Supplementary information S1 | **High-resolution sequencing strategies to study dynamics of and interactions between co-transcriptional processes**

In recent years, next generation sequencing became the tool of choice to investigate gene expression regulation. Researchers developed a multitude of sample preparation strategies to obtain binding profiles of DNA- and RNA-binding proteins and to monitor transcription dynamics at nucleotide resolution.

Chromatin immunoprecipitation approaches. Traditionally, chromatin immunoprecipitation (ChIP) was developed to monitor localization of DNA- or nucleosome-binding proteins, such as transcription factors and RNA polymerase[1]. Formaldehyde crosslinking between DNA and proteins followed by fragmentation of genomic DNA enables specific pulldown of the protein of interest and associated genomic DNA. Additions to the classic protocol increase resolution, specificity and detection range of the procedure substantially[2,3]. With an extension of the ChIP assay to RNA-binding proteins tethered to RNA polymerase by nascent RNA[4], stepwise and co-transcriptional spliceosome assembly was shown to occur *in vivo*[5-7].

Nascent RNA sequencing at nucleotide resolution. Nascent transcripts provide a snapshot of the progress of transcription and its associated processing steps, such as pre-mRNA 5' end capping, splicing, folding and editing. The 3' end of nascent RNA corresponds to the position of elongating RNA polymerase II (Pol II) along the gene. Nascent transcripts can be enriched from total RNA and isolated in multiple ways: 1) isolation from a chromatin fraction[8-10], 2) immunoprecipitation of Pol II core subunits or subsets of the Pol II C-terminal domain (CTD) modified with specific post-translational modifications (PTMs)[11-14] and 3) pulse-labeling of nascent RNA with modified nucleotides such as 4-thio-UTP, followed by modification-specific purification[15-20]. Several advances highlighted in this Review have been made using those global methods to enrich for nascent RNAs.

Enriching for processing intermediates. Co-transcriptional pre-mRNA processing intermediates can inform about the dynamics of individual steps in pre-mRNA splicing and the fate of the transcripts. Pre-mRNA splicing intermediates, such as the step I products or the excised intron lariat, can be detected using next generation sequencing[11,12,21-24]. Mapping the branchpoint sequence became possible by enriching the intron lariats prior to sequencing[21-23] and by combining total RNA-seq with U2 snRNP–pre-mRNA base-pairing modeling[25]. The combination of native elongating transcript sequencing (NET-seq) with immunopurification of specific PTMs of the Pol II CTD revealed that splicing intermediates are enriched in Pol II phosphorylated on Serine 5[12,14].

Alternative methods to monitor nascent RNAs. Sequence analysis of full-length nascent transcripts is a promising strategy to quantitatively assess transcription and nascent RNA processing. Full-length nascent transcript sequencing by long-read sequencing (LRS) was used to identify the minimal length of nascent RNA at which step II of pre-mRNA splicing can be completed[8]. The co-occurrence of alternative and constitutive splicing events on single transcripts can also be analyzed using LRS[26,27]. Direct sequencing of RNA will be another promising avenue to explore in future[28].

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