistry*, 273, 5858–5868. [PubMed: 9488723]
- Roh TY, Cuddapah S, Cui K, & Zhao K (2006). *Proceedings of the National Academy of Sciences of the United States of America*, 103, 15782–15787.
- Romito A, & Cobellis G (2016). *Stem Cells International*, 2016, 9451492. [PubMed: 26798367]
- Rufiange A, Jacques PE, Bhat W, Robert F, & Nourani A (2007). *Molecular Cell*, 27, 393–405. [PubMed: 17679090]
- Ruhl DD, Jin J, Cai Y, Swanson S, Florens L, Washburn MP, et al. (2006). *Biochemistry*, 45, 5671–5677. [PubMed: 16634648]
- Runge JS, Raab JR, & Magnuson T (2018). *G3: genes—genomes—genetics*, 8, 1095–1102. [PubMed: 29432129]
- Saladi SV, & de la Serna IL (2010). *Stem Cell Reviews and Reports*, 6, 62–73.
- Santisteban MS, Hang M, & Smith MM (2011). *Molecular Cell Biology*, 31, 1848–1860.
- Santoro SW, & Dulac C (2012). *eLife*, 1, e00070. [PubMed: 23240083]
- Sarma K, Cifuentes-Rojas C, Ergun A, Del Rosario A, Jeon Y, White F, et al. (2014). *Cell*, 159, 869–883. [PubMed: 25417162]
- Schaniel C, Ang Y-S, Ratnakumar K, Cormier C, James T, Bernstein E, et al. (2009). *Stem Cells (Dayton, Ohio)*, 27, 2979–2991.
- Schenk R, Jenke A, Zilbauer M, Wirth S, & Postberg J (2011). *Chromosoma*, 120, 275–285. [PubMed: 21274551]
- Schlesinger S, Kaffe B, Melcer S, Aguilera JD, Sivaraman DM, Kaplan T, et al. (2017). *Nucleic Acids Research*, 45, 12181–12194. [PubMed: 29036702]
- Schneider J, Bajwa P, Johnson FC, Bhaumik SR, & Shilatifard A (2006). *The Journal of Biological Chemistry*, 281, 37270–37274. [PubMed: 17046836]
- Schnetz MP, Handoko L, Akhtar-Zaidi B, Bartels CF, Pereira CF, Fisher AG, et al. (2010). *PLoS Genetics*, 6, e1001023. [PubMed: 20657823]
- Schwabish M, & Struhl K (2004). *Molecular Cell Biology*, 24, 10111–10117.
- Seto E, & Yoshida M (2014). *Cold Spring Harbor Perspectives in Biology*, 6, a018713. [PubMed: 24691964]
- Shan Y, Liang Z, Xing Q, Zhang T, Wang B, Tian S, et al. (2017). *Nature Communications*, 8, 672.
- Shao Z, Raible F, Mollaaghababa R, Guyon JR, Wu CT, Bender W, et al. (1999). *Cell*, 98, 37–46. [PubMed: 10412979]
- Sharma A, Singh K, & Almasan A (2012). *Methods in Molecular Biology*, 920, 613–626. [PubMed: 22941631]
- Shen Z, Formosa T, & Tantin D (2018). *Stem Cells and Development*, 27, 1693–1701. [PubMed: 30319048]
- Shi Y (2007). *Nature Reviews Genetics*, 8, 829.
- Shinagawa T, Takagi T, Tsukamoto D, Tomaru C, Huynh LM, Sivaraman P, et al. (2014). *Cell Stem Cell*, 14, 217–227. [PubMed: 24506885]
- Shivaswamy S, Bhinge A, Zhao Y, Jones S, Hirst M, & Iyer VR (2008). *PLoS Biology*, 6, e65. [PubMed: 18351804]
- Shivaswamy S, & Iyer V (2008). *Molecular and Cell Biology*, 28, 2221–2234.
- Shogren-Knaak M, Ishii H, Pazin M, Davie J, & Peterson CL (2006). *Science*, 311, 4.

- Shuaib M, Ouararhni K, Dimitrov S, & Hamiche A (2010). Proceedings of the National Academy of Sciences of the United States of America, 107, 1349–1354.
- Shukla S, Kavak E, Gregory M, Imashimizu M, Shutinoski B, Kashlev M, et al. (2011). Nature, 479, 74. [PubMed: 21964334]
- Simic R, Lindstrom DL, Tran HG, Roinick KL, Costa PJ, Johnson AD, et al. (2003). The EMBO Journal, 22, 1846–1856. [PubMed: 12682017]
- Siuti N, Roth MJ, Mizzen CA, Kelleher NL, & Pesavento JJ (2006). Journal of Proteome Research, 5, 233–239. [PubMed: 16457587]
- Skene PJ, & Henikoff S (2013). Development, 140, 2513–2524. [PubMed: 23715545]
- Skene PJ, & Henikoff S (2017). eLife, 6, 1–35.
- Smith E, & Shilatifard A (2010). Molecular Cell, 40, 689–701. [PubMed: 21145479]
- Smith S, & Stillman B (1989). Cell, 58, 15–25. [PubMed: 2546672]
- Solomon MJ, Larsen PL, & Varshavsky A (1988). Cell, 53, 937–947. [PubMed: 2454748]
- Squarrito M, Gorrini C, & Amati B (2006). Trends in Cell Biology, 16, 433–442. [PubMed: 16904321]
- Stanton BZ, Hodges C, Calarco JP, Braun SMG, Ku WL, Kadoch C, et al. (2017). Nature Genetics, 49, 282–288. [PubMed: 27941795]
- Stern M, Jensen R, & Herskowitz I (1984). Journal of Molecular Biology, 178, 853–868. [PubMed: 6436497]
- Stillman B (1986). Cell, 45, 555–565. [PubMed: 3011272]
- Stopka T, & Skoultschi AI (2003). Proceedings of the National Academy of Sciences of the United States of America, 100, 14097–14102.
- Studitsky VM, Clark DJ, & Felsenfeld G (1994). Cell, 76, 371–382. [PubMed: 8293470]
- Studitsky VM, Kassavetis GA, Geiduschek EP, & Felsenfeld G (1997). Science, 278, 1960–1963. [PubMed: 9395401]
- Stuwe T, Hothorn M, Lejeune E, Rybin V, Bortfeld M, Scheffzek K, et al. (2008). Proceedings of the National Academy of Sciences of the United States of America, 105, 8884–8889.
- Subramanian V, Mazumder A, Surface LE, Butty VL, Fields PA, Alwan A, et al. (2013). PLoS Genetics, 9, e1003725. [PubMed: 23990805]
- Sullivan SA, & Landsman D (2003). Proteins, 52, 454–465. [PubMed: 12866056]
- Sun Z, Filipescu D, Andrade J, Gaspar-Maia A, Ueberheide B, & Bernstein E (2018). Nature Structural & Molecular Biology, 25, 958–970.
- Surface LE, Fields PA, Subramanian V, Behmer R, Udeshi N, Peach SE, et al. (2016). Cell Reports, 14, 1142–1155. [PubMed: 26804911]
- Suto R, Clarkson M, Tremethick D, & Luger K (2000). Nature Structural Biology, 7, 1121–1124. [PubMed: 11101893]
- Szerlong H, Hinata K, Viswanathan R, Erdjument-Bromage H, Tempst P, & Cairns BR (2008). Nature Structural & Molecular Biology, 15, 469–476.
- Tachiwana H, Kagawa W, Shiga T, Osakabe A, Miya Y, Saito K, et al. (2011). Nature, 476, 232. [PubMed: 21743476]
- Tachiwana H, Osakabe A, Shiga T, Miya Y, Kimura H, Kagawa W, et al. (2011). Acta Crystallographica. Section D, Biological Crystallography, 67, 578–583. [PubMed: 21636898]
- Tagami H, Ray-Gallet D, Almouzni G, & Nakatani Y (2004). Cell, 116, 51–61. [PubMed: 14718166]
- Takahashi YH, Westfield GH, Oleskie AN, Trievel RC, Shilatifard A, & Skiniotis G (2011). Proceedings of the National Academy of Sciences of the United States of America, 108, 20526–20531.
- Takahashi K, & Yamanaka S (2006). Cell, 126, 663–676. [PubMed: 16904174]
- Takaku M, Grimm SA, Shimbo T, Perera L, Menafrá R, Stunnenberg HG, et al. (2016). Genome Biology, 17, 36. [PubMed: 26922637]
- Takeuchi JK, & Bruneau BG (2009). Nature, 459, 708–711. [PubMed: 19396158]
- Tan Y, Xue Y, Song C, & Grunstein M (2013). Proceedings of the National Academy of Sciences of the United States of America, 110, 11493–11498.

- Tang Y, Poustovoitov MV, Zhao K, Garfinkel M, Canutescu A, Dunbrack R, et al. (2006). *Nature Structural & Molecular Biology*, 13, 921–929.
- Tavares L, Dimitrova E, Oxley D, Webster J, Poot R, Demmers J, et al. (2012). *Cell*, 148, 664–678. [PubMed: 22325148]
- Timinszky G (2009). *Nature Structural & Molecular Biology*, 16, 7.
- Tirosh I, Sigal N, & Barkai N (2010). *Genome Biology*, 11, 1–13 R49.
- Tjeertes JV, Miller KM, & Jackson SP (2009). *The EMBO Journal*, 28, 1878–1889. [PubMed: 19407812]
- Tomioka M, Nishimoto M, Miyagi S, Katayanagi T, Fukui N, Niwa H, et al. (2002). *Nucleic Acids Research*, 30, 3202–3213. [PubMed: 12136102]
- Tomson BN, & Arndt KM (2013). *Biochimica et Biophysica Acta*, 1829, 116–126. [PubMed: 22982193]
- Tong JK, Hassig CA, Schnitzler GR, Kingston RE, & Schreiber SL (1998). *Nature*, 395, 917–921. [PubMed: 9804427]
- Tsai PH, Chien Y, Wang ML, Hsu CH, Laurent B, Chou SJ, et al. (2019). *Nucleic Acids Research*, 47, 10115–10133. [PubMed: 31555818]
- Tsubota T, Berndsen CE, Erkmann JA, Smith CL, Yang L, Freitas MA, et al. (2007). *Molecular Cell*, 25, 703–712. [PubMed: 17320445]
- Turinetto V, Orlando L, Sanchez-Ripoll Y, Kumpfmüller B, Storm MP, Porcedda P, et al. (2012). *Stem Cells*, 30, 1414–1423. [PubMed: 22628289]
- Tutter AV, Kowalski MP, Baltus GA, Iourgenko V, Labow M, Li E, et al. (2009). *The Journal of Biological Chemistry*, 284, 3709–3718. [PubMed: 19036726]
- Ueda J, Harada A, Urahama T, Machida S, Maehara K, Hada M, et al. (2017). *Cell Reports*, 18, 593–600. [PubMed: 28099840]
- Urahama T, Harada A, Maehara K, Horikoshi N, Sato K, Sato Y, et al. (2016). *Epigenetics & Chromatin*, 9, 2. [PubMed: 26779285]
- Van Attikum H, Fritsch O, Hohn B, & Gasser S (2004). *Cell*, 119, 11.
- van den Berg DL, Snoek T, Mullin NP, Yates A, Bezstarosti K, Demmers J, et al. (2010). *Cell Stem Cell*, 6, 369–381. [PubMed: 20362541]
- van Mierlo G, Veenstra GJC, Vermeulen M, & Marks H (2019). *Trends in Cell Biology*, 29, 660–671. [PubMed: 31178244]
- Varshavsky AJ, Sundin O, & Bohn M (1979). *Cell*, 16, 453–466. [PubMed: 222461]
- Vastenhouw NL, & Schier AF (2012). *Current Opinion in Cell Biology*, 24, 374–386. [PubMed: 22513113]
- Venkatesh S, & Workman JL (2015). *Nature Reviews. Molecular Cell Biology*, 16, 178–189. [PubMed: 25650798]
- Vierbuchen T, Ling E, Cowley CJ, Couch CH, Wang X, Harmin DA, et al. (2017). *Molecular Cell*, 68, 1067–1082.e12. [PubMed: 29272704]
- Vincenz C, & Kerppola TK (2008). *Proceedings of the National Academy of Sciences of the United States of America*, 105, 16572–16577.
- Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, et al. (2006). *Nature*, 439, 871–874. [PubMed: 16357870]
- Voigt P, Tee WW, & Reinberg D (2013). *Genes & Development*, 27, 1318–1338. [PubMed: 23788621]
- Vostrov AA, & Quitschke WW (1997). *The Journal of Biological Chemistry*, 272, 33353–33359. [PubMed: 9407128]
- Vostrov AA, Taheny MJ, & Quitschke WW (2002). *Journal of Biological Chemistry*, 277, 1619–1627. [PubMed: 11706010]
- Wade PA, Jones PL, Vermaak D, & Wolffe AP (1998). *Current Biology*, 8, 843–846. [PubMed: 9663395]
- Wan Y, Saleem RA, Ratushny AV, Roda O, Smith JJ, Lin CH, et al. (2009). *Molecular Cell Biology*, 29, 2346–2358.

- Wang W, Côté J, Xue Y, Zhou S, Khavari PA, Biggar SR, et al. (1996). *The EMBO Journal*, 15, 5370–5382. [PubMed: 8895581]
- Wang L, Du Y, Ward JM, Shimbo T, Lackford B, Zheng X, et al. (2014). *Cell Stem Cell*, 14, 575–591. [PubMed: 24792115]
- Wang Y, Long H, Yu J, Dong L, Wassef M, Zhuo B, et al. (2018). *BMC Biology*, 16, 107. [PubMed: 30249243]
- Wang X, Wang S, Troisi EC, Howard TP, Haswell JR, Wolf BK, et al. (2019). *Nature Communications*, 10, 1–11 1881.
- Weiner A, Hughes A, Yassour M, Rando OJ, & Friedman N (2010). *Genome Research*, 20, 90–100. [PubMed: 19846608]
- West MH, & Bonner WM (1980). *Biochemistry*, 19, 3238–3245. [PubMed: 7407044]
- Whetstine JR, Nottke A, Lan F, Huarte M, Smolikov S, Chen Z, et al. (2006). *Cell*, 125, 467–481. [PubMed: 16603238]
- White CL, & Luger K (2004). *Journal of Molecular Biology*, 342, 1391–1402. [PubMed: 15364568]
- Whyte WA, Bilodeau S, Orlando DA, Hoke HA, Frampton GM, Foster CT, et al. (2012). *Nature*, 482, 221–225. [PubMed: 22297846]
- Whyte WA, Orlando DA, Hnisz D, Abraham BJ, Lin CY, Kagey MH, et al. (2013). *Cell*, 153, 307–319. [PubMed: 23582322]
- Wiechens N, Singh V, Gkikopoulos T, Schofield P, Rocha S, & Owen-Hughes T (2016). *PLoS Genetics*, 12, e1005940. [PubMed: 27019336]
- Wiedemann SM, Mildner SN, Bonisch C, Israel L, Maiser A, Matheisl S, et al. (2010). *The Journal of Cell Biology*, 190, 777–791. [PubMed: 20819935]
- Wirbelauer C, Bell O, & Schubeler D (2005). *Genes & Development*, 19, 1761–1766. [PubMed: 16077006]
- Wong MM, Cox LK, & Chrivia JC (2007). *The Journal of Biological Chemistry*, 282, 26132–26139. [PubMed: 17617668]
- Wu JI, Lessard J, Olave IA, Qiu Z, Ghosh A, Graef IA, et al. (2007). *Neuron*, 56, 94–108. [PubMed: 17920018]
- Wysocka J, Swigut T, Xiao H, Milne TA, Kwon SY, Landry J, et al. (2006). *Nature*, 442, 86–90. [PubMed: 16728976]
- Xia W, & Jiao J (2017). *Cell Death and Differentiation*, 24, 1548–1563. [PubMed: 28524856]
- Xie W, Song C, Young NL, Sperling AS, Xu F, Sridharan R, et al. (2009). *Molecular Cell*, 33, 417–427. [PubMed: 19250903]
- Xu M, Barnard MB, Rose SM, Cockerill PN, Huang SY, & Garrard WT (1986). *The Journal of Biological Chemistry*, 261, 3838–3845. [PubMed: 3081510]
- Xue Y, Van C, Pradhan SK, Su T, Gehrke J, Kuryan BG, et al. (2015). *Genes & Development*, 29, 350–355. [PubMed: 25691465]
- Xue Y, Wong J, Moreno GT, Young MK, Côté J, & Wang W (1998). *Molecular Cell*, 2, 851–861. [PubMed: 9885572]
- Yadon AN, & Tsukiyama T (2011). *Cell*, 144, 453–453.e1. [PubMed: 21295704]
- Yan W, Ma L, Burns KH, & Matzuk MM (2003). *Proceedings of the National Academy of Sciences of the United States of America*, 100, 10546–10551.
- Yan Y, Zhao W, Huang Y, Tong H, Xia Y, Jiang Q, et al. (2017). *Scientific Reports*, 7, 46276. [PubMed: 28393894]
- Yang B, Zwaans BM, Eckersdorff M, & Lombard DB (2009). *Cell Cycle*, 8, 2662–2663. [PubMed: 19597350]
- Ye B, Liu B, Yang L, Huang G, Hao L, Xia P, et al. (2017). *Nature Communications*, 8, 1518.
- Ye B, Liu B, Yang L, Zhu X, Zhang D, Wu W, et al. (2018). *The EMBO Journal*, 37, 1–15 (2018). [PubMed: 29212815]
- Yelagandula R, Stroud H, Holec S, Zhou K, Feng S, Zhong X, et al. (2014). *Cell*, 158, 98–109. [PubMed: 24995981]

- Yen K, Vinayachandran V, Batta K, Koerber RT, & Pugh BF (2012). *Cell*, 149, 1461–1473. [PubMed: 22726434]
- Yen K, Vinayachandran V, & Pugh BF (2013). *Cell*, 154, 1246–1256. [PubMed: 24034248]
- Yildirim O, Li R, Hung J-H, Chen PB, Dong X, Ee L-S, et al. (2011). *Cell*, 147, 1498–1510. [PubMed: 22196727]
- Yin J-W, & Wang G (2014). *Development*, 141, 977–987. [PubMed: 24550107]
- Yoda K, Ando S, Morishita S, Houmura K, Hashimoto K, Takeyasu K, et al. (2000). *Proceedings of the National Academy of Sciences of the United States of America*, 97, 7266–7271.
- Yoshida T, Hazan I, Zhang J, Ng SY, Naito T, Snippert HJ, et al. (2008). *Genes & Development*, 22, 1174–1189. [PubMed: 18451107]
- Youdell ML, Kizer KO, Kisseleva-Romanova E, Fuchs SM, Duro E, Strahl BD, et al. (2008). *Molecular Cell Biology*, 28, 4915–4926.
- Young RA (2011). *Cell*, 144, 940–954. [PubMed: 21414485]
- Zalensky AO, Tomilin NV, Zalenskaya IA, Teplitz RL, & Bradbury EM (1997). *Experimental Cell Research*, 232, 29–41. [PubMed: 9141618]
- Zaret KS, & Carroll JS (2011). *Genes & Development*, 25, 2227–2241. [PubMed: 22056668]
- Zaret KS, Lerner J, & Iwafuchi-Doi M (2016). *Molecular Cell*, 62, 665–667. [PubMed: 27259199]
- Zawadzki K, Morozov A, & Broach J (2009). *Molecular Cell Biology*, 20, 3451–3617.
- Zee BM, Levin RS, Xu B, LeRoy G, Wingreen NS, & Garcia BA (2010). *The Journal of Biological Chemistry*, 285, 3341–3350. [PubMed: 19940157]
- Zentner G, Tesar P, & Scacheri P (2011). *Genome Research*, 21, 10.
- Zhang R, Erler J, & Langowski J (2017). *Biophysical Journal*, 112, 450–459. [PubMed: 27931745]
- Zhang Y, LeRoy G, Seelig H-P, Lane WS, & Reinberg D (1998). *Cell*, 95, 279–289. [PubMed: 9790534]
- Zhang X, Li B, Li W, Ma L, Zheng D, Li L, et al. (2014). *Stem Cell Reports*, 3, 460–474. [PubMed: 25241744]
- Zhang H, Roberts D, & Cairns BR (2005). *Cell*, 123, 219–231. [PubMed: 16239141]
- Zhao H, Han Z, Liu X, Gu J, Tang F, Wei G, et al. (2017). *The Journal of Biological Chemistry*, 292, 8507–8519. [PubMed: 28298436]
- Zhao W, Huang Y, Zhang J, Liu M, Ji H, Wang C, et al. (2017). *The Journal of Biological Chemistry*, 292, 21527–21537. [PubMed: 29054931]
- Zhao R, Nakamura T, Fu Y, Lazar Z, & Spector D (2011). *Nature Cell Biology*, 13, 9.
- Zhao W, Tong H, Huang Y, Yan Y, Teng H, Xia Y, et al. (2017). *The Journal of Biological Chemistry*, 292, 2773–2784. [PubMed: 28049731]
- Zhou R, Bonneaud N, Yuan CX, de Santa Barbara P, Boizet B, Schomber T, et al. (2002). *Nucleic Acids Research*, 30, 3245–3252. [PubMed: 12136106]
- Zhou BR, Feng H, Kato H, Dai L, Yang Y, Zhou Y, et al. (2013). *Proceedings of the National Academy of Sciences of the United States of America*, 110, 19390–19395.
- Zhou HY, Katsman Y, Dhaliwal NK, Davidson S, Macpherson NN, Sakthidevi M, et al. (2014). *Genes & Development*, 28, 2699–2711. [PubMed: 25512558]
- Zhu D, Fang J, Li Y, & Zhang J (2009). *PLoS One*, 4, e7684. [PubMed: 19888462]
- Zhu F, Farnung L, Kaasinen E, Sahu B, Yin Y, Wei B, et al. (2018). *Nature*, 562, 76–81. [PubMed: 30250250]
- Zink LM, Delbarre E, Eberl HC, Keilhauer EC, Bonisch C, Punzeler S, et al. (2017). *Nucleic Acids Research*, 45, 5691–5706. [PubMed: 28334823]

Fig. 1.

Model of the crystal structure of the nucleosome. The nucleosome is comprised of 147 base pairs of double-stranded DNA wrapped around an octamer of histone proteins, including two copies each of H2A, H2B, H3, and H4. Nucleosomes contain numerous structural features, including the nucleosome dyad, the DNA entry/exit sites, the histone acidic patch, and the histone fold domain. PDB ID: 1AO1, Luger et al., 1997.

Fig. 2.

Chromatin packaging regulates DNA-templated processes. Nucleosome remodeling factors facilitate transitions between accessible chromatin (euchromatin) and closed chromatin (heterochromatin). Open chromatin is permissive of transcription factor binding and recruitment of other factors, in turn promoting DNA-templated processes such as transcription.

Fig. 3.

Non-canonical nucleosome structures are generated through the action of chromatin modifying proteins. Overlapping dinucleosomes are 14-mers of histone proteins generated when the sliding action of a nucleosome remodeler (e.g., yeast RSC) forces a nucleosome to crash into the neighboring nucleosome, thereby displacing an H2A/H2B dimer. Subnucleosomes are generated when H2A/H2B dimers are removed from the octasome (canonical nucleosome), a process often carried out by H2A/H2B chaperones (e.g., the FACT complex). This dimer removal can occur once, leaving behind a hexasome composed of the H3/H4 tetramer and one H2A/H2B dimer, or twice, leaving behind the H3/H4 tetramer alone.

Fig. 4.

ATPase domains of the four nucleosome remodeling factor families. ATPases in the SWI/SNF, ISWI, CHD, and INO80 nucleosome remodeling factor families all share related DEXDc (RecA-like Lobe 1) and HELICc (RecA-like Lobe 2) domains. SWI/SNF family ATPases are defined by an N-terminal HSA domain and a C-terminal bromodomain. ISWI family ATPases are defined by a C-terminal module consisting of HAND, SANT, and SLIDE domains. CHD family ATPases are defined by an N-terminal dual tandem chromodomain. INO80 family ATPases feature an N-terminal HSA domain like SWI/SNF remodelers, but not a C-terminal bromodomain.

Fig. 5.

Nucleosome remodeling factor complexes are assembled in a stepwise manner. The assembly process of the SWI/SNF family nucleosome remodeler esBAF is depicted. An initial homodimerization event between BAF155 dimers is necessary before any other esBAF subunits can be loaded. Following this dimerization, a BAF60 subunit is loaded onto the complex to form the “BAF initial core complex.” Core subunits BAF47 and BAF57 are subsequently assembled to form the BAF core complex, at which point an ATPase module containing BRG1, BAF53A, ARID1A, a BAF45 subunit, and SS18 is added to form the full esBAF complex.

Fig. 6. Histones are post-translationally modified as a mechanism of epigenetic regulation. Histone proteins have N- and C-terminal tails that are highly modified, although residues within the globular domains can also be modified. The most common histone post-translational modifications are methylation and acetylation of lysine residues, although numerous modifications not listed in this figure have been identified.

Fig. 7.

Bivalent promoters mark lowly expressed but poised genes in stem cells. Between 3000 and 4000 promoters in embryonic stem cells are marked by both an activation-associated modification (H3K4me3) and a repression-associated modification (H3K27me3), and are thus known as bivalent promoters. Bivalent genes remain accessible to transcription factors, but lowly expressed until the H3K27me3 mark is removed (often upon a cellular differentiation event).

Fig. 8.

3D chromatin interactions occur in defined regions of the nucleus. The chromatin that compacts to form each chromosome occupies a defined region of space within the nucleus, known as a chromosome territory. Within these chromosome territories are compartments of active and inactive chromatin that house topologically-associating domains, or TADs. 3D interaction frequency (such as promoter-enhancer looping or CTCF-CTCF looping) is heightened within, but not across, TADs. PDB IDs used: 5N9J, 5SVA.

Table 1

Families of nucleosome remodeling factor ATPases.

Nucleosome remodeler family	Gene (ATPase)	Protein (ATPase)
SWI/SNF	<i>ATRX</i>	Atrx
	<i>RAD54B</i>	Rad54B
	<i>RAD54L</i>	Rad54
	<i>RAD54L2</i>	Arip4
	<i>SMARCA2</i>	Brm
	<i>SMARCA4</i>	Brg 1
	<i>HELLS</i>	Hells
	<i>ERCC6</i>	Eccc6
	<i>ERCC6L</i>	Eccc6L
	<i>ZRANB3</i>	Zranb3
	<i>SMARCAL</i>	Harp
	<i>SMARCAD</i>	Hel 1
	<i>BTAFL</i>	Btaf1
	<i>HLTF</i>	Hltf
	<i>TTF2</i>	Ttf2
	<i>SHPRH</i>	Shprh
ISWI	<i>SMARCA1</i>	Snf2h
	<i>SMARCA5</i>	Snf2l
INO80	<i>INO80</i>	Ino80
	<i>SRCAP</i>	Srcap
	<i>EP400</i>	p400
CHD	<i>CHD1</i>	Chd1
	<i>CHD1L</i>	Chd1L
	<i>CHD2</i>	Chd2
	<i>CHD3</i>	Chd3
	<i>CHD4</i>	Chd4
	<i>CHD5</i>	Chd5
	<i>CHD6</i>	Chd6
	<i>CHD7</i>	Chd7
	<i>CHD8</i>	Chd8
	<i>CHD9</i>	Chd9

Mammals express 31 nucleosome remodeling factor ATPases, each of which belongs to one of the following families (with number of ATPases listed in parentheses): SWI/SNF (16), ISWI (2), INO80 (3), and CHD (10). Familial classifications are primarily based on structural characteristics; as such, nucleosome remodeling factors within the same family can have wildly different mechanisms of action and functional outcomes.

Table 2

Common histone proteins, chaperones that exchange them, and associated localization and processes.

Histone dimer	Exchanging chaperones	Localization	Associated processes
H2A/H2B	FACT, NPM1/2, Nap1, APLF, SET/TAF1b, CINAP, Nucleolin, ISWI, HSP90A/B, HSC70, IPO9, NPM2	Genome-wide	Core histones
H2A.X/H2B	FACT; high sequence similarity so likely many canonical H2A chaperones	DNA damage sites	DNA damage repair
H2A.Z/H2B	FACT, Tip60/p400, SRCAP, IPO9, Nap1, Anp32e	Sites of active transcription, heterochromatin/euchromatin boundaries	Active transcription, regulatory regions
macroH2A/H2B macroH2A.2/H2B	ATRX; unknown but maybe APLF	Deposited genome-wide, but condensed to repressed regions	Heterochromatin compaction
H3/H4	MCM2–7, HAT, IPO4, CAF1, HDAC, NURF, NURD, PRC2, HIRA, ATRX, DAXX, EP400, ASF1a/b, Cabin1, DEK	Genome-wide	Core histones
H3.3/H4	ATRX, DAXX, HIRA	Sites of active transcription, regulatory regions, telomeres, pericentric heterochromatin	Heterochromatin compaction, active transcription, differentiation
CENP-A/H4	HJURP, RBBP4, DAXX	Centromeres	Core histones
H3/H4G	Unknown	Nucleolus, rDNA	Breast cancer progression
H1	HSP90A/B, HAT, IPO9	Heterochromatin	Higher-order chromatin compaction

Core histone proteins conserved throughout eukaryotes are listed, as well as selected histone variants that are highly conserved and/or have important functions in gene regulation. Generally, core histones are synthesized in a replication-dependent manner, while variant histones are synthesized and incorporated throughout the cell cycle.

Table 3

Uncommon histone variants and their associated processes.

Histone variant	Implicated processes	Associated references
H1.1-H1.5	Replication-dependent Histone H1 variants found in somatic cells	Izzo et al. (2017)
H1.0	Replication-independent linker histone found in somatic cells	Izzo et al. (2017)
H1.10	Replication-independent linker histone found in somatic cells	Izzo et al. (2017)
H1LS1	Spermatid-specific; important in chromatin remodeling during spermatogenesis	Yan, Ma, Burns, and Matzuk (2003)
H1T2	Spermiogenesis, DNA compaction; exclusive to male haploid germ cells	Martianov et al. (2005)
H1x	Enriched in closed chromatin, accumulates in nucleolus during G1	Happel, Schulze, and Doenecke (2005)
H1oo	Oocyte-specific; maintains expression of pluripotency genes like Nanog, MYC, and KLF9; essential for mouse meiosis	Hayakawa, Ohgane, Tanaka, Yagi, and Shiota (2012) and Furuya et al. (2007)
H3T/H3.4	Development; essential for spermatogenesis	Ueda et al. (2017)
H3.5	Spermatogenesis; transcription initiation; forms an unstable nucleosome	Urahama et al. (2016) and Schenk, Jenke, Zilbauer, Wirth, and Postberg (2011)
H3.X	Primate-specific; transcribed, but not detectable at protein level <i>in vivo</i>	Wiedemann et al. (2010)
H3.Y	Primate-specific, differentiates between HIRA and DAXX chaperones and features many novel amino acids near DNA entry/exit sites	Kujirai et al. (2017), Wiedemann et al. (2010), and Zink et al. (2017)
H2B.W	Spermiogenesis, testis-specific	Siuti, Roth, Mizzen, Kelleher, and Pesavento (2006) and Zalensky, Tomilin, Zalenskaya, Teplitz, and Bradbury (1997)
H2B.Z	H2AZ interaction; apicomplexan specific	Hoeijmakers et al. (2013) and Petter et al. (2013)
Sperm H2B	Development; Echinoidea-specific	Oliver et al. (2002)
subH2B	Higher eukaryotes; spermiogenesis-specific	Aul and Oko (2001)
H2B.E	Murine olfactory neurons	Santoro and Dulac (2012)
TH2A	Testis, oocyte, and zygote-specific	Shinagawa et al. (2014)
H2A.B	Associated with transcription upregulation, active gene TSSs in testes, arginine-rich	Arimura et al. (2015) and Molaro, Young, and Malik (2018)
H2A.Bbd	Human-specific (mouse H2A. Lap1); expressed in testis and brain	Arimura et al. (2015)
H2A.L	Pericentric chromatin organization in spermatids and spermiogenesis	Molaro et al. (2018)
H2A.P	Placental mammal-specific; putative variant similar to H2A.B and H2A.L	Molaro et al. (2018)
H2A.Q	Testis-specific, X-chromosome localized; may be involved in sex chromosome-related genetic conflicts	Molaro et al. (2018)
H2A.W	Plant-specific, SPKK motif-containing	Yelagandula et al. (2014)

Histone variants not listed in Table 2 are compiled here; most uncommon histone variants are highly cell-type-specific, and the overwhelming majority are specific to oocytes or testes. Uncommon histone variants have not been extensively studied; seminal papers and comprehensive reviews have been listed here for further information.