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Genome-wide analysis of chromatin composition and dynamics at human transcription termination regions

Transcription termination of protein-coding genes represents the last step of nascent RNA synthesis by RNA polymerase II (RNAPII), following initiation and elongation. Because transcription occurs in a dynamic nuclear environment, chromatin architecture and epigenetic factors strongly influence its progression, and transcription and chromatin organization mutually shape each other. To date, most studies have focused on initiation and elongation, leaving termination comparatively overlooked due to the assumption that RNAPII had already completed its most important tasks. Yet accumulating evidence demonstrates that termination is a crucial regulatory step, essential for safeguarding transcriptional integrity and fine-tuning gene expression.

This work investigates how chromatin organization and dynamics affect transcription termination. In the first part, I show that termination regions can be successfully defined genome-wide as windows marked by threonine 4 phosphorylation (T4ph) of the RNAPII C-terminal domain (CTD). T4ph acts as a universal marker of RNAPII terminal pausing, present at both annotated gene ends and premature transcription termination (PTT) sites within gene bodies. Genome-wide definition of termination windows further reveals their association with the occupancy of chromatin looping factors and their association with a gradual decline of H3K36me3, a histone modification characteristic of active transcription.

In the second part, I explore the role of the H3K36me3-depositing methyltransferase SETD2. While SETD2 is generally required for correct transcription initiation, I demonstrate that in ~15–25% of genes it is additionally essential for proper termination. At these loci, SETD2's catalytic activity promotes efficient pre-mRNA 3' end cleavage and prevents cryptic transcription initiation within gene bodies, particularly near gene ends. Importantly, this function of SETD2 in termination occurs independently of alternative polyadenylation (APA).

Finally, I address the clinical relevance of accurate termination by showing how PTT contributes to transcriptome diversity in pontocerebellar hypoplasia type 10, a rare neurodevelopmental disorder. Since PTT regulates isoform balance and attenuates gene expression, its role in disease pathogenesis merits further investigation.

Taken together, this work emphasizes that gene ends represent important regulatory regions influencing transcript stability, localization and the eventual function of encoded proteins. Genome-wide definition of termination windows offers a valuable resource for improving genomic annotations and for identifying pathogenic variants in noncoding regions. Moreover, the discovery of a novel role for the tumor suppressor SETD2 in 3' end processing underscores the intricate interplay between epigenetic regulation and transcription, and highlights termination as a one of central safeguards of transcriptional stability in both normal physiology and disease.