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Specific DNA Methylation Pattern in Chicken Lampbrush

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Abstract

Involved in the control of transcription and replication, developmental reprogramming, retroelement silencing and other genomic activities, DNA methylation is a crucial epigenetic regulatory mechanism. For embryonic development to occur during mammalian development, a certain DNA methylation pattern in germ cells must be created. In other animals, DNA methylation in germ cells is less well understood. We examined the single-cell methylome of chicken diplotene oocytes to fill this gap. We developed a methylation-based segmentation of the chicken genome and discovered methylated gene promoters exclusive to oocytes after thoroughly characterising the methylation patterns in these cells. Our results demonstrate that methylation patterns in these cells closely reflect chromosomal distribution seen in somatic tissues, despite the creation of a particular transcriptionally hyperactive genome architecture in chicken diplotene oocytes.

Keywords: DNA methylation • Chicken oocyte • Chicken genome • Lampbrush chromosomes

Introduction

In vertebrates, DNA methylation plays a role in DNA repair, endogenous and exogenous gene silencing, heterochromatin creation, chromatin architecture establishment and maintenance and other genomic activities. Contrary to other epigenetic changes, DNA methylation can be passed down through cell divisions, allowing somatic cells to transfer epigenetic information throughout the mitotic cycle. There are unique processes driving epigenetic reprogramming and the establishment of germ-line-specific methylation patterns in germ cells and early embryos that can be used to erase this epigenetic memory.

Mammalian sperm progenitors go through two rounds of epigenetic reprogramming during development and by the time gametogenesis is complete, the mature sperm genome displays 80-90% CpG methylation. Despite having a somewhat greater DNA methylation content, the genomic pattern of DNA methylation in sperm is generally similar to that of somatic cells [1]. Beginning with essentially unmethylated, non-growing oocytes present in the primordial follicle prior to folliculogenesis, oocyte methylation gradually rises during development, reaching a level of about 40-50% in mature gametes. Since transcription is necessary for the creation of methylation patterns in oocytes, methylated regions are primarily restricted to the transcribed gene bodies, leaving intergenic regions and genes that are not transcribed hypomethylated. Mammalian gametes exhibit certain DNA methylation patterns and amounts overall, which is necessary to preserve these species' evolutionary plans [2].

The knowledge of DNA methylation in the gametes of non-mammalian vertebrate species is far less. Here, we concentrate on the epigenetic profile of domestic chicken (Gallus gallus) germ cells. One of the most well-known non-mammalian vertebrate species is the chicken and as a substitute and superior experimental species, chickens play a crucial role in animal research. Additionally, the most significant source of commercially produced

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meat worldwide is domestic chicken. Modern genomic techniques were used to conduct substantial research on chicken models, yielding abundant data on somatic cell methylation patterns, transcription dynamics and genome architecture [3-5]. Additionally, a recent analysis of the DNA methylation in chicken sperm cells revealed that the sperm DNA is hypomethylated, which is consistent with the absence of the DNMT3L cofactor in the chicken genome. To investigate DNA methylation in developing chicken oocytes, no genomic data are available. The chicken oocyte genome acquires a characteristic organisation during the diplotene stage of prophase I of meiosis, which is marked by increased transcription and the creation of lampbrush chromosomal structures. Although there are no molecular data available, cytological evidence suggests that DNA methylation in developing chicken oocytes correlates with chromatin arrangement and transcription.

Here, we examine the DNA methylation of chicken oocyte diplotene at two stages. We demonstrate that methylation patterns in oocytes at both stages, including hypomethylation of CpG islands and hypermethylation of transposons and other repetitive DNA elements, are similar to those seen in somatic cells. Average DNA methylation levels in oocytes are comparable to somatic cells, in contrast to sperm. We created a brand-new HMM-based segmentation algorithm that locates hypomethylated areas in the chicken genome and shown that there are just a few hypomethylated gene promoters that are particular to oocytes. Overall, the evidence from our research points to a little role for DNA methylation in the developmental reprogramming of the oocyte and lampbrush chromosome production in birds.

Results

We selected the SWF (small white follicles) and LWF (large white follicles) stages, which can be clearly identified using microscopy studies, to profile oocyte DNA methylation in adult chickens and analyse its dynamics. Both SWF and LWF are diplotene phases, which correspond to mammalian oocyte development stages that are around 10-15 days post-partum [2]. Based on the size of the follicle, we established the SWF and LWF stages (1-2 mm follicle for the SWF stage, 4-7 mm follicle for the LWF stage). We obtained oocyte nuclei and examined their size and chromosomal shape to confirm that follicles of various sizes contain oocytes at various developmental stages. A typical avian diplotene nucleus with evenly scattered lampbrush bivalents can be seen during the SWF stage, which is consistent with previously reported results. Later stages see a minor increase in nuclei size, although chromosomal shape is the primary distinction between SWF and LWF phases.

We consistently analysed publically available data for fibroblasts, spleen, jejunum, ileum, muscles and sperm cells to compare levels and patterns of

DNA methylation in oocytes with other chicken cell types. The average CpG methylation in oocytes (53.2%) was found to be equivalent to that in somatic cells (52.45-62.8%). Additionally, we validated the previously observed hypomethylation (40.5%) of the sperm genome.

The levels of methylation on micro and macrochromosomes, as well as the methylation patterns of various genomic characteristics, were then compared. Between chicken lampbrush chromosomes and somatic cells, the DNA methylation patterns are often similar. The distribution of promoters and CpG islands was bimodal: whereas the majority of these sites had low levels of methylation, a small subset had high levels. Exons similarly displayed a bimodal distribution of methylation, with few exons showing no methylation and the majority of the locations being substantially methylated. Similarly, promoterproximal 5' untranslated regions were almost entirely demethylated, but 3' untranslated regions were heavily methylated. Increased methylation levels were seen in intergenic sequences, repetitive elements and introns. Except for slightly greater average methylation levels on macrochromosomes, which could be explained by a higher proportion of introns, repetitive sequences and intergenic features, we did not find any significant differences between microand macrochromosomes. Sperm cells exhibit lower levels of methylation for all examined genomic characteristics, reiterating the hypomethylation of the sperm genome that has previously been noted.

Discussion and Conclusion

Here, we describe a thorough analysis of the oocyte methylome at the chicken lampbrush stage. The results of computational analysis and agreement between our findings and those of earlier cytological analyses point to the high quality of the collected data. For instance, prior cytological results where the distribution of 5-methylcytosine along the axes of chicken lampbrush chromosomes was defined by immunostaining coincide with a high amount of methylation of transposons and other repetitive DNA. The general regulation of these genomic components during oogenesis was shown to be caused by the enrichment of 5 mC in compact chromomeres linked to the clusters of specific tandem repeats.

Despite the distinct genome architecture of diplotene oocytes, we were surprised to find that there was no significant variation in the methylome between oocytes and somatic cells across the whole genome. All of the changes we found were concentrated at a small number of loci and the range of somatic cell types was comparable to the number of loci with variable methylation. Comparable to this, the methylation pattern that seemed to be fairly similar at the SWF and LWF phases is not altered by transcriptional suppression that takes place during the SWF to LWF transition. Thus, we deduced that the creation of lampbrush chromosomes is influenced by epigenetic processes other than DNA methylation. One of these factors was the hyperacetylation of histone H4, which was shown both on transcription loops and at the locations where they are attached to chromomeres. Enhanced chromatin accessibility followed by the start of hypertranscription may cause prolonged lateral loops to become clearly apparent. We further point out that these findings are completely at odds with what is observed in mammals, where DNA methylation rises and imprinted loci have a particular methylation profile during the oocyte growth phase.

After fertilisation, it has been hypothesised that CpG methylation along lampbrush chromosomes may contain specific epigenetic information and control gene expression. It was unknown, nevertheless, whether bivalent condensation preserves the DNA methylation signature. We found a few gene promoters with oocyte-specific methylation patterns, however they were few in number. Demethylation of transcription start sites may hypothetically serve as a marker for early development gene activation during zygotic activation. This theory can be tested by conducting additional epigenetic and transcriptome studies of SWF, LWF and later oocyte stages.

The new HMM-based method we created to split the genome onto differentially methylated states is the last thing we want to draw attention to. Although HMM is a well-known method for segmenting the genome, we made certain special adjustments in this case that are crucial for the analysis of methylation data. This enabled us to segment the chicken genome based on methylation. We think that this segmentation can be utilised as a reference in future research on chicken genomics even if it was based on data from oocytes given the very low variability of methylation levels between cell types.

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