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Review

RNA Editing: A Contributor to Neuronal Dynamics in the Mammalian Brain

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Post-transcriptional RNA modification by adenosine to inosine (A-to-I) editing expands the functional output of many important neuronally expressed genes. The mechanism provides flexibility in the proteome by expanding the variety of isoforms, and is a requisite for neuronal function. Indeed, targets for editing include key mediators of synaptic transmission with an overall significant effect on neuronal signaling. In addition, editing influences splice-site choice and miRNA targeting capacity, and thereby regulates neuronal gene expression. Editing efficiency at most of these sites increases during neuronal differentiation and brain maturation in a spatiotemporal manner. This editing-induced dynamics in the transcriptome is essential for normal brain development, and we are only beginning to understand its role in neuronal function. In this review we discuss the impact of RNA editing in the brain, with special emphasis on the physiological consequences for neuronal development and plasticity.

Trends

Recent analyses show a global developmental regulation of A-to-I editing during neuronal maturation.

Local distribution of both coding and non-coding RNA editing substrates in dendrites promotes local variability in levels of edited proteins and RNAs within the neuron.

Recent advances in NGS transcriptome analyses will make it possible to study activity-induced variations in RNA editing that may further explain the complexity of neurotransmission.

Post-Transcriptional RNA Editing by Adenosine Deaminases Acting on RNA (ADARs) in the Brain

RNA editing in the brain was initially observed when studying the main players of fast excitatory neurotransmission. Cloning of the mammalian AMPA receptor subunit GluA2 revealed a discrepancy between the encoded genomic sequence and the mRNA, giving rise to an amino acid substitution (Q/R). This is caused by the enzymatic deamination of an adenosine (A) into the base analog inosine (I) in the double-stranded (ds) pre-mRNA, altering the CAG codon to CIG [1]. Inosine prefers to base-pair with cytosine and is thus interpreted as guanosine (G) during translation [2].

This finding initiated a decade of work focused on identifying and characterizing the editing enzymes. Two conserved ADAR enzymes (ADAR1 and ADAR2) have been found to catalyze A-to-I deamination in dsRNA (Box 1) [3–8]. The full understanding of how ADARs determine which dsRNA structure to edit and to what frequency is still under investigation, although both *cis*- and *trans*-regulatory factors have been described [9–12]. Editing efficiency varies, allowing the simultaneous presence of unedited and edited isoforms, which is especially important for brain function. In this way, the cell utilizes editing to expand their proteome from a limited set of genes.

Initially only serendipitously identified single sites of editing were found by comparisons between the cDNA and the genomic sequence. However, the recent bloom of next-generation sequencing (NGS) has provided a powerful tool in detecting reliable levels of editing at numerous sites simultaneously. Currently, editing is predicted to occur at over a hundred million sites, with the vast majority positioned in primate-specific inverted repetitive elements of non-coding *Alu* sequences [13]. When transcribed as inverted repeats, *Alu*s frequently form long stem-loop

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Box 1. A-to-I RNA Editing Players

Perturbing expression levels and functions in multiple animal models have collaterally proved that the ADAR enzymes are essential proteins. In mammals, there are two enzymatically active proteins: ADAR1 (*ADAR*) and ADAR2 (*ADARB1*). The specificity of these enzymes differs but is also overlapping. For example, the Q/R site in the transcript coding for the key subunit GluA2 of the AMPA receptor is only edited by ADAR2, while the R/G site within the same transcript can be edited by both ADAR1 and ADAR2 [21]. Ablating ADAR2 results in a lethal phenotype within 3 weeks after birth as a result of seizures [21–23]. Interestingly, this phenotype can be rescued by replacing the A with a G at the Q/R site in GluA2, mimicking editing at this site [21].

ADAR1 mutant mice are embryonic lethal at embryonic (E) day E12.5 owing to defects in erythropoiesis, stress-induced apoptosis, liver disintegration, and overproduction of type I interferon [110,111]. The direct cause of this phenotype is not completely understood but it can be partly rescued at birth by the additional removal of a main player in the innate immune response, the MAVS (mitochondrial antiviral signaling) protein [112]. This result was recently confirmed by the rescue of an ADAR1 editing-deficient knock-in mouse that survived until adulthood by concurrent deletion of the cytosolic sensor of dsRNA, MDA5 (melanoma differentiation-associated protein), another factor involved in the same innate immunity signaling pathway [113].

structures that are largely double-stranded. Although the number of possible editing sites in these elements is immense, the efficiency of editing at individual sites is often low, and their functional importance is currently under active investigation and debate ([14–16] for recent reviews). We have chosen to highlight the role of single sites of editing for neuronal function during early development and in the control of synaptic plasticity in the adult nervous system.

RNA Editing as a Regulator of Neurotransmission

A-to-I RNA editing plays a significant physiological role in neuronal function by modifying several transcripts coding for proteins involved in neurotransmission. The consequences of these A-to-I editing events are often amino acid changes in the translated protein. Subunits of the excitatory AMPA and kainate glutamate receptors are subjected to editing at a site located in the ion-selection filter of the inner channel pore. Here, mRNA editing leads to replacement of a glutamine (Q) for an arginine (R) at the Q/R site in GluA2, GluK1, and GluK2. This renders the assembled receptors impermeable to Ca^{2+} (Figure 1) [1,17–19]. GluK2 Q/R editing-deficient mice reveal that the unedited subunit may be involved in synaptic plasticity via long-term potentiation (LTP) [20], while failure to edit the GluA2 Q/R site results in epileptic seizures and death [21–23]. This severe postnatal phenotype is most likely due to developmental aberrations because of excessive Ca^{2+} influx. Complete rescue of the excitotoxic phenotype was observed when pre-edited GluA2 alleles ($GluA2^{R/R}$) were introduced (Box 1) [21]. Q/R site editing is also detected in close to 100% of the GluA2 transcripts from embryogenesis to adulthood [1,21,24]. Interestingly, recent studies of Q/R editing levels during the early stages of *in vitro* neuronal differentiation have shown that neural progenitor cells (NPCs) express Ca^{2+}-permeable receptors. However, upon initiation of differentiation into neurons, GluA2 transcripts become rapidly and efficiently edited (Figure 2, Key Figure) [25,26]. Interestingly, delivery of ADAR2 into NPCs prevents neuronal differentiation [26], indicating the importance of the temporal regulation of this editing event. Moreover, the presence of unedited GluA2 has been described early during pig brain embryogenesis. Here, the extent of Q/R editing was remarkably low with a near-equal distribution of the Q and R isoforms in the early embryo [27]. At this stage the AMPA receptors are also Ca^{2+}-permeable. Thereafter, editing increased to virtually 100%, similar to observations made during mouse brain maturation (Figure 2) [24]. Together, these studies indicate that unedited GluA2 plays a role during early neurogenesis by giving rise to Ca^{2+}-permeable receptors which may contribute to dendritic arbor, synapse, and circuit formation, whereas the fully edited isoform is crucial for neuronal and organism survival at later stages.

In addition to altered membrane excitability by ion flow, RNA editing also contributes to plasticity by fine-tuning firing patterns via changes in the speed of inactivation after a depolarization event. In the GluA2, GluA3, and GluA4 subunits, an arginine is altered to glycine (R/G) in the extracellular