A set of genes critical to development is epigenetically poised in mouse germ cells from fetal stages through completion of meiosis

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In multicellular organisms, germ cells carry the hereditary material from one generation to the next. Developing germ cells are unipotent gamete precursors, and mature gametes are highly differentiated, specialized cells. However, upon gamete union at fertilization, their genomes drive a totipotent program, giving rise to a complete embryo as well as extraembryonic tissues. The biochemical basis for the ability to transition from differentiated cell to totipotent zygote is unknown. Here we report that a set of developmentally critical genes is maintained in an epigenetically poised (bivalent) state from embryonic stages through the end of meiosis. We performed ChIP-seq and RNA-seq analysis on flow-sorted male and female germ cells during embryogenesis at three time points surrounding sexual differentiation and female meiotic initiation, and then extended our analysis to meiotic and postmeiotic male germ cells. We identified a set of genes that is highly enriched for regulators of differentiation and retains a poised state (high H3K4me3, high H3K27me3, and lack of expression) across sexes and across developmental stages, including in haploid postmeiotic cells. The existence of such a state in embryonic stem cells has been well described. We now demonstrate that a subset of genes is maintained in a poised state in the germ line from the initiation of sexual differentiation during fetal development and into postmeiotic stages. We propose that the epigenetically poised condition of these developmental genes is a fundamental property of the mammalian germ-line nucleus, allowing differentiated gametes to unleash a totipotent program following fertilization.

Significance

Germ cells, meaning the mature sperm and egg and their developmental precursors, carry the DNA that is passed from one generation to the next. Although the sperm and egg are highly specialized, differentiated cells, they meet at fertilization to produce a totipotent zygote, a cell that can generate any other cell type. We report the finding that a set of developmentally important genes is kept in a “poised” state in the germ cells; although these genes are never expressed in the germ cells themselves, they maintain a chromatin state usually associated with the potential for rapid gene activation. We propose that maintenance of this poised state in the germ cells contributes to the generation of totipotency in the fertilized zygote.
and housekeeping genes for H3K4me3 and developmental and transcriptional regulators for H3K27me3, consistent with their association with active and repressed promoters, respectively (Table S3). As expected, highly expressed genes had high H3K4me3 and low H3K27me3 signal (Fig. S3).

Between E12.5 and E13.5, germ cells of both sexes lose the ability to give rise to pluripotent cell lines in culture and begin to down-regulate pluripotency-associated genes. Both male and female germ cells begin to express components of the meiotic program, and at E14.5 female germ cells enter the first stages of meiotic prophase. Male cells do not initiate meiotic prophase, but instead arrest at G1 until after birth. We asked whether our data were consistent with these known aspects of germ cell biology. As expected, expression of the pluripotency regulators Oct4 (Pou5f1), Sox2, and Nanog decreased over time, with a corresponding decrease in H3K4me3 signal (Fig. 1B). Conversely, expression and H3K4me3 signal increased over time at the promoters of the meiosis-associated genes Rec8, Stra8, and Sycp3, with more dramatic increases in female compared with male germ cells (Fig. 1B).

In general, there were statistically significant increases in H3K4me3 signal at promoters of genes included in the “meiosis” GO category (GO:0071300) between E12.5 and E14.5 in both sexes [P = 0.0065 (female) and P = 0.00017 (male), Mann–Whitney U test], with more dramatic increases in females (Fig. S4 and Table S3). H3K27me3 signal associated with these promoters (Fig. S4) did not change significantly. In contrast, H3K27me3 signal associated with genes included in the GO category “cellular response to retinoic acid” (GO:0071300) exhibited significant differences between time points [P = 0.016 (female) and P = 0.026 (male), Mann–Whitney U test], but the direction of change varied by gene: ChIP signal increased at some promoters and decreased at others (Fig. S4 and Table S4). Retinoic acid is integral to meiotic initiation in the germ cells (24, 25), and also plays a critical role in regulation of pluripotency and differentiation (26, 27); it is likely to have multiple regulatory roles in this cell population.

Identification of Poised Promoters in Fetal Germ Cells. We next examined genome-wide chromatin states across sexes and developmental time points. First, to look for similarities across datasets, we treated all six conditions (two sexes and three time points) as equivalent, and used k-means clustering to group all genes according to similarity of H3K4me3 and H3K27me3 profile near their transcriptional start sites. In this analysis, no parameters regarding ChIP profile are initially specified to define the clusters; results therefore represent an unbiased description of common promoter chromatin states in the cell population. We observed that in both sexes and at all three time points, one cluster of genes was characterized by high H3K4me3 and high H3K27me3 signal, a characteristic of bivalent or poised genes in ESCs (Fig. 2A, cluster 4). Furthermore, this class of genes displayed markedly lower overall expression at all ages and in both sexes, compared with each of the other classes identified (Fig. 2B). Cluster 4 was significantly enriched for functional categories corresponding to development and organogenesis (Fig. 2C). The combination of high H3K4me3 signal, high H3K27me3 signal, low expression, and functional enrichment for developmental regulators led us to hypothesize that this class of genes exists in a poised state, similar to the poised state identified in ESCs (8).

A Class of Poised Genes Does Not Resolve Between E12.5 and E14.5. The presence of poised genes in E12.5 germ cells is consistent with current models for the function of poised genes in stem cells. In ESCs, poised genes are thought to retain H3K4me3, despite their transcriptionally repressed state, to facilitate transcriptional activation after receiving a differentiation signal (8). At E12.5, both male and female germ cells are thought to be relatively undifferentiated, and they can form pluripotent cell lines in culture. Between E12.5 and E14.5, however, germ cells of both sexes undergo major changes in cell state, as both transition to highly sex-specific transcriptional programs and begin the process of differentiation into mature sperm or oocytes (18). In
particular, female germ cells at E14.5 have initiated meiotic pro-
phase, a clear departure from an undifferentiated state. We
therefore asked if genes that are poised in E12.5 or E13.5 germ
cells tended to resolve toward H3K4me3 or H3K27me3 by E14.5.

Because the clustering described above treated all six con-
tions as equivalent, it did not account for changes in the state of
each promoter over time. To track changes in poised pro-
moter state over time at individual genes, we established a set of
criteria that could be used to call a single promoter as poised or
not at a given time point. We defined poised genes as having high
H3K4me3 signal (>75th percentile of ChIP signal) within 2 kb
upstream or downstream of the transcriptional start site, as well as
absent transcription (fragments per kilobase per million reads ≤1).

To start, we analyzed data separately for male and female
germ cells. We found 513 genes in male and 727 genes in female
that met our criteria for the poised state at E12.5, and these
numbers decreased to 291 and 270 genes at E14.5 in male and
female, respectively (Fig. 3A). Among the genes meeting criteria
for the poised state at E14.5, 247 in male (85%) and 254 in fe-
male (94%) were also poised at E12.5, E13.5, or both, indicating
that genes that are poised at E14.5 had likely retained this state
over time (Table S5). Of those genes that are poised in either sex
at both E14.5 and earlier time points, 147 were shared between
sexes, relative to the genome as a whole. We
found that this gene set was overwhelmingly enriched for tran-
scriptional regulators of somatic differentiation, including both
general regulators of differentiation (“positive regulation of stem
cell differentiation,” GO:0000078, fold-enrichment 58.72), and
regulators of tissue-specific differentiation (including kidney, lung,
heart, liver, pancreas, skeletal system, and brain; fold-enrichment
6.8–38.29) (Table 1). Of the 147 genes, 89 were associated with
“developmental process” (GO:0002502, fold-enrichment 3.76). We
found a similar set of enriched categories when the 147 poised genes
were compared with all genes that lacked expression in fetal germ
cells.

Germ-Line-Poised Genes Include Critical Regulators of Early Development.
To better understand the biological function of this gene class, we
looked for GO categories enriched in the set of 147 genes that is
maintained in the poised state between E12.5/E13.5 and E14.5
and is shared between sexes, relative to the genome as a whole. We
found that this gene set was overwhelmingly enriched for tran-
scriptional regulators of somatic differentiation, including both
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cells.

In contrast, only 21 genes that resolved toward a repressed state (low
H3K4me3, high H3K27me3, and low expression) were shared between males and females (28% and
18% of genes resolving toward the repressed state at E14.5 in
males and females, respectively), and none of those that resolved
toward an active state (high H3K4me3, low H3K27me3, and high
expression) were shared between sexes. We conclude that a class
of genes is maintained in a poised state in both male and female
germ cells during the period encompassing sexual differentiation
and female initiation of meiosis. This shared collection of germ-
line–poised genes is a subset of cluster 4, the class of H3K4me3+/
H3K27me3+ genes initially identified by genome-wide clustering
of promoter ChIP signals (Fig. 3B). Notably, a subset of genes
appeared poised in one sex but not the other. It is possible that
these genes represent a true sex-specific set of germ-cell–poised
genesis; however, because our filtering criteria were designed to
exclude false positives, it is likely that many of these genes are
true positives in both sexes but met the filtering threshold in only
one sex. The set of true shared, poised genes may therefore be
larger than reported here.

To see if this set of genes is also poised at earlier times in
development, we compared our data to published H3K4me3 and
H3K27me3 ChIP-seq data from mixed male and female germ
cells at E11.5 (11). (The published dataset did not include corre-
spanding transcriptional data.) We identified 805 genes at
E11.5 with high H3K4me3 (>75th percentile) and high
H3K27me3 (>95th percentile). Of the 147 poised genes that we
identified as shared across fetal male and female germ cells be-
tween E12.5 and E14.5, 142 (97%) were included in this set of
805 genes at E11.5, supporting the continuity of the poised state
at these genes in fetal germ cells.
cells. The set of poised genes retained in male, female, or both during fetal development included essential regulators of ectoderm (Sox1, Olig2), mesoderm (T, Ntx2-5), and embryonic endoderm (Sox17, Foxa2), as well as primitive endoderm (Gata6, Pdxfra) and trophectoderm (Cd82, Haud1) (Table S5). We evaluated expression of each of these genes during mouse embryogenesis, using publicly available data. Of the 147 genes, 127 are somatically expressed during mouse embryogenesis, and 52 are expressed before E8.75, implying that they are involved in early germ layer formation. Only 32 were expressed in mESCs. We conclude that the genes maintained in a transcriptionally poised state in fetal germ cells are developmental regulators. This class includes genes essential for specification of a complete conceptus, including the embryo proper, trophectoderm, and primitive endoderm (28).

**Germ-Line–Poised Genes Remain Poised During and After Meiosis in Males.** We next asked whether these germ-line–poised genes remain poised during and after meiosis. During meiosis, germ cells segregate homologous chromosomes to generate haploid gamete precursors, a highly specialized process requiring tight control of transcription and specialized chromosome packaging. Female germ cells initiate chromosome condensation and meiotic prophase at E14.5 and then arrest in late prophase until ovulation, whereas male cells initiate chromosome condensation and meiotic prophase at transcription and specialized chromosome packaging. Female germ cells are poised during and after meiosis. During meiosis, germ cells segregate chromosomes, conferring a continuous germ-cell life cycle, allowing meristems to continue forming totipotent cells throughout much of the life cycle in germ cells of both sexes. We collected high-throughput ChIP and RNA sequencing data from sorted male and female germ cells at three fetal time points surrounding sex determination and female meiotic initiation, as well as male meiotic and postmeiotic germ cells. Under each of these developmental conditions, we identified genes with high H3K4me3 and high H3K27me3 signal near their transcriptional start sites, as well as absent gene expression. A subset of poised genes, representing a range of critical developmental transcription factors and signaling molecules, was common to all germ-cell states examined; these genes were maintained in the poised state not only through the onset of sexual differentiation in fetal germ cells but also during and after meiosis in the adult male. The poised state has been well described in ESCs, where it is thought to allow more efficient entry into induced differentiation programs (8), and it has recently been suggested that its presence in the germ cells provides an in vivo correlate for this feature of pluripotent cells in vitro (11). Our data point to a broader biological function for the poised state: transmission through the germ line of the ability to generate a totipotent zygote at fertilization.

We propose a model wherein central regulators of embryonic and extraembryonic development are maintained in a poised state throughout the germ-cell life cycle, conferring a continuous germ-

<table>
<thead>
<tr>
<th>Description</th>
<th>False-discovery rate q-value</th>
<th>Enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developmental process</td>
<td>2.35E-31</td>
<td>3.76</td>
</tr>
<tr>
<td>Regulation of nucleobase compound metabolic process</td>
<td>3.53E-29</td>
<td>3.97</td>
</tr>
<tr>
<td>Regulation of transcription, DNA-dependent</td>
<td>2.73E-29</td>
<td>4.4</td>
</tr>
<tr>
<td>Regulation of RNA biosynthetic process</td>
<td>2.28E-29</td>
<td>4.4</td>
</tr>
<tr>
<td>Single-organism developmental process</td>
<td>2.70E-29</td>
<td>4.45</td>
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**Discussion**

We report here the presence of a class of essential developmental regulators that is maintained in a poised chromatin state throughout much of the life cycle in germ cells of both sexes. We collected high-throughput ChIP and RNA sequencing data from sorted male and female germ cells at three fetal time points surrounding sex determination and female meiotic initiation, as well as male meiotic and postmeiotic germ cells. Under each of these developmental conditions, we identified genes with high H3K4me3 and high H3K27me3 signal near their transcriptional start sites, as well as absent gene expression. A subset of poised genes, representing a range of critical developmental transcription factors and signaling molecules, was common to all germ-cell states examined; these genes were maintained in the poised state not only through the onset of sexual differentiation in fetal germ cells but also during and after meiosis in the adult male. The poised state has been well described in ESCs, where it is thought to allow more efficient entry into induced differentiation programs (8), and it has recently been suggested that its presence in the germ cells provides an in vivo correlate for this feature of pluripotent cells in vitro (11). Our data point to a broader biological function for the poised state: transmission through the germ line of the ability to generate a totipotent zygote at fertilization.

We propose a model wherein central regulators of embryonic and extraembryonic development are maintained in a poised state throughout the germ-cell life cycle, conferring a continuous germ-

Table 1. GO categories enriched in genes maintained in a poised state in both male and female fetal germ cells

<table>
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<tr>
<th>Description</th>
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<th>Enrichment</th>
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</thead>
<tbody>
<tr>
<td>Positive regulation of stem cell differentiation</td>
<td>3.30E-05</td>
<td>58.72</td>
</tr>
<tr>
<td>Formation of primary germ layer</td>
<td>1.64E-03</td>
<td>13.85</td>
</tr>
<tr>
<td>Retinoic acid receptor signaling pathway</td>
<td>7.78E-03</td>
<td>27.52</td>
</tr>
<tr>
<td>Nervous system development</td>
<td>3.94E-08</td>
<td>7.63</td>
</tr>
<tr>
<td>Brain development</td>
<td>9.65E-06</td>
<td>11.29</td>
</tr>
<tr>
<td>Sensory organ development</td>
<td>7.83E-06</td>
<td>11.59</td>
</tr>
<tr>
<td>Eye development</td>
<td>2.12E-04</td>
<td>11.17</td>
</tr>
<tr>
<td>Inner ear morphogenesis</td>
<td>1.89E-06</td>
<td>17.27</td>
</tr>
<tr>
<td>Cartilage development</td>
<td>1.22E-05</td>
<td>13.5</td>
</tr>
<tr>
<td>Skeletal system development</td>
<td>1.42E-05</td>
<td>10.74</td>
</tr>
<tr>
<td>Connective tissue development</td>
<td>7.42E-05</td>
<td>10.48</td>
</tr>
<tr>
<td>Kidney development</td>
<td>2.66E-08</td>
<td>14.68</td>
</tr>
<tr>
<td>Lung development</td>
<td>3.05E-02</td>
<td>6.8</td>
</tr>
<tr>
<td>Heart morphogenesis</td>
<td>1.64E-02</td>
<td>11.51</td>
</tr>
<tr>
<td>Liver development</td>
<td>2.35E-02</td>
<td>10.3</td>
</tr>
<tr>
<td>Pancreas development</td>
<td>1.11E-03</td>
<td>24.46</td>
</tr>
<tr>
<td>Endocrine pancreas development</td>
<td>8.04E-07</td>
<td>38.29</td>
</tr>
<tr>
<td>Limb morphogenesis</td>
<td>4.86E-07</td>
<td>13.11</td>
</tr>
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</table>
Promoters that are poised during fetal development remain poised during and after meiosis. (A) Schematic of meiosis in male germ cells. (B) Number of germ-line–poised genes retained across fetal time points in male only (blue) or in both male and female (purple), compared with genes identified as poised in both pachynete spermatocytes and round spermatids (“male meiotic,” green). (C) Sample gene tracks for three genes maintained in a poised state across germ cell development.

The concept of “latent pluripotency,” or continuous retention of a state conducive to establishment of tissues belonging to the three germ layers, has recently been proposed as a unifying feature of the germ line. It raises several questions central to understanding the nature and regulation of the germ line.

First, whether the poised state is maintained in mature sperm or oocytes, during fertilization, and in the mammalian early embryo and epiblast, remains an area of active investigation. Even if a core set of poised genes is required in the germ line for generation of a totipotent zygote, loss and reestablishment of that state might occur immediately following fertilization. In mammals, cooccurrence of H3K4me3 and H3K27me3 at promoters of select developmental regulators has been reported in epiblast cells, but genome-wide chromatin data are not available (30).

In mature mammalian sperm, histones are largely replaced by protamines, presumably eliminating information carried by histone modifications. However, a few modified histones are retained in mature sperm in mice (31, 32) and humans (31, 33). Of the 92 genes that we identified as poised at fetal through postmeiotic stages, 47 have been reported to retain histones marked by H3K4me3 and H3K27me3 after protamine replacement in mouse sperm (32). Notably, we observed that H3K4me3 signal in round spermatids sometimes spreads into broad domains around poised promoters, similar to the relatively broad H3K4me3 domains reported in mature sperm (31, 33). It will be intriguing to further explore this correlation.

Second, in addition to its association with poised or active gene promoters, the H3K4me3 histone modification is enriched at sites of recombination and crossing over during meiosis (34).

In mammals, the methyltransferase PRDM9 (PR domain containing 9) directs recombination to defined hotspots, which are H3K4me3+ but are not associated with gene promoters; as a result, most recombination in wild-type individuals occurs in intergenic regions (35). In the absence of PRDM9, recombination is redirected toward H3K4me3+ promoter regions. This inappropriate recruitment of the recombination machinery to coding or regulatory regions might be expected to have deleterious effects, especially at developmentally critical genes. It is possible that the presence of the poised state in meiotic chromatin has an additional protective effect at gene promoters with which it is associated, acting as a failsafe for the targeting activity of PRDM9. In fact, we found a statistically significant depletion of recombination at germ-line–poised gene promoters in Prdm9 mutants, using previously published data (SI Text) (35). It will be interesting to determine whether a meiosis-specific set of histone modifications or chromatin factors accumulates around poised sites in meiotic germ cells.

Third, the factors that regulate maintenance of the poised state in the germ line remain unknown. Unlike ESCs (9), germ cells must fully differentiate into gametes while simultaneously protecting a complete set of poised genes and retaining the ability to give rise to a totipotent zygote. As a result, two independently targeted modes of regulation may be required. It was recently shown that two alternative components of Polycomb repressive complex 1, Ring1 and Rnf2, have genetically separable functions in regulating the poised state (36). One possible model is that, in addition to its association with poised promoters, H3K4me3 is enriched at sites of recombination and crossing over during meiosis (34). In this model, H3K4me3 may function as a failsafe for the targeting activity of PRDM9, acting as a failsafe for the targeting activity of PRDM9. It will be interesting to determine whether a meiosis-specific set of histone modifications or chromatin factors accumulates around poised sites in meiotic germ cells.

Critical developmental genes are continuously maintained in a poised state (purple and orange circles), with transcription repressed. Loss of either H3K4me3 (resolution toward the active state, with associated increases in gene expression) or H3K27me3 (resolution toward the repressed state) at specific promoters results in terminal differentiation. Those cells that maintain a complete set of poised developmental regulators throughout development constitute the germ cells.

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roles in female germ-cell maintenance and differentiation: both are required for survival of female fetal germ cells, but only **Rnf2** is required to direct differentiation of these cells (36). If there are two classes of poised genes, those that resolve in germ cells and those that do not, then elucidation of how these classes are established and controlled could provide insight into modes of regulation of poised promoters both in vivo and in vitro. Furthermore, the role of factors involved in germ cell specification, such as **Blimp1** and **Prdm14**, and differentiation, such as **Dazl**, can now be reevaluated in the context of their effects on regulation of germ-line–poised genes.

Finally, the fate of poised promoters in the meiotic and postmeiotic female germ line remains unknown. Analysis of germ-line–poised genes in the female compared with the male germ line will help to assess the universality of this gene class, and may illuminate sex-specific differences in regulation of the poised state. In both male and female gametates, the state of poised promoters during and after fertilization may be differentially regulated, with implications for maternal and paternal influences on inheritance and on development of offspring. In both sexes, a better understanding of the factors influencing maintenance of the poised state during and after fertilization will have broad implications for human development and disease.

### Materials and Methods

Further details can be found in [SI Materials and Methods](#).