The Chromatin State during Gonadal Sex Determination

Shannon Dupont · Blanche Capel

Department of Cell Biology, Duke University Medical Center, Durham, NC, USA

Keywords

Sex determination · Chromatin · Histone modifications

Abstract

At embryonic day (E) 10.5, prior to gonadal sex determination, XX and XY gonads are bipotential and able to differentiate into either a testis or an ovary. At this point, they are transcriptionally and morphologically indistinguishable. Sex determination begins around E11.5 in the mouse when the supporting cell lineage commits to either Sertoli or granulosa cell fate. Testis-specific factors such as SRY and SOX9 drive differentiation of bipotential-supporting cells into the Sertoli cell pathway, whereas ovary-specific factors like WNT4 and FOXL2 guide differentiation into granulosa cells. It is known that these 2 pathways are mutually antagonistic, and repression of the alternative fate is critical for maintenance of the testis or ovary programs. While we understand much about the transcription factor networks guiding the process of sex determination, it is only more recently that we have begun to understand how this process is epigenetically controlled. Studies in the past decade have demonstrated the importance of the chromatin state for gene expression and cell fate commitment, with histone modifications and DNA accessibility having a direct role in gene regulation. It is now clear that the chromatin state during sex determination is dynamic and likely critical for the establishment and/or

maintenance of the transcriptional programs. Prior to sex determination, supporting cells have similar chromatin structure and histone modification profiles, reflecting the bipotential nature of these cells. After differentiation to Sertoli or granulosa cells, the chromatin state acquires sex-specific profiles. The proteins that regulate the deposition of histone modifications or the opening of compact chromatin likely play an important role in Sertoli and granulosa cell fate commitment and gonad development. Here, we describe studies profiling the chromatin state during gonadal sex determination and one example in which depletion of *Cbx2*, a member of the Polycomb Repressive Complex 1 (PRC1), causes male-to-female sex reversal due to a failure to repress the ovarian pathway.

© 2021 S. Karger AG, Basel

Introduction

Gonadal sex determination is the process by which the initially bipotential gonad initiates differentiation into either the testis or ovary. This process begins in the mouse at around embryonic (E)11.5 within the supporting cell lineage of the gonad. Prior to sex determination, bipotential supporting cells maintain identical transcriptional programs regardless of genetic sex [Nef et al., 2005; Jameson et al., 2012b; Munger et al., 2013; Stévant et al., 2018],

which likely reflects the early fate plasticity that allows these bipotential cells to commit to either Sertoli or granulosa cell fate. In XY supporting cells, sex determination begins with the expression of the Y-linked factor *Sry*. SRY activates its downstream target SOX9 and guides the differentiation of bipotential supporting cells into the Sertoli cells of the testis. In XX cells, upregulation of ovary-specific factors like WNT4 and FOXL2 guides the differentiation of bipotential supporting cells into the granulosa cells of the ovary.

While we understand much about the gene regulatory networks that govern Sertoli or granulosa fate determination, the role epigenetic factors play in shaping and stabilizing the fate of supporting cells is not well understood. As cells differentiate, they acquire heritable changes to their chromatin that establish and maintain cell type specific patterns of gene expression [Lee et al., 2004a; Azuara et al., 2006; Greer et al., 2012]. Histone-modifying complexes regulate chromatin structure by post-translationally modifying histones. The type and placement of these chemical modifications dictate the activity of the overlapping promoter or regulatory region. However, despite increasing evidence that cell differentiation is epigenetically regulated, little is known about the epigenetic mechanisms that regulate sex-determining genes during cell fate commitment and differentiation within the supporting cell lineage. Here, we outline studies that have provided insight into the chromatin state in the supporting cell lineage during and after sex determination.

Sex-Specific Chromatin Accessibility Patterns

The first attempt to understand the global chromatin state within the supporting cell lineage was a study by Maatouk et al. [2017] where DNaseI-seq was performed on purified Sertoli cells at E13.5 and E15.5, just after sex determination. Prior to this study, only a few cis regulatory elements important for Sertoli differentiation were known through investigations of the ability of candidate enhancers to regulate expression in transgenic mice [Arango et al., 1999; Sekido and Lovell-Badge, 2008]. To achieve a genome-wide understanding of the location of enhancer regions and the epigenetic landscape of Sertoli cells, Maatouk et al. [2017] purified E15.5 Sertoli cells by fluorescence activated cell sorting (FACS) using a *Sox9-EGFP* line and DNaseI-seq to identify about 81,000 DNaseI hypersensitive sites (DHSs). DHSs are open regions of chromatin which house putative regulatory sites that are accessible to transcription factors, repressors, or

RNA polymerase [Song et al., 2011]. About 60% of the total genes, or ~13,000 genes, had DHS peaks within their transcription start site (TSS) (Fig. 1a). Because the experiment was performed in isolated Sertoli cells, it was expected that regions of open chromatin would correlate with Sertoli-specific genes, whereas granulosa-specific genes would have closed chromatin domains near their TSSs. However, both Sertoli and granulosa genes had open chromatin upstream of their TSSs. For example, both *Sox9* (Sertoli-specific) and *Rspo1* (granulosa-specific) genes had DHS signals at their TSSs (Fig. 1b), although RNA-seq confirmed that the magnitude of the DHS peak at the promoter region correlated with the level of gene expression [Maatouk et al., 2017].

Histone modifications are post-translational modifications to histone proteins that can affect gene expression through direct changes to chromatin structure or through the recruitment of additional factors that recognize these marks. For example, acetylation of Histone 3 at Lysine 27 (H3K27ac) shows a strong association with active enhancers [Creyghton et al., 2010; Rada-Iglesias et al., 2011]. ChIP-seq for H3K27ac in Sertoli cells showed that 70% of H3K27ac+ peaks overlapped a DHS peak, were enriched around Sertoli-specific genes, and usually absent from granulosa genes (Fig. 1c, d). These results suggested that the open regions of chromatin neighboring granulosa genes in Sertoli cells were binding sites for repressive transcription factors or epigenetic regulators that block gene expression at these loci. In total, these data implied that both activation and repression of genes that determine the fate of supporting cells are mediated by epigenetic mechanisms [Maatouk et al., 2017].

García-Moreno et al. [2019a] generated a map of the chromatin landscape in XX and XY gonadal supporting cells during the bipotential stage and after commitment to the Sertoli or granulosa fate. To define chromatin accessibility patterns, the authors employed ATAC-seq, a more sensitive method to identify nucleosome-depleted regions (NDRs). They also used ChIP-seq for H3K27ac to identify active enhancers across the genome. They found that over time the chromatin landscape in the supporting cell lineage acquires sex-specific patterns in nucleosome-depleted regions (NDRs) which, similar to DHSs, are open regions of chromatin which house gene regulatory elements [Lee et al., 2004b]. ATAC-seq revealed just over 40,000 NDRs common to both XX and XY supporting cells prior to sex determination (E10.5). In supporting cells after sex determination (E13.5), the number of NDRs increased in both sexes. Interestingly, at E10.5, when XX and XY supporting cells are nearly indistinguishable [Nef

Fig. 1. Regulatory elements in Sertoli cells. **a** E15.5 Sertoli cells were put through DNaseI-seq, identifying 81,000 DNase hypersensitive sites (DHSs). About 60% of total genes had DHS peaks within their transcription start sites (TSS). These sites correspond to putative regulatory sites, where transcription factors or RNA polymerase can access chromatin to regulate gene expression. **b** Example of DNase and H3K27ac ChIP-seq tracks with corresponding significance indicated by solid black bars above each peak. It was expected that only Sertoli-specific genes would maintain DHSs in Sertoli cells, but instead both granulosa- and Sertoli-

specific genes (*Rspo1* and *Sox9*, respectively) showed open chromatin near the TSS (black boxed arrows). However, Sertoli genes showed higher DHS peaks at the promoter region (note difference in y axes). Note also that the H3K27ac peak (green) was significant near the Sertoli gene (*Sox9*) but not near the granulosa gene (*Rspo1*). **c** Overall, there was no significant difference in the number of gonad-specific (GS) DHSs per gene regulatory domain for Sertoli and granulosa genes, but H3K27ac marks (**d**) were highly enriched around Sertoli genes, and levels at granulosa genes were similar to all genes. Modified after Maatouk et al. [2017].

Fig. 2. Sex-specific chromatin patterns. **a** Graph showing the average number of NDRs per transcription start site in XX and XY supporting cells at timepoints indicated on the x axis. NDRs represented here are GS NDRs, filtered from ubiquitous NDRs based on cell type specificity. GS NDRs are more enriched near granulosa genes in both XX and XY cells at E10.5. At E13.5, GS NDRs increased in both XX and XY cells. Of the newly formed NDRs at E13.5, one-third were shared between the sexes, while two-thirds were specific for either Sertoli or granulosa cells. In XX cells GS NDRs were strongly biased toward granulosa genes, whereas in XY cells levels were similar between granulosa and Sertoli genes. **b** At E10.5 there are similar numbers of H3K27ac+ NDR between XX and XY cells, and by E13.5 the number of H3K27ac+ active enhancers also increased in both sexes, with a larger increase found in Sertoli cells. **c** By E13.5 in XX cells there was an equal distribution of H3K27ac- NDRs around Sertoli- and gran-

ulosa-promoting genes. In XY cells, H3K27ac- NDRs are significantly enriched around granulosa-promoting genes. **d** Transcription factor motif analysis of GS H3K27ac- NDRs in XY cells showed that the DMRT and SOX motifs were highly enriched near granulosa genes, whereas Sertoli genes in XX cells possessed only motifs for general gonad development factors. TSSs are indicated with black boxed arrows. **e** Venn diagrams showing the proportion of GS NDRs that were H3K27ac- in XX supporting cells (outlined in pink) and H3K27ac+ in XY supporting cells (outlined in blue). For each overlapping region example ChIP-seq tracks are shown below the Venn diagrams to demonstrate the chromatin landscape where both an enhancer or silencer could exist, depending on the sex. Note the lack of H3K27ac enrichment in XX cells near Sertoli genes and in XY cells near granulosa genes. Gene TSSs are indicated with black boxed arrows. Modified after García-Moreno et al. [2019a].

(For figure see next page.)

et al., 2005, Jameson et al., 2012b, Munger et al., 2013; Stévant et al., 2018], 90% of XX and 86% of XY ubiquitous NDRs are shared between the sexes. This suggests that bipotential supporting cells are not only morphologically and transcriptionally indistinguishable but also share similar chromatin landscapes. However, when only those NDRs that were gonad-specific (GS) were considered, it was found that GS NDRs were more enriched near granulosa-promoting genes at E10.5 (Fig. 2a). This suggests an epigenetic mechanism for the reported predisposition to granulosa fate in bipotential supporting cells [Jameson et al., 2012b]. After sex determination, at E13.5, only ~40% of NDRs were retained and those shared between XX and XY cells decreased to 55%. Of the newly formed NDRs at E13.5, one-third were shared between the sexes, while two-thirds were specific for either Sertoli or granulosa cells. This ATAC-seq data indicated that differentiation of bipotential supporting cells is accompanied by an increase in sex-specific NDRs that likely promote sex-specific transcriptional programs characteristic of Sertoli or granulosa cell fate.

This group also investigated how potential enhancer or silencer regions are acquired during sex determination. ChIP-seq results demonstrated that the number of NDRs marked with H3K27ac at E10.5 was similar in XX and XY cells. This number increased in both sexes by E13.5, with a larger increase found in Sertoli cells (Fig. 2b). In granulosa cells at E13.5, there were similar numbers of H3K27ac⁺ NDRs around Sertoli- and granulosa-promoting genes, whereas H3K27ac⁺ NDRs were significantly enriched in Sertoli cells around granulosa-promoting genes (Fig. 2c). Transcription factor (TF) motif analysis indicated that the SOX and DMRT motifs were highly represented in H3K27ac⁺ NDRs in XX cells, with only general gonad development motifs identified in XY cells (Fig. 2d). Since DMRT and SOX factors are known to block the activation of granulosa genes in Sertoli cells [Barrionuevo et al., 2006; Matson et al., 2011], the authors concluded that the H3K27ac⁺ NDRs enriched at granulosa genes house silencer regions bound by Sertoli factors

that repress the alternative pathway. One interesting finding from this study was that there were few shared GS NDRs that were H3K27ac⁺ in one sex and H3K27ac⁺ in the opposite sex. Near Sertoli genes, ~8.3% (308/3,698) of GS NDRs were H3K27ac⁺ in XX cells and H3K27ac⁺ in XY cells (shown in left Venn diagram of Fig. 2e). Near granulosa genes, ~4.3% (104/2,391) of GS NDRs were H3K27ac⁺ in XX cells and H3K27ac⁺ in XY cells (shown in right Venn diagram of Fig. 2e). This means that only ~8.3% of GS NDRs near Sertoli genes and ~4.3% of GS NDRs near granulosa genes could house both an enhancer and silencer depending on the sex. Together this would imply most cis regulatory elements near gonadal genes occupy distinct genomic regions [García-Moreno et al., 2019a].

Other Histone Modifications Associated with Gene Transcription

Histone 3 Lysine 4 (H3K4) methylation is highly enriched at active promoters, while H3K27 methylation is classically associated with transcriptional repression [Margueron et al., 2011; Shilatifard et al., 2012]. Despite their antagonistic nature, many developmental genes are marked by overlapping H3K27me₃ and H3K4me₃ in pluripotent cells. This “bivalency” plays an important role in maintaining pluripotency and is proposed to maintain developmental genes in a poised state for either activation or repression upon differentiation [Bernstein et al., 2006; Pan et al., 2007; Sachs et al., 2013]. Interestingly we see that the promoters of key sex-determining genes are bivalent prior to sex determination. García-Moreno et al. [2019b] showed through ChIP-seq analysis that in both pre-Sertoli and pre-granulosa cells the promoters of sex-determining genes, such as *Sox9*, *Fgf9*, *Wnt4*, and *Foxl2*, have both the H3K27me₃ and H3K4me₃ marks. Overall, about 30% of genes that become either Sertoli- or granulosa-specific were bivalent in both sexes at the bipotential stage (Fig. 3a). No significant differences in mean enrich-

Fig. 3. Histone modification landscape in supporting cells. **a** Pie charts for each sex at E10.5 and E13.5 indicating the percentage of sex-determining genes that have H3K27me₃ only (red), H3K4me₃ only (green), or both H3K27me₃ and H3K4me₃ (yellow) histone marks. **b** Toy diagrams of ChIP-seq tracks showing that Sertoli (*Sox9*) and granulosa (*Wnt4*) genes are bivalent at E10.5 in both XX and XY cells (TSS indicated below tracks with black boxed arrow). At E13.5, bivalency is maintained at genes associated with

the alternative pathway and resolved into H3K4me₃ only at active genes. **c** Model for epigenetic regulation of sex-determining genes. Sex-determining genes are initially bivalent. When activated, the histone demethylases KDM6B and UTX may be recruited to remove the repressive histone modifications to resolve bivalency into H3K4me₃ only. Alternatively, gene repression is maintained via Polycomb proteins, which regulate the deposition of H3K27me₃ by PRC2 followed by chromatin compaction by PRC1.

(For figure see next page.)

ment levels of H3K27me3 and H3K4me3 between XX and XY E10.5 cells were identified, indicating that the deposition of these histone modifications contributes to their bipotential state, rather than serving to prime cells for one specific fate.

After sex determination, at E13.5, the promoters of genes that were activated lost the repressive H3K27me3 mark in that sex, while genes that were silenced retained their bivalency (Fig. 3b). About 68% of Sertoli genes that were bivalent at E10.5 lost the H3K27me3 mark to become H3K4me3+ only in XY cells. In XX cells, only 45% of the bivalent ovary-determining genes were resolved into H3K4me3-positive only. While H3K27me3 was significantly lost by E13.5, enrichment of H3K4me3 did not increase significantly at these genes but remained at levels similar to the bipotential stage. These results suggest that (1) sex-determining genes are poised for activation (or repression) at the bipotential stage, (2) activation of genes is associated with removal of H3K27me3 marks, while (3) repression of the alternative supporting cell fate requires maintenance of existing H3K27me3 repressive marks rather than de novo deposition of these marks after commitment to the Sertoli or granulosa program (although in some instances, the domain of H3K27me3 marks is expanded) [Garcia-Moreno et al., 2019b].

Removal of the H3K27me3 mark may be critical for gene activation. While passive loss of this mark through proliferation could explain the loss of H3K27me3 in XY cells, this cannot explain the loss of H3K27me3 in XX cells, which are in cell cycle arrest throughout this period of development. Mammals have 2 known H3K27me3-specific demethylases: KDM6B and UTX (KDM6A). A homologue of *Utx*, called *Uty*, exists on the Y chromosome, but a mutation has eliminated its demethylase activity [Shpargel et al., 2012]. Whether one or more of these genes is required to activate Sertoli or granulosa genes during sex determination is currently unknown (Fig. 3c). Provocatively, KDM6B plays an important role in sex determination in the red-eared slider turtle, *Trachemys scripta* [Ge et al., 2018; Weber et al., 2020]

Role of PRC1 in Supporting Cells

H3K27 methylation is deposited by Polycomb group proteins (PcG), which form the Polycomb Repressive Complex 1 and 2 (PRC1/2) (Fig. 3c). CBX2, a component of PRC1, is required to establish the male pathway in mice [Kato-Fukui et al., 1998, 2012] and in humans [Biaison-Lauber et al., 2009]. It was proposed that sex reversal was

caused by an inability to activate *Sry* [Kato-Fukui 1998, 2012]; however, whether this activation was direct or indirect was unknown. The fact that CBX2 is a repressor and not an activator and accumulated evidence indicating that male sex determination is established in part by stable repression of genes in the female pathway that oppose male development [Kim et al., 2006; Maatouk et al., 2008; Jameson et al., 2012a; Munger et al., 2013] suggested that the downregulation of *Sry* expression in *Cbx2*^{-/-} mutants might be secondary to a failure to repress the female pathway. In support of this idea, Garcia-Moreno and colleagues [2019b] showed that cells in the *Cbx2*^{-/-} XY gonad express SOX9 and FOXL2 simultaneously, with a reduction in SOX9-positive cells over time. *Sry* was detected in the *Cbx2*; *Wnt4* double-knockout gonads, indicating that CBX2 is not required for its activation. Stabilization of SOX9 and the male pathway could be rescued by deleting *Wnt4*, the main driver of the female pathway. These results are consistent with the idea that the initiation phase of male sex determination occurred normally, but the stabilization phase, in which the female pathway is silenced by epigenetic chromatin regulation, was disrupted in the absence of CBX2. Furthermore, at E13.5 genes associated with the female pathway (e.g., *Lef1* and *Foxl2*) acquire extended domains of H3K27me3 in wild type XY cells. In a parallel manner, *Sox9* and *Dmrt1* acquired extended H3K27me3 domains in XX cells [Garcia-Moreno et al., 2019b]. Although some male pathway genes are repressed in XX gonads [Munger et al., 2013], loss of *Cbx2* does not cause female-to-male sex reversal. This might mean that repression of the male pathway is not critical for ovary development, or alternatively, that another Cbx paralog is a critical component of the Polycomb complex in XX gonads. The role of Polycomb-mediated repression in the maintenance of supporting cell identity was further supported by a recent study that found that loss of PRC1 in neonatal XY mice causes derepression of granulosa genes. In this study the authors depleted PRC1 in postnatal Sertoli cells using loss-of-function mouse models for the PRC1 components RING1A and RING1B. These PRC1-deficient neonatal Sertoli cells exhibited increased cell death and decreased proliferation compared with wild type cells, accompanied by a loss of repressive histone modifications at granulosa genes [Maezawa et al., 2021]. It is thus possible that disruption of an invariant component of the PRC1 complex prior to sex determination causes female-to-male sex reversal. This remains to be investigated.

Conclusion

Evidence gathered so far supports a critical but underexplored role for chromatin dynamics during fate determination and stabilization within the supporting cell lineage. However, it is currently unclear whether changes to the chromatin state are more important for the establishment of Sertoli and granulosa identity or in stabilization and maintenance of fate commitments [Uhlenhaut et al., 2009; Matson et al., 2011]. The supporting cell lineage is a unique and biologically rich model to study the interplay between cell fate commitment and transcriptional and epigenetic changes. Each bipotential supporting cell maintains plasticity throughout the differentiation process and has the capability to become 1 of 2 differentiated cell types. We understand that the transcriptional changes that occur within supporting cells are closely mirrored by changes in histone modifications near sex-determining genes. However, in developing systems it is not clear whether changes in histone modifications occur first with changes in transcription following soon after or whether histone modifications are deposited to reflect and maintain transcriptional activation or repression. It remains possible that the acquisition of active or silent histone marks is secondary to transcription through the locus (or lack thereof). Armed with a better understanding of dynamic sex differences in chromatin state, the field can begin to address how the acquisition of unique chromatin landscapes relates to changes in transcription and cell fate commitment.

Of particular interest are the mechanisms underlying the sex specificity of epigenetic changes. As illustrated by the resolution of bivalent genes during sex determination, there are mechanisms in place to remove H3K27me3 specifically from Sertoli or granulosa genes, depending on which pathway the cell elects to follow. This sex specificity could be the result of transcription factor interactions with epigenetic modifying complexes through changes in the 3D conformation of the genome which lead to sequestration of Sertoli and granulosa genes in active or silent domains, or through other mechanisms. For example, it has been argued that the XX or XY chromosome constitution affects the way an individual cell reads the genome, leading to epigenetic differences between XX and XY cells [Maatouk and Capel, 2008; Xu et al., 2008; Maatouk and Capel, 2010; Arnold, 2020]. It is clear that any differences between XX and XY cells can be overridden by expression of *Sry*, because expression of *Sry* in the XX gonad leads to commitment to the Sertoli pathway [Koopman et al., 1991]. Nonetheless, differences in the epigenetic land-

scape in XX and XY cells could predispose the cells to the female or male pathway. No direct evidence for this has been found at this time, however, further studies on histone modifications in models of sex reversal could provide answers.

Acknowledgements

We thank current and former members of the Capel lab for helpful discussions, especially Danielle Maatouk, who was the first to initiate global epigenetic studies during sex determination, and Alex Garcia-Moreno, who performed many of the studies reported in this review and whose figures we have redrawn and summarized here.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

S.D. was supported by a T32 for the Development and Stem Cell Biology Training Program, and S.D. and B.C. are currently supported by a grant from the National Institutes of Health (1R01HD090050-0 to BC).

Author Contributions

S.D. and B.C. contributed equally to the creation of this manuscript.

References

- Arango NA, Lovell-Badge R, Behringer RR. Targeted mutagenesis of the endogenous mouse *Mis* gene promoter: in vivo definition of genetic pathways of vertebrate sexual development. *Cell*. 1999;99(4):409–19.
- Arnold AP. Four Core Genotypes and XY* mouse models: Update on impact on SABV research. *Neurosci Biobehav Rev*. 2020;119:1–8.
- Azuara V, Perr P, Sauer S, Spivakov M, Jørgensen HF, John RM, et al. Chromatin signatures of pluripotent cell lines. *Nat Cell Biol*. 2006;8(5):532–8.
- Barriónuevo F, Bagheri-Fam S, Klattig J, Kist R, Taketo MM, Englert C, et al. Homozygous inactivation of *Sox9* causes complete XY sex reversal in mice. *Biol Reprod*. 2006;74:195–201.
- Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell*. 2006;125(2):315–26.

- Biason-Lauber A, Konrad D, Meyer M, deBeaufort C, Schoenle EJ. Ovaries and female phenotype in a girl with 46,XY karyotype and mutations in the CBX2 gene. *Am J Hum Genet*. 2009;84:658–63.
- Creyghton MP, Cheng AW, Welstead GG, Kooistra T, Carey BW, Steine EJ, et al. Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc Natl Acad Sci U S A*. 2010;107:21931–6.
- Garcia-Moreno SA, Futtner CR, Salamone IM, Gonen N, Lovell-Badge R, Maatouk DM. Gonadal supporting cells acquire sex-specific chromatin landscapes during mammalian sex determination. *Dev Biol*. 2019a;446(2):168–79.
- Garcia-Moreno SA, Lin YT, Futtner CR, Salamone IM, Capel B, Maatouk DM. CBX2 is required to stabilize the testis pathway by repressing Wnt signaling. *PLoS Genet*. 2019b;15(5):e1007895.
- Ge C, Ye J, Weber C, Sun W, Zhang H, Zhou Y, et al. The histone demethylase KDM6B regulates temperature-dependent sex determination in a turtle species. *Science*. 2018;360(6389):645–8.
- Greer EL, Shi Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet*. 2012;13(5):343–57.
- Jameson SA, Lin YT, Capel B. Testis development requires the repression of Wnt4 by Fgf signaling. *Dev Biol*. 2012a;370(1):24–32.
- Jameson SA, Natarajan A, Cool J, Defalco T, Maatouk DM, Mork L, et al. Temporal transcriptional profiling of somatic and germ cells reveals biased lineage priming of sexual fate in the fetal mouse gonad. *PLoS Genet*. 2012b;8(3):e1002575.
- Katoh-Fukui Y, Tsuchiya R, Shiroishi T, Nakahara Y, Hashimoto N, Noguchi K, et al. Male-to-female sex reversal in M33 mutant mice. *Nature*. 1998;393(6686):688–92.
- Katoh-Fukui Y, Miyabayashi K, Komatsu T, Owaki A, Baba T, Shima Y, et al. Cbx2, a polycomb group gene, is required for Sry gene expression in mice. *Endocrinology*. 2012;153(2):913–24.
- Kim Y, Kobayashi A, Sekido R, DiNapoli L, Brennan J, Chaboissier MC, et al. Fgf9 and Wnt4 act as antagonistic signals to regulate mammalian sex determination. *PLoS Biol*. 2006;4(6):e187.
- Koopman P, Gubbay J, Vivian N, Goodfellow P, Lovell-Badge R. Male development of chromosomally female mice transgenic for Sry. *Nature*. 1991;351(6322):117–21.
- Lee JH, Hart SR, Skalnik DG. Histone deacetylase activity is required for embryonic stem cell differentiation. *Genesis*. 2004a;38(1):32–8.
- Lee CK, Shibata Y, Rao B, Strahl BD, Lieb JD. Evidence for nucleosome depletion at active regulatory regions genome-wide. *Nat Genet*. 2004b;36:900–5.
- Maatouk DM, Capel B. Sexual development of the soma in the mouse. *Curr Top Dev Biol*. 2008;83:151–83.
- Maatouk DM, Capel B. Unmasking a role for sex chromosomes in gene silencing. *Genome Biol*. 2010;11(9):134.
- Maatouk DM, DiNapoli L, Alvers A, Parker KL, Taketo MM, Capel B. Stabilization of beta-catenin in XY gonads causes male-to-female sex-reversal. *Hum Mol Genet*. 2008;17(19):2949–55.
- Maatouk DM, Natarajan A, Shibata Y, Song L, Crawford GC, Ohler U, et al. Genome-wide identification of regulatory elements in Sertoli cells. *Development*. 2017;144(4):720–30.
- Maizawa S, Yukawa M, Hasegawa K, Sugiyama R, Hu M, Vidal M. Polycomb suppresses a female gene regulatory network to ensure testicular differentiation. *bioRxiv*. 2021. <https://doi.org/10.1101/2021.01.19.427322>.
- Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. *Nature*. 2011;469(7330):343–9.
- Matson CK, Murphy MW, Sarver AL, Griswold MD, Bardwell VJ, Zarkower D. DMRT1 prevents female reprogramming in the postnatal mammalian testis. *Nature*. 2011;476:101–4.
- Munger SC, Natarajan A, Looger LL, Ohler U, Capel B. Fine time course expression analysis identifies cascades of activation and repression and maps a putative regulator of mammalian sex determination. *PLoS Genet*. 2013;9:e1003630.
- Nef S, Schaad O, Stallings NR, Cederroth CR, Pitettti JL, Schaer G, et al. Gene expression during sex determination reveals a robust female genetic program at the onset of ovarian development. *Dev Biol*. 2005;287:361–77.
- Pan G, Tian S, Nie J, Yang C, Ruotti V, Wei H, et al. Whole-genome analysis of histone H3 lysine 4 and lysine 27 methylation in human embryonic stem cells. *Cell Stem Cell*. 2007;1(3):299–312.
- Rada-Iglesias A, Bajpai R, Swigut T, Brugmann SA, Flynn RA, Wysocka J. A unique chromatin signature uncovers early developmental enhancers in humans. *Nature*. 2011;470:279–83.
- Sachs M, Onodera C, Blaschke K, Ebata KT, Song JS, Ramalho-Santos M. Bivalent chromatin marks developmental regulatory genes in the mouse embryonic germline in vivo. *Cell Rep*. 2013;3(6):1777–84.
- Sekido R, Lovell-Badge R. Sex determination involves synergistic action of SRY and SF1 on a specific Sox9 enhancer. *Nature*. 2008;453(7197):930–4.
- Shilatifard A. The COMPASS family of histone H3K4 methylases: mechanisms of regulation in development and disease pathogenesis. *Annu Rev Biochem*. 2012;81:65–95.
- Shpargel KB, Sengoku T, Yokoyama S, Magnuson T. UTX and UTY demonstrate histone demethylase-independent function in mouse embryonic development. *PLoS Genet*. 2012;8(9):e1002964.
- Song L, Zhang Z, Gräsfeder LL, Boyle AP, Giresi PG, Lee B-K, et al. Open chromatin defined by DNaseI and FAIRE identifies regulatory elements that shape cell-type identity. *Genome Res*. 2011;21:1757–67.
- Stévant I, Neirijnck Y, Borel C, Escoffier J, Smith LB, Antonarakis SE, et al. Deciphering cell lineage specification during male sex determination with single-cell RNA sequencing. *Cell Rep*. 2018;6:1589–99.
- Uhlenhaut NH, Jakob S, Anlag K, Eisenberger T, Sekido R, Kress J, et al. Somatic sex reprogramming of adult ovaries to testes by FOXL2 ablation. *Cell*. 2009;139:1130–42.
- Weber C, Zhou Y, Lee JG, Looger LL, Qian G, Ge C, et al. Temperature-dependent sex determination is mediated by pSTAT3 repression of Kdm6b. *Science*. 2020;368(6488):303–6.
- Xu J, Deng X, Watkins R, Distèche CM. Sex-specific differences in expression of histone demethylases Utx and Uty in mouse brain and neurons. *J Neurosci*. 2008;28:4521–7.