Playing inside the genes

Intragenic histone acetylation after membrane depolarization of neural cells opens a path for alternative splicing regulation

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Regulation of alternative splicing is coupled to transcription quality, the polymerase elongation rate being an important factor in modulating splicing choices. In a recently published work, we provide evidence that intragenic histone acetylation patterns can be affected by neural cell excitation in order to regulate alternative splicing of the neural cell adhesion molecule (NCAM) mRNA. This example illustrates how an extracellular stimulus can influence transcription-coupled alternative splicing, strengthening the link between chromatin structure, transcriptional elongation and mRNA processing.

Alternative splicing is known to be the main contributor to the expansion of proteome expression potentials in metazoans. The number of protein variants generated by alternative splicing is particularly high in the nervous system, affecting proteins such as ion channels, cell adhesion molecules, components of the cytoskeleton, proteins involved in signaling and trafficking, transcription factors and even splicing regulators. The alternative variants have usually different functions and regulatory features, which in some cases may even be antagonistic. Furthermore, since the process is tightly regulated, alternative splicing provides a whole new level for fine control of gene expression, in addition to the extensively studied regulation of transcription initiation. The cellular functions where this mechanism plays a role in neurons include nearly all the aspects of both developing and mature cells.

Cellular components of the nervous system require complex functional regulation, with responses varying in different neuronal types, during development and in response to different stimuli. Given the expansion of functions of alternative splicing in neuronal cells, the precise control of the splicing choices is expected to be of crucial importance for proper cellular functionality. Combinatorial control, including additive and cooperative interactions between different regulatory components, is believed to be responsible for such fine tuning of alternative splicing. It implies that a change in the activity of a single splicing regulator can affect splicing of specific mRNAs to different degrees in the same cell or influence alternative splicing of one mRNA in one cell type and not in others, depending on the action of other regulatory elements.

In extreme cases, malfunction of the complex network of alternative splicing regulation, and the consequent alterations in splicing patterns, is associated with severe neurological disorders such as ataxia, frontotemporal dementia, myotonic dystrophy and spinal muscular atrophy.

Transcription and mRNA processing are part of a growing list of nuclear and cytoplasmic mRNA-protein biogenesis steps that need to be taken into account in order to describe gene expression in an integrative way. Most importantly, these steps are usually coupled, influence each other and can be co-regulated in a concerted way to achieve specific cellular responses. In particular, the notion that eukaryotic mRNAs are first fully transcribed and then processed is now abandoned, since most mRNA processing steps are known to occur co-transcriptionally in vivo.

In a recent report, we characterized how a dynamical modulation of transcription quality can regulate alternative splicing of an endogenous gene in response to an external cue, such as neural cell excitation. We determined that exon 18 of the gene coding for the neural cell adhesion molecule (NCAM) is preferentially included in the mature mRNA when a slow RNA polymerase II (pol II) mutant is used to drive transcription. This exon is also regulated in response to excitation of neuroblastoma cells and hippocampal neurons: upon cell membrane depolarization a switch is detected from the NCAM 180 isoform mRNA (typical of mature neurons.

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Intragenic histone acetylation regulates alternative splicing

Figure 1. Model of neuronal excitation-induced regulation of alternative splicing for the NCAM exon 18. Intragenic histones located between exons 17 and 19 are basally hypo-acetylated, while chromatin adopts a closed structure (top). Upon depolarization with high extracellular KCl concentration, histone acetylation and chromatin relaxation is detected in this region (bottom). Concomitantly, RNA pol II processivity increases, a situation which is associated with alternative exon exclusion in co-transcriptional splicing since less time is available for recognition of the weak exon (exon 18, which is shown in dark gray). The transcriptional changes affect only elongation, since no changes are detected at the promoter region (marked with a blue arrow).

In particular, histone acetylation is known to be crucial for many processes that rely on neuronal excitation, learning being a clear example; with a well supported role of histone acetyl-transferases (HATs) like CBP.26,27 The molecular mechanism that mediates the acetylation changes observed in the NCAM regulation remains to be deciphered. Between HAT and HDAC activities will affect the acetylation patterns observed, but how they can be recruited differentially to intragenic region is a matter that has not been explored in detail yet.

Evidence indicating that both of these activities can be associated with the RNA pol II can provide a clue.28 Since histone modifications posed by the transcription machinery recruits splicing factors,29 and alternative splicing factors such as the SR protein SC35 can regulate pol II elongation,30 the elucidation of the interplay between chromatin, elongation and mRNA processing seems a complex and exciting task to be undertaken. In this direction, the regulation of intragenic histone acetylation patterns is claiming for attention as an actor in this play.

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