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Update Article

Spatio-temporal regulations and functions of neuronal alternative RNA splicing in developing and adult brains

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ABSTRACT

Alternative pre-mRNA splicing is a fundamental mechanism that generates molecular diversity from a single gene. In the central nervous system (CNS), key neural developmental steps are thought to be controlled by alternative splicing decisions, including the molecular diversity underlying synaptic wiring, plasticity, and remodeling. Significant progress has been made in understanding the molecular mechanisms and functions of alternative pre-mRNA splicing in neurons through studies in invertebrate systems; however, recent studies have begun to uncover the potential role of neuronal alternative splicing in the mammalian CNS. This article provides an overview of recent findings regarding the regulation and function of neuronal alternative splicing. In particular, we focus on the spatio-temporal regulation of neurexin, a synaptic adhesion molecule, by neuronal cell type-specific factors and neuronal activity, which are thought to be especially important for characterizing neural development and function within the mammalian CNS. Notably, there is increasing evidence that implicates the dysregulation of neuronal splicing events in several neurological disorders. Therefore, understanding the detailed mechanisms of neuronal alternative splicing in the mammalian CNS may provide plausible treatment strategies for these diseases.

1. Introduction

Alternative RNA splicing through the selective exclusion or inclusion of pre-mRNA sequences is a powerful system for transcriptomic and proteomic diversity. The resulting molecular repertories are thought to be essential for generating biological complexity in mammalian systems. Therefore, splicing regulation is likely prominent in the vertebrate central nervous system (CNS) (Barbosa-Morais et al., 2012; Jelen et al., 2007; Merkin et al., 2012). The mammalian brain is the most complicated but well-organized system. Neuronal cell development is controlled by a highly organized sequence of developmental events that consist of proliferation, differentiation, migration, and maturation (Wang and Zoghbi, 2001). Neuronal alternative splicing events may contribute to each step of this developmental sequence, which is expected to contribute greatly to the complexity and specificity of neural circuits (Li et al., 2007; Raj and Blencowe, 2015).

Neuronal alternative splicing is dynamically controlled via spatio-temporal regulation. Alternative splicing decisions are highly altered during neural development in a neuronal tissue- or cell type-specific fashion (Calarco et al., 2011; Kalsotra and Cooper, 2011). Furthermore, neuronal activity modulates alternative splicing via Ca^{2+}-dependent signaling pathways (Razanau and Xie, 2013). However, uncovering the dynamic regulation of neuronal alternative splicing raises further questions. First, what mechanisms underlie neuronal alternative splicing and regulate it in a spatio-temporal manner? While a majority of previous studies have highlighted the *cis*-acting RNA elements and *trans*-acting RNA-binding proteins (RBPs) that regulate global alternative splicing

Abbreviations: AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ASD, autism-spectrum disorder; AS4, alternatively spliced segment 4; CaMKIV, Ca^{2+}/calmodulin-dependent kinase IV; CCK, cholecystokinin; CNS, central nervous system; DSCAM, Down syndrome cell adhesion molecule; GC, cerebellar granule cell; hnRNP, heterogeneous ribonucleoprotein; LRRTMs, leucine-rich repeated transmembrane proteins; L-VDCC, L-type voltage-dependent Ca^{2+} channel; NMDAR, *N*-methyl-*D*-aspartic acid receptor; NMD, nonsense-mediated decay; Nrxn, neurexin gene; NRX, neurexin protein; PC, Purkinje cell; PF, parallel fiber; PPA, perforant path associated; RBP, RNA-binding protein; SAM68, Src-associated in mitosis of 68 kDa protein; SCA, Schaffer collateral associated; SLM1, SAM68-like mammalian protein 1; SLM2, SAM68-like mammalian protein 2; S.L.M., stratum lacunosum moleculare; SMA, spinal muscular atrophy; S.O., stratum oriens; S.R., stratum radiatum; STAR, signal transduction and activation of RNA; VIP, vasoactive intestinal peptide.

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events, the specific signaling pathways and molecules that govern neuronal alternative splicing in developmental, tissue-specific, and cell type-specific fashions are not well characterized. Second, to what extent do neuronal alternative splicing programs impact neural complexity and diversity in the mammalian CNS? Overall, the utilization of alternative splicing has expanded, particularly in the CNS, over the course of evolution. However, the functional aspects of alternative splicing have not been clearly demonstrated in mammalian brains.

The present review summarizes recent studies on neuronal alternative splicing. We mainly focus on spatio-temporal regulation by neuronal cell type-specific factors and neuronal activity. These forms of regulation are especially important for neural development and other functions within the mammalian CNS. Here, we will introduce and discuss the history and recent findings regarding the molecular mechanisms of spatio-temporal regulation of neuronal alternative splicing, including some of our own recent work. We will then provide new insights into relevant mechanisms and potential roles for alternative splicing in the mammalian CNS.

2. Neuronal activity-dependent alternative splicing regulation

Neuronal activity-dependent signaling pathways in the brain play a critical role in circuit assembly and plasticity processes (Davis, 2006; Wong and Ghosh, 2002). Among the signals that regulate splicing, Ca^{2+}-mediated signals are the most critical for the control of various physiological processes, from neural development and differentiation to synaptic plasticity in mature neurons. Indeed, neuronal activity-dependent alternative splicing is regulated by the influx of Ca^{2+}, which is mediated through *N*-methyl-*D*-aspartic acid receptors (NMDARs) and/or L-type voltage-dependent calcium channels (L-VDCC). Ca^{2+}-mediated signals serve as a powerful mechanism for the dynamic modification of neuronal function, where the production of specific splice variants modifies cellular trafficking, signaling properties, or synaptic protein function (An and Grabowski, 2007; Li et al., 2007; Xie and Black, 2001).

2.1. Ca^{2+}-dependent regulation via CaMKIV responsible cis-acting elements

High K^+-induced depolarization triggers a shift in alternative splicing within cultured neuronal cells via Ca^{2+}/calmodulin-dependent kinase IV (CaMKIV) (An and Grabowski, 2007; Li et al., 2007; Xie and Black, 2001). Most of these activity-regulated exons are in genes known to be critical for neuronal function and development such as neural cell adhesion molecule (NCAM), synapse-associated protein of 25 kDa (SNAP25), the NMDA receptor type I (NMDAR1), Big Potassium (BK) and Ca^{2+} channels, and the inositol triphosphate receptor type 1 (IP_3R1) (Xie, 2008). An initial finding revealed the alternative splicing of the BK channel STREX exon, which is 1 of more than ten alternative exons of the vertebrate *Slo* gene. BK channels contain both Ca^{2+} and voltage-sensitive domains and are important for coupling Ca^{2+} transients with the electrical properties of excitable cells by participating in the repolarization and post-hyperpolarization action potential phases (Salkoff et al., 2006). Black and colleagues identified the *cis*-acting elements for CAMKIV-mediated alternative splicing, referred to as CaMKIV-responsive elements (CaRRE1 and 2: CACAUNRUUAU and GUGGUAGA), on the STREX exon and neighboring intronic sequence (Xie and Black, 2001). Similarly, another CaMKIV-responsive element has been revealed, the UAGG motif, through which exon21 of the NMDAR1 gene is included in a depolarization-dependent manner (An and Grabowski, 2007). CaMKIV or depolarization enhances interaction of these elements with heterogeneous ribonucleoproteins (hnRNPs), hnRNPA1 and hnRNPL, suggesting that these hnRNPs modulate depolarization-dependent alternative splicing during CaMKVI signaling (An and Grabowski, 2007; Liu et al., 2012) (Fig. 1A). However, the aforementioned RNA elements are not necessarily observed in all pre-mRNAs subject to depolarization-dependent alternative splicing. Therefore, given that a significant number of alternatively spliced pre-mRNAs do not contain CaRREs or the UAGG motif, additional mechanisms are likely active in neuronal activity-dependent alternative splicing.

2.2. Ca^{2+}-dependent regulation via a KH-domain protein, SAM68

We recently proposed a novel Ca^{2+}-dependent regulation system that is independent of CaRRE or the UAGG motif. We observed alternative splicing of the cell adhesion molecule, neurexin (*Nrxn*) exon20, at alternative splice site 4 (AS4) in response to neuronal activity (Iijima et al., 2011). Neither CaRREs nor the UAGG motif was observed at *Nrxn* AS4, yet, there was a dependence on CaMKIV activity. Neurexin proteins (NRX) are synapse organizers localized to the presynapse, and they induce synapse formation through *trans*-synaptic interactions with several post-synaptic receptors, including neuroligins, leucine-rich repeated transmembrane proteins (LRRTMs), and the Cbln1-GluD2 complex (Krueger et al., 2012). The resulting molecular repertories of NRX complexes allow each type of neuron to exert different synaptic properties, and are thought to contribute to the complexity and diversity of neural circuits. Importantly, *trans*-synaptic interaction with post-synaptic ligands depends on the insertion or exclusion of exon20; neuroligins and LRRTMs preferentially interact with AS4(−) lacking exon20 (Boucard et al., 2005; Chih et al., 2006; Ko et al., 2009; Siddiqui et al., 2010), whereas the Cbln1-GluD2 complex specifically binds to AS4(+) containing exon20 (Matsuda and Yuzaki, 2011; Uemura et al., 2010). Thus, an alternative splice choice at AS4 is critical for a selective *trans*-synaptic interaction with these postsynaptic ligands (Krueger et al., 2012; Reissner et al., 2013).

Our recent study showed that SAM68 (the Src-associated substrate in mitosis of 68 kDa) is a key player in the activity-dependent alternative splicing of *Nrxn1* (Iijima et al., 2011). SAM68 is a member of the STAR (signal transduction and activation of RNA) family of RBPs containing the KH-domain (Volk et al., 2008). SAM68 is ubiquitously and abundantly expressed in the CNS, but does not constitutively influence *Nrxn* splicing. Instead, high K^+-induced depolarization or increased neuronal activity promotes the ability of SAM68 to affect *Nrxn* splicing through CaMKIV signaling and direct phosphorylation, which causes the skipping of exon20 through an association with AU-rich intronic elements in the pre-mRNA (Iijima et al., 2011) (Fig. 1A). Although several substrates for SAM68 were identified previously (Chawla et al., 2009; Huot et al., 2012), our findings further elucidate a novel SAM68-mediated splicing mechanism and the target pre-mRNA in mature neurons.

3. Neuronal cell type-specific alternative RNA splicing

Neural networks for all brain tissues are composed of several neuron types. Neuronal cell types are characterized by unique morphological and functional properties that shape signal processing within a given network (Masland, 2004; Okaty et al., 2011a,b). Given that the remarkable diversity of neurons is achieved via neuronal cell type-specific gene expression programs, neuronal cell type-specific alternative RNA splicing programs are likely one of the crucial mechanisms for generating the morphological and functional identities of individual cell populations.