Catching mammalian RNA polymerase II in act

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RNA processing is tightly connected to RNA polymerase II (Pol II) transcription. The C-terminal domain (CTD) of Pol II largest subunit is dynamically phosphorylated during transcription to allow efficient and regulated recruitment of RNA processing factors. Recently I have developed native elongating transcript-sequencing technique for mammalian cells (mNET-seq) that allows to map Pol II active site and co-transcriptional RNA cleavage at a single nucleotide resolution genome-wide[1,2]. In addition precise Pol II CTD code in nascent RNA synthesis can be analyzed using a selection of CTD phosphorylation specific Pol II antibodies[1,3]. Interestingly, mNET-seq revealed a splicing intermediate are specifically associated with serine 5-phosphorylated (S5P) CTD Pol II[1].

I have applied this sequencing method to proteomics analysis, named mNET-mass spectrometry (mass spec). Combined with mNET-seq and -mass spec technologies of human Pol II elongation complexes, I have defined co-transcriptional splicing kinetics in human and mouse (unpublished). Additionally I found distinctive patterns of transcription for protein-coding and long intergenic noncoding RNA (lincRNA) in terms of Pol II CTD isoforms, RNA stability and RNA processing. I have shown lincRNAs are less processed and co-transcriptionally cleaved, causing premature termination followed by post-transcriptional RNA degradation[3].

Overall mNET-seq and -mass spec are powerful techniques to explore the complex life of nascent RNA in mammalian cells. I would like to present here in this symposium what mNET-based techniques could do for gene expression study.

References

1. Nojima et al. ***Cell*** 161, 526-540, 2015
2. Nojima et al., ***Nature Protocols*** 11, 413-428, 2016
3. Schlakow, Nojima (equally contributed) et al., ***Molecular Cell***, in press