Resolving CNS mRNA Heterogeneity: Examining mRNA Alternative Polyadenylation at a Cell-Type-Specific Level

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http://dx.doi.org/10.1016/j.neuron.2017.08.045

Alternative polyadenylation often regulates mRNA isoform usage. In this issue of *Neuron*, Hwang et al. (2017) describe a powerful new cell-type-specific methodology, cTag-PAPERCLIP, which can be used to study alternative polyadenylation in the CNS.

The production of mature mRNA species involves several processing steps, including the linked endonucleolytic cleavage and polyadenylation of a nascent transcript. Alternative cleavage and polyadenylation (APA) is a phenomenon in which alternate polyadenylation sites are targeted in order to generate different mRNA isoforms from the same gene (Figure 1A). As this usage of distinct polyadenylation sites within a nascent transcript can lead to alterations in exon incorporation, mRNA stability, and mRNA localization, APA is an essential and broadly conserved process that can influence the encoded protein's sequence, levels, and localization (Tian and Manley, 2017).

Several global transcriptomic studies have found that APA is a highly dynamic process that differs across cell types and developmental stages, for example, with rapidly proliferating cells having the shortest 3′ UTRs and differentiated cells, including neurons, possessing longer 3′ UTRs at a genome-wide level (Miura et al., 2013). In neurons, APA has been linked to several mRNA isoform properties, including localization (Taliaferro et al., 2016), and encoded protein sequence alterations (Flavell et al., 2008). These studies have shown that cell-type-specific data are essential to not only accurately profile APA, but also to understand its significance and gain mechanistic insights into its regulation. Thus, in order to study the role of APA in each developmental context and cell type, a methodology has been needed that can faithfully report cell-type-specific APA *in situ*. In the central nervous system (CNS), this is a particularly daunting challenge given the heterogeneity of cell types.

Hwang and colleagues recently developed a methodology that can be used to interrogate the 3′ ends of mRNAs called poly(A) binding protein (PABP)-mediated mRNA 3′ end retrieval by cross-linking immunoprecipitation (CLIP) (PAPERCLIP) (Hwang et al., 2016). PAPERCLIP uses the CLIP approach to target PABP and retrieve its associated mRNAs' 3′ ends for RNA sequencing (RNA-seq). This methodology allows unbiased, quantitative, nucleotide-level resolution of mRNA 3′ ends, since it eliminates artifacts due to the internal mRNA mispriming that are inherent in other 3′ UTR sequencing methodologies that rely on the use of reverse transcription with oligo(dT) primers. Using PAPERCLIP, Hwang et al. (2016) not only detected APA shifts during mouse brain development, but also importantly showed that PAPERCLIP can be used to gain mechanistic insights into the regulation of APA. However, even though the PAPERCLIP methodology is ideal for high-performance identification of mRNA 3′ ends, and thus APA, the near-universal expression of PABP limits the resolution of PAPERCLIP data in tissues constituted of many heterogeneous cell types, such as in the CNS. In this issue of *Neuron*, Hwang et al. (2017) report a new version of the PAPERCLIP methodology that can be used in a cell-type-specific manner. For this purpose, they generated a knockin mouse line in which PABP is tagged with GFP in a conditional Cre-dependent fashion. This version of PAPERCLIP is called conditionally tagged poly(A) binding protein-mediated mRNA 3′ end retrieval by cross-linking immunoprecipitation (cTag-PAPERCLIP) (Figure 1B). The use of Cre-based conditional expression makes this approach highly selective and versatile, in theory allowing access to any genetically defined cell type. The fact that cTag-PAPERCLIP purifications can be performed from whole tissue is extremely important to obviate stress-induced effects (e.g., upon tissue dissociation and cell purification), which themselves have been well documented to lead to APA shifts.

In this first application of cTag-PAPERCLIP, Hwang et al. (2017) identify differences in APA between excitatory neurons, inhibitory neurons, astrocytes, and microglia. Among all of the genes expressed across the four genetically defined cell types, about one-third displayed APA by cTag-PAPERCLIP analysis. And among these third, over two dozen showed marked differences in APA between neurons and glia. Studying how these differences may be regulated, Hwang et al. (2017) focus on the role of RNA-binding proteins NOVA2 and PTBP2 and directly show that NOVA2 promotes neuronal-type APA, while PTBP2 suppresses neuronal-type APA for some of these genes. In a final example of the power of cTag-PAPERCLIP to obviate stress-induced changes to APA profiles, Hwang et al. (2017) showed that cTag-PAPERCLIP can isolate microglial transcripts with minimal induction of microglial activation markers. This ability allowed them to

discover a role for APA during microglial activation following lipopolysaccharide (LPS) challenge, in which they observed not only biased usage of proximal APA sites, but also, as one example, a switch to full-length ARAF protein expression during microglial activation that they link to inflammatory cytokine production.

Thus, Hwang et al. (2017) not only show the power of cTag-PAPERCLIP to accurately report APA in distinct cell types, but also show the requirement for such high-performance data in order to make mechanistic and functional predictions. Application of new CLIP techniques (Van Nostrand et al., 2016; Zarnegar et al., 2016) may make cTag-PAPERCLIP more sensitive when applied to rare cell types, and the combination of cTag-PAPERCLIP data, cell-type-specific translational profiling (Heiman et al., 2008), and cell-type-specific miRNA profiling (He et al., 2012) has great potential to reveal further mechanistic and functional insights regarding APA in neurons. Finally, as APA has been shown to be altered during several disease contexts, application of the cTag-PAPERCLIP methodology also has great potential to yield new insights into CNS disease biology. For example, amyotrophic lateral sclerosis (ALS)-linked pathophysiology has been associated with various aspects of RNA processing, and the ALS-linked proteins C9ORF72 and HNRNPA2B1 have both recently been suggested to affect APA. A more specific characterization of APA in ALS models using cTag-PAPERCLIP may yield new insights into the APA-linked consequences of ALS-linked mutations. The cTag-PAPERCLIP methodology shows great promise for leading the way to many novel insights into the phenomenology and mechanistic basis of APA in both normal and disease contexts.

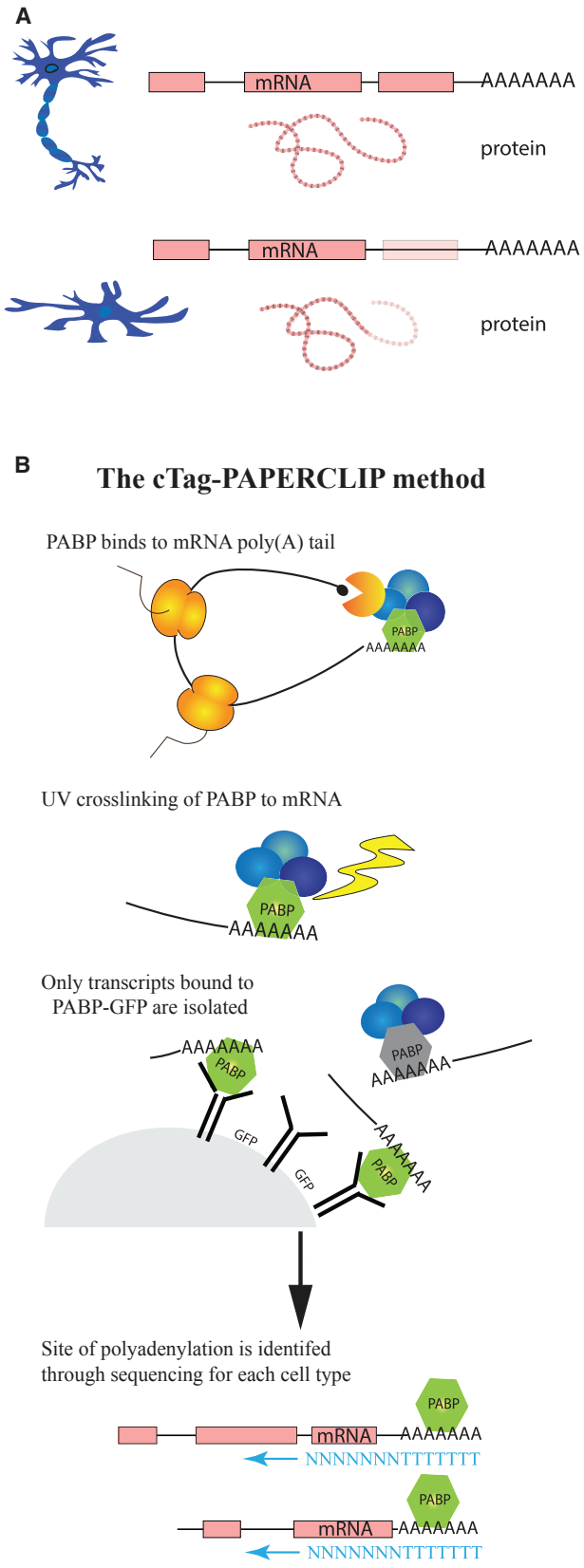

Figure 1. cTag-PAPERCLIP Can Be Used to Evaluate APA in a Cell-Type-Specific Manner

(A) Alternative cleavage and polyadenylation (APA) can produce mRNA diversity not only in the 3′ UTR, but also upstream of the 3′ UTR, leading to shifts in protein expression level, localization, and function between cell types (protein sequence variation shown; neuron, top; glia bottom).

(B) cTag-PAPERCLIP combines conditional genetic tagging of PABP protein (GFP tag; green PABP) with CLIP (UV crosslinking, yellow) to retrieve 3′ mRNA ends in a cell-type-specific manner. Cell-type-specific cTag-PAPERCLIP-isolated RNA can then be sequenced to accurately identify APA sites with high resolution.

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