VISUALIZING LIVE-CELL EPIGENETIC MODIFICATIONS OF ENDOGENOUS RNA POLYMERASE II AND HISTONES AT AN ACTIVATED GENE ARRAY

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KEY WORDS: Living cells, in vivo labeling, chromatin dynamics, transcription, histone modifications

Epigenetic protein modifications play a fundamental role in gene regulation, but the dynamics of these modifications remain a mystery in living cells. Part of the problem is that standard labeling techniques based on permanent fluorescent fusion tags such as GFP are unable to distinguish modified forms of the same protein. For example, although GFP has been used to visualize the live-cell dynamics of RNA polymerase II (pol II) [1], it has been difficult to distinguish actively elongating forms of pol II (phosphorylated at Serine 2) from freely diffusing (unphosphorylated) or initiated but stationary forms (phosphorylated at Serine 5). This complicates the analysis of pol II transcription dynamics and leaves some doubt about deduced results [2]. In this talk I will describe how this difficulty can be overcome with FabLEM (Fab-based Live Endogenous Modification labeling), a recently developed technique utilizing fluorescent antigen binding fragments (Fab) to reversibly label protein modifications in living cells with minimal disturbance [3-4]. Specifically, I will discuss our recent experiments visualizing pol II phosphorylation in conjunction with histone acetylation/methylation at an activated gene array in single living cells. By timing the recruitment of these epigenetic marks to the gene array, this work is the first to distinguish in vivo transcription initiation kinetics from recruitment and elongation kinetics. I will conclude by discussing how this data constrains quantitative models for transcription dynamics.

[1] X. Darzacq, J. Yao, D.R. Larson, S.Z. Causse, L. Bosanac, V. de Turris, V.M. Ruda, T. Lionnet, d. Zenklusen, B. Guglielmi, R. Tjian, R.H. Singer, "Imaging transcription in living cells," *Annu. Rev. Biophys.,* **38**, 173-196 (2009).
[2] T. J. Stasevich and J. G. McNally, "Assembly of the Transcription Machinery: Ordered and Stable, Random and Dynamic, or Both?," *Chromosoma*, **120**, 533-545 (2011).
[3] H. Kimura, Y. Hayashi-Takanaka, and K. Yamagata, "Visualization of DNA methylation and histone modifications in living cells," *Curr. Opin. Cell Biol.,* **22**, 412-418 (2010).
[4] Y. Hayashi-Takanaka, K. Yamagata, T. Wakayama, T.J. Stasevich, T. Kainuma, T. Tsurimoto, M. Tachibana, Y. Shinkai, H. Kurumizaka, N. Nozaki, and H. Kimura, "Tracking epigenetic histone modifications in single cells using Fab-based live endogenous modification labeling," *Nucleic Acids Res.,* **39**, 6475-6488 (2011).