Abstract 1298

## Control of gene outputs by the Integrator complex and premature transcription termination events

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Keywords: Integrator complex, Transcription termination, Protein phosphatase 2A (PP2A), RNA, Gene regulation

The metazoan-specific Integrator complex catalyzes 3' end processing of small nuclear RNAs (snRNAs) as well as premature transcription termination events that attenuate expression of many protein-coding genes, in some cases by more than 100 fold. The Integrator complex has RNA endonuclease and protein phosphatase activities, but how Integrator activity can be toggled on/off at a given gene locus depending on cellular transcriptional needs remains poorly understood. We recently showed that the Integrator phosphatase module is critical at only a subset of Drosophila protein-coding genes and that PP2A recruitment is a tunable step that modulates transcription termination efficiency at these loci. By profiling the transcriptome after individual depletion of each Integrator subunit, we now further find that gene loci can have highly distinct dependencies on Integrator subunits. For example, some protein-coding loci are controlled by only one Integrator subunit, while others require most of the subunits. We will present ongoing work that is exploring the underlying regulatory mechanisms that enable gene-specific control by Integrator, as well as how disease-associated mutations in Integrator subunits can lead to dysregulation of only a subset of Integrator regulated genes.

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**Theme: Signaling Mechanisms in the Nucleus**

Abstract 1308

## Engineering Signaling Pathways for Selective Tracking of Individual Epigenetic Writers in Mammalian Cells

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Keywords: Enzyme engineering, Epigenomics, DNA methyltransferase, Biorthogonal covakent labeling, Genome engineering

Enzymatic methylation of cytosine to 5-methylcytosine in DNA is a fundamental epigenetic mechanism involved in mammalian development and disease. DNA methylation is brought about by collective action of three independently expressed and regulated AdoMet-dependent proteins (Dnmt1, Dnmt3A and Dnmt3B). However, the catalytic interactions and temporal interplay of these epigenetic "writers" in establishing and maintaining genomic DNA methylation profiles characteristic to each cell type are poorly understood. To achieve selective tracking of the catalytic action of an individual Dnmt enzyme, we used structure-guided engineering of the mouse Dnmt1 or Dnmt3A to achieve preferential transfer of bio-orthogonal chemical moieties containing functional azide groups onto DNA from a synthetic cofactor analog. We then established endogenous expression of the engineered enzymes in a series of mouse and human cell lineages by installing the corresponding codons in the Dnmt1 or Dnmt3A alleles using CRISPR-Cas9 genome editing. Further, we elaborated an electroporation-based procedure for pulse-internalization of the azide cofactor into the engineered cells to enable selective catalysis-dependent azide-tagging of Dnmt-specific DNA targets in vivo. The deposited azide groups were exploited as 'click' handles for reading adjoining sequences and precise mapping of the tagged methylation sites in the genome using TOP-seq profiling [1]. Altogether, we present the first general approach that produces high-resolution genome-wide temporal "tracks" of the Dnmt-dependent catalysis in live mammalian cells [2]. Pilot studies of differentiation in model stem cell systems unveiled detailed interplay of the Dnmt activities during cell state transitions paving the way to unprecedented inroads into temporal aspects of genomic methylation in eukaryotic systems. [1] Staševskij et al. Mol. Cell, 2017, 65: 554–564. [2] Stankevičius et al. Mol. Cell, 2022, 82:1053–1065.

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