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The Functional Role of Testis-Specific Histone Variants in the Regulation of Spermatogenesis

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https://hdl.handle.net/2324/7363604

出版情報：Kyushu University, 2024, 博士（理学）, 課程博士
バージョン：
権利関係：Public access to the fulltext file is restricted for unavoidable reason (3)

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（様式３）

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論 文 名　：The Functional Role of Testis-Specific Histone Variants in the Regulation of Spermatogenesis（精子形成における精巣特異的ヒストンバリアントの機能的役割）

区　　分　：甲

論 文 内 容 の 要 旨

A body is formed by the fertilized egg proliferating through cell division and differentiation, and acquiring its own unique gene expression. Proliferation and gene expression are regulated by changes in chromatin structure, involving various epigenetic mechanisms such as chromatin remodeling, histone modification, and histone variant replacement. For example, transcription factors induce chromatin modifiers to directly activate or inhibit gene transcription, and histone modifications and histone variant incorporation alter the state of chromatin structure, acting as a receptacle for transcription factors and indirectly controlling processes such as DNA repair and chromosome segregation. In particular, in germ cell development, analysis of changes in chromatin structure during spermatogenesis, which involves the cell cycle and meiosis, has progressed and it has become clear that specific chromatin structures are involved in appropriate gene expression and the transition from a histone-based to a protamine-based structure. Abnormalities in the regulation of these processes can lead to spermatogenesis failure, infertility, and other disorders. However, it is still unclear how specific histone variants are involved in chromatin remodeling and how dysregulation of these processes affects normal spermatogenesis and tissue homeostasis.

The development of several cutting-edge omics has greatly facilitated the study of epigenetic regulatory processes. In this work, we used Chromatin Integration Labeling (ChIL)-seq and RNA-seq to investigate how epigenetic regulation affects gene expression. In addition, we focused on specific cells and used Photo-Isolation Chemistry (PIC), a spatial transcriptomics technique, to ensure the accuracy of the target cells and to clarify their interactions with other cells.

In Chapter 3 of this thesis, the function of the histone H3 variant H3mm15, which is specifically expressed in the testis, was analyzed. The establishment of knockout mice showed that H3mm15 is essential for maintaining the structural organization of the seminiferous tubules and for the appropriate expression of genes related to spermatogenesis. H3mm15 forms unstable nucleosomes and it has been suggested that it regulates gene expression differently from other histone variants such as H3.3 by affecting incorporation into chromatin, especially in promoter regions. In Chapter 4, the effects of spermatogenesis arrest were studied using H3t knockout mice. This study found that in the absence of germ cells, Leydig cells in the testicular stroma proliferate and increase overall testosterone production to compensate for the loss of germ cells. This adaptive response includes an increase in the expression of stress-related genes, particularly those involved in testosterone synthesis, suggesting that the somatic cells of the testis can sense the state of spermatogenesis and respond accordingly. In Chapter 5, we discuss these findings and provide a perspective for future

research.

In Chapter 6, we analyzed epigenetic regulation in the cell cycle using proliferating myoblasts as a model. Although the master transcription factor MyoD is known to play an important role in muscle differentiation, its function in undifferentiated myoblasts remains unclear. In this chapter, we report on the function of MyoD in epigenetic regulation, such as histone modification and gene expression, in undifferentiated myoblasts.

Although cutting-edge omics can be used to study a wide variety of biological processes, when addressing specific research questions, it is necessary to carefully consider the unique advantages and limitations of each omics to ensure that reliable and meaningful data are obtained. In Chapter 7, we focus on the technical limitations of RNA-seq, in particular the way that enzymatic processing during cell preparation introduces bias into gene expression data. By systematically evaluating enzyme digestion conditions such as temperature and time, we optimized the RNA-seq protocol to minimize these biases. We also identified a set of "bias genes" that consistently respond to enzyme treatment, providing a valuable resource for improving the accuracy of RNA-seq data.