CDB SEMINAR

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16:00~17:00 Seminar Room A7F

Dynamics of transcription and histone modifications in living cells and organisms

Summary

Posttranslational histone modification plays a critical role in genome functions such as the epigenetic gene regulation and the maintenance of genome integrity (1). These modifications change locally and globally during the cell cycle, development and differentiation, and in response to external stimuli. However, it remains largely unknown how these modifications are regulated at single cell levels (2). We have developed a method to visualize histone modifications in living cells by loading the fluorescently labeled antigen-binding fragments (Fabs) from the specific monoclonal antibodies (3). This Fab-based live endogenous modification labeling (FabLEM) technique has revealed the differential regulation of H3S10 phosphorylation between normal and cancerous cells (4) and the distinct behaviors of H3K9 and H3K27 acetylation in mouse preimplantation embryos produced by in vitro fertilization and somatic cell nuclear transfer (5). We now applied the FabLEM technique to measure the kinetics of RNA polymerase II, which is assembled into the preinitiation complex as unphosphorylated form, and becomes phosphorylated at Ser5 and Ser2 in the C-terminal domain repeats. By timing the recruitment of these different phosphorylation marks to a gene array upon the stimulation of transcription and by fitting the data to a mathematical model, it was revealed that the transition from the initiation to elongation is quite efficient on the array. This high elongation efficiency is correlated with the preexisting histone H3K27 acetylation level. I will also present a recent development of a genetically encoded modification-specific intracellular antibody (Mintbody) system to track the posttranslational modifications in living organisms (6).

References

1) Kimura H. Histone modification for human epigenome analysis. J Hum Genet 58, 439-445 (2013).
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3) Kimura H et al. The organization of histone H3 modifications as revealed by a panel of specific monoclonal antibodies. Cell Struct Funct 33, 61-73 (2008).
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5) Hayashi-Takanaka, Y. et al. Tracking epigenetic histone modifications in single cells using Fab-based live endogenous modification labeling. Nucleic Acids Res. 39, 6475-6488 (2011).
6) Sato, Y et al. Genetically encoded system to track histone modification in vivo. Sci Rep 3, 2436 (2013).

This seminar is held as part of the 'Epigenetic seminar series' associated with a RIKEN-wide funding program, and is broadcast to other RIKEN centers.

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