Title of Project : Unveiling the mechanism of action of Blimp1, a transcriptional regulator that governs fates and homeostasis of diverse cell lineages

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Research Area : Developmental biology

Keyword : Cellular Differentiation, Stem Cells, Germ Cells, Epigenetics

【Purpose and Background of the Research】

Understanding the mechanism of cell fate specification and maintenance is one of the most important subjects in developmental biology and life science. Generally, cellular fates and functions are determined by the transcription factors and epigenetic states that each cell type harbors. There are almost no reports, however, in which both two aspects are resolved at a genome-wide level in in vivo cellular differentiation processes. This is mainly because cell fate specification in vivo involves a small number of precursor cells and there has so far been no methods that quantitatively measure these aspects. This research aims to develop a method that enables chromatin immunoprecipitation-DNA chip (ChIP-Chip) or ChIP-Sequence analysis from a small number of cells (~1,000 cells) and using the developed methodology, to uncover the mechanisms of actions of a unique transcriptional regulator, Blimp1 both in the germ cell lineage and B-cell lineage. Since Blimp1 plays a critical role in fate specification and homeostasis of diverse cell lineages, this research will lead to a general understanding of the mechanisms involved in cellular differentiation.

【Research Methods】

In order to develop a methodology that enables ChIP-Chip or ChIP-Seq from a small numbers of cells, we use ES cells expressing Oct4 bearing EGFP or biotin ligase recognition sequence tag. Using these cells, we explore and improve many conditions that are involved in ChIP. We employ a method that we developed for single-cell cDNA amplification for the quantitative amplification of ChIP-ed DNAs. Since the genome-wide binding sites of Oct4 in ES cells are well characterized, we evaluate our methodology using published data as controls. We then generate knock-in mice that express Blimp1 bearing appropriate tags and analyze the genome-wide binding sites of Blimp1 in the process of germ cell specification and subsequent development as well as B-cell differentiation into plasma cells and plasma cell maintenance. The synthetic analysis of genome-wide transcriptions and their impairment by Blimp1 knockout and genome-wide Blimp1 binding will lead to understanding of Blimp1 function at an unprecedented resolution.

【Expected Research Achievements and Scientific Significance】

The successful development of a methodology for ChIP-Chip or ChIp-Seq from a small numbers of cells enables measurements of epigenetic states of any cell types resent in our body. Among many other potential applications, this will lead to better understandings of the mechanisms involved in the maintenance of stem cells and development of methods for their long term culture. This will also contribute to the evaluation of the quality of the cells generated in vitro from pluripotent stem cells for therapic purposes. Understanding the mechanisms of action of Blimp1 both in the germ and B-cell lineages will be a critical contribution to a general understanding of cell fate specification and maintenance. Especially, the mechanism of genome-wide epigenetic reprogramming associated with germ cell development will be resolved very finely.

【Publications Relevant to the Project】

・Ohinata, Y., Ohta, H., Shigeta, M., Yamanaka, K., Wakayama, T., and Saitou, M. (2009). A signaling principle for the specification of the germ cell lineage in mice. Cell, 137, 571-584.
・Kurimoto, K., Yabuta, Y., Ohinata, Y., Shigeta, M., Yamanaka, K., and Saitou, M. (2008). Complex genome-wide transcription dynamics orchestrated by Blimp1 for the specification of the germ cell lineage in mice. Genes & Development, 22, 1617-1635.

【Term of Project】 FY2009-2013

【Budget Allocation】 76,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.med.kyoto-u.ac.jp/J/grad_school/introduction/1103/