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Distinct DNA Methylation Patterns Found in Chicken Lampbrush Chromosomes

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Abstract

Playing a pivotal role in transcription and replication control, developmental reprogramming, retroelement silencing, and various other genomic activities, DNA methylation is an essential epigenetic regulatory mechanism. In the context of mammalian development, the establishment of a specific DNA methylation pattern in germ cells is a prerequisite for embryonic development. However, our understanding of DNA methylation in germ cells of other animals remains limited. To address this gap in knowledge, we conducted a comprehensive analysis of the single-cell methylome of chicken diplotene oocytes. Through the development of a methylation-based segmentation approach for the chicken genome, we identified methylated gene promoters exclusive to oocytes. This extensive characterization of methylation patterns in these cells revealed that they closely mirror the chromosomal distribution observed in somatic tissues, despite the presence of a distinct transcriptionally hyperactive genome architecture in chicken diplotene oocytes. Our findings shed light on the intricate role of DNA methylation in these cells.

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

Introduction

In vertebrates, DNA methylation serves various essential roles in genomic activities, encompassing DNA repair, gene silencing, heterochromatin formation, establishment and maintenance of chromatin architecture, and more. Unlike other epigenetic modifications, DNA methylation can be transmitted through cell divisions, enabling somatic cells to transmit epigenetic information during mitotic cycles. Distinct processes govern epigenetic reprogramming and the creation of germ-line-specific methylation patterns in germ cells and early embryos, which can be employed to reset this epigenetic memory.

In the context of mammalian sperm progenitors, two rounds of epigenetic reprogramming occur during development. By the end of gametogenesis, the mature sperm genome typically exhibits 80-90% CpG methylation. Although the DNA methylation content is somewhat higher, the genomic methylation pattern in sperm closely resembles that of somatic cells [1]. In contrast, in primordial follicles before folliculogenesis, oocytes are essentially unmethylated and non-growing. Methylation gradually increases during oocyte development, reaching approximately 40-50% in mature gametes. This methylation primarily occurs within transcribed gene bodies, leaving intergenic regions and non-transcribed genes hypomethylated. Mammalian gametes display specific DNA methylation patterns and levels crucial for preserving evolutionary plans of these species [2].

However, knowledge about DNA methylation in the gametes of non-mammalian vertebrates is limited. This study focuses on the epigenetic profile of germ cells in domestic chickens (Gallus gallus), a well-established and valuable species in animal research. Domestic chickens are not only a superior experimental model but also a major global source of meat production. Recent advances in genomic techniques have generated abundant data on somatic cell methylation patterns, transcription dynamics, and genome architecture in chicken models [3-5]. Notably, an analysis of chicken sperm cells revealed hypomethylation, consistent with the absence of the DNMT3L cofactor in the chicken genome. Unfortunately, there is no available genomic data to investigate DNA methylation in developing chicken oocytes. The chicken oocyte genome undergoes distinctive organization during the diplotene stage of prophase I of meiosis, marked by increased transcription and the formation of lampbrush chromosomal structures. Although molecular data are lacking, cytological evidence suggests a correlation between DNA methylation in developing chicken oocytes and chromatin arrangement and transcription.

Description

This study delves into the DNA methylation of chicken oocyte diplotene at two developmental stages. Our findings demonstrate that methylation patterns in oocytes at both stages are akin to those in somatic cells, featuring hypomethylation of CpG islands and hypermethylation of transposons and other repetitive DNA elements. The average DNA methylation levels in oocytes resemble those in somatic cells, in contrast to sperm. We have developed a novel HMM-based segmentation algorithm to pinpoint hypomethylated regions in the chicken genome, revealing only a few hypomethylated gene promoters specific to oocytes. Overall, our research provides evidence suggesting a limited role for DNA methylation in the developmental reprogramming of oocytes and the production of lampbrush chromosomes in birds.

Conclusion

In this study, we present a comprehensive examination of the oocyte methylome during the chicken lampbrush stage. Our findings from computational analysis align remarkably well with earlier cytological investigations, indicating the robustness of our dataset. For instance, our results corroborate prior cytological studies that used immunostaining to define the distribution of 5-methylcytosine along the axes of chicken lampbrush chromosomes, revealing a high degree of methylation in transposons and other repetitive DNA elements. The regulation of these genomic components during oogenesis appears to be influenced by the enrichment of 5-mC in compact chromomeres associated with specific tandem repeat clusters.

Surprisingly, despite the distinct genome architecture of diplotene

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oocytes, we observed minimal variation in the methylome compared to somatic cells across the entire genome. Instead, the changes we identified were concentrated at a limited number of loci, with the magnitude of variation comparable to that among different somatic cell types. Moreover, the methylation pattern, which appeared relatively stable during the transition from SWF to LWF phases despite transcriptional suppression, suggests that the creation of lampbrush chromosomes is influenced by epigenetic processes other than DNA methylation. One such factor identified was the hyperacetylation of histone H4, observed both on transcription loops and their attachment sites to chromomeres. Increased chromatin accessibility, followed by enhanced transcription, may contribute to the prominent appearance of prolonged lateral loops. Notably, these findings deviate from observations in mammals, where DNA methylation levels increase and imprinted loci display distinctive methylation profiles during oocyte growth.

After fertilization, it has been proposed that CpG methylation along lampbrush chromosomes may carry specific epigenetic information and regulate gene expression. However, it remains unclear whether bivalent condensation maintains the DNA methylation signature. While we identified a small number of gene promoters with oocyte-specific methylation patterns, they were relatively scarce. Demethylation of transcription start sites could potentially serve as a marker for early developmental gene activation during zygotic activation. This hypothesis warrants further investigation through additional epigenetic and transcriptome studies focusing on SWF, LWF, and subsequent oocyte stages.

Finally, we wish to highlight the novel HMM-based method we developed for genome segmentation based on differential methylation states. Although HMM is a well-established tool for genome segmentation, our unique adjustments tailored for methylation data analysis are pivotal. This segmentation approach can serve as a valuable reference for future research in chicken genomics, even when derived from oocyte data, given the remarkably consistent methylation levels observed across various cell types.

Acknowledgement

None.

Conflict of Interest

None.

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