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Symposium

Novel RNA Modifications in the Nervous System: Form and Function

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Modified RNA molecules have recently been shown to regulate nervous system functions. This mini-review and associated mini-symposium provide an overview of the types and known functions of novel modified RNAs in the nervous system, including covalently modified RNAs, edited RNAs, and circular RNAs. We discuss basic molecular mechanisms involving RNA modifications as well as the impact of modified RNAs and their regulation on neuronal processes and disorders, including neural fate specification, intellectual disability, neurodegeneration, dopamine neuron function, and substance use disorders.

Introduction

Chemical modifications play a crucial role in the regulation of biological processes. For example, the function of a protein is often modulated by its stable tagging with different chemical groups (phosphates, sugars, or lipids), whereas specific chemical marks made along the chromatin (the DNA and/or its packaging proteins) can influence gene expression. A variety of post-transcriptional modifications of RNA are also found in all organisms. The RNA Modification Database indicates that there are at least 65 RNA modifications that arise in eukaryotic cells (Cantara et al., 2011). Historically, transfer RNA (tRNA) and ribosomal RNA (rRNA) have been shown to be heavily modified, but some of these modifications also occur in messenger RNA (mRNA) (Meyer et al., 2012; Li and Church, 2013; Russell and Limbach, 2013; Wang et al., 2014c). Most recently, RNA modifications have also been found in noncoding RNAs (ncRNAs) (Storz, 2002; Matera et al., 2007; Yu and Chen, 2010; Meyer et al., 2012; Squires et al., 2012).

A few covalent RNA modifications, such as 5′mRNA capping, alternative splicing, and polyadenylation, have been studied extensively. To date, however, most RNA modifications have not been well characterized for two major technical reasons. The first reason is that many modified RNA bases are recognized by reverse transcriptases the same way as their unmodified counterparts. Because a common step in many RNA experiments is to reverse transcribe the RNA into cDNA, this effectively "erases" any information concerning the types and locations of RNA modifications that might have been present (Behm-Ansmant et al., 2011). A second reason is that our technical ability to detect and quantitate RNA modifications has been limited until recently (Yan et al., 2013; Kullolli et al., 2014). Both of these issues have severely impaired our ability to systematically characterize the "epitranscriptome," which can be defined as all of the chemical modifications of RNA molecules (both coding and noncoding) (Saletore et al., 2012; Hussain et al., 2013a; Li and Mason, 2014; Meyer and Jaffrey, 2014). Thus, the functional roles of many post-transcriptional RNA modifications remain unknown, although they could potentially influence parameters, such as RNA stability, translation, trafficking, localization, enzymatic or sensing activity, regulatory capabilities, or patterns of interactions with other molecules.

The purpose of this mini-review and the associated SFN mini-symposium is to highlight the types and known functions of several novel modified RNAs in the nervous system. We will discuss the two most well-studied mammalian mRNA modifications, including N6-methyladenosine (m^6A) and 5-methylcytosine (m^5C) as well as evolving technologies to identify and quantify other less well-characterized RNA modifications in mRNA and regulatory RNA. We will also discuss the role of adenosine to inosine-edited RNAs in brain function as well as the properties of a new topological class of RNA (circular RNA). The known and postulated functional roles of these modifications in neuronal processes and diseases including neural fate specification, dopamine neuron function, neurological disorders, intellectual disability, and substance use disorders, will be described.

m^6A

m^6A is an RNA modification that was recently discovered to be a widespread feature of mammalian mRNAs (Dominissini et al.,

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2012; Meyer et al., 2012). Although m^6A has been found in mRNAs from diverse tissue types, the brain is among the tissues with the highest levels of m^6A. This finding, coupled with the fact that m^6A is known to be dynamically regulated (Dominissini et al., 2012; Schwartz et al., 2013), suggests that adenosine methylation could potentially mediate the intracellular response to neuronal signaling events by regulating the function of neuronal mRNAs. Additionally, recent studies have identified two members of the 2-oxoglutarate-dependent dioxigenase family of proteins as m^6A demethylases (Jia et al., 2011; Zheng et al., 2013). One of these, FTO, has been linked to a variety of human diseases, including cancer (Garcia-Closas et al., 2013; Iles et al., 2013), obesity (Tung and Yeo, 2011), attention-deficit/hyperactivity disorder (Choudhry et al., 2013), and Alzheimer's disease (Keller et al., 2011; Reitz et al., 2012). Additionally, humans with a nonsynonymous mutation in the FTO enzymatic domain exhibit brain malformation and impaired brain function (Reitz et al., 2012), and intronic FTO single nucleotide polymorphisms have been associated with abnormal brain volumes in both adolescents (Melka et al., 2013) and healthy elderly subjects (Ho et al., 2010). These findings suggest that FTO-mediated m^6A demethylation might contribute to neuronal signaling pathways that regulate brain development and function.

FTO is highly expressed within the brain, and its expression level and subcellular localization within distinct brain regions are susceptible to dynamic regulation (Boender et al., 2012; Vujovic et al., 2013). Thus, targeted m^6A demethylation directed by FTO is a potential mRNA regulatory mechanism through which neurons might regulate their response to various signaling events. Indeed, recent studies have shown that FTO knock-out mice have an abnormal behavioral and electrophysiological response to cocaine (Hess et al., 2013). Targeted deletion of FTO in dopaminergic neurons revealed impaired presynaptic dopamine receptor signaling, suggesting that FTO is necessary for the proper presynaptic response to extracellular dopamine levels (Hess et al., 2013). Additionally, analysis of mRNA methylation in dopaminergic neurons following FTO loss of function identified a subset of mRNAs whose m^6A levels were influenced by FTO. Many of these transcripts encode proteins involved in the response to dopamine, suggesting that FTO-mediated dynamic methylation of neuronal mRNAs is necessary for proper dopaminergic signaling. Given the multitude of neuronal pathways that involve dopaminergic transmission, it is likely that fine-tuning of neuronal m^6A levels regulates a variety of pathways contributing to mental health and disease. Further research into the mechanisms through which m^6A regulates mRNAs in response to neuronal signaling events will likely reveal additional roles for this widespread modification in neuronal function.

m^5C

Although the existence of m^5C in DNA and RNA was described over 5 decades ago, its precise regulatory functions in RNA remain unclear (Gold et al., 1963; Garcia-Closas et al., 2013). Recent advances in high-throughput techniques to globally map m^5C in RNA and the association of mutations in genes encoding m^5C methyltransferases with intellectual disability in humans have provided important insights into the function of this modification. Bisulfite sequencing was the first methodology adapted to globally detect m^5C in RNA (Schaefer et al., 2009; Squires et al., 2012). The development of three more transcriptome-wide approaches followed (Hussain et al., 2013a). Using these high-throughput methods, m^5C has been identified in coding as well as noncoding RNAs, such as vault RNAs (vRNAs) and tRNAs (Motorin et al., 2010; Squires et al., 2012; Amort et al., 2013; Edelheit et al., 2013; Hussain et al., 2013a, b; Khoddami and Cairns, 2013). Functionally, m^5C has been shown not only to affect degradation and stress-induced ribonuclease cleavage of tRNAs but also to change global protein translation (Alexandrov et al., 2006; Chow et al., 2007; Chernyakov et al., 2008; Schaefer et al., 2010; Chan et al., 2012; Tuorto et al., 2012; Metodiev et al., 2014). In addition, hypomethylation of vRNAs alters their processing into microRNA-like RNAs (Hussain et al., 2013b). In rRNA, m^5C is also thought to play a role in translation (Chow et al., 2007; Metodiev et al., 2014). m^5C modification has been proposed to affect mRNA stability; however, its function is still controversial (Zhang et al., 2012; Hussain et al., 2013a).

The two best described m^5C RNA methyltransferases in higher eukaryotes are DNMT2 and NSUN2 (Brzezicha et al., 2006; Frye and Watt, 2006; Goll et al., 2006). Although no gross phenotype has been observed in DNMT2-deficient mice or plants (Goll et al., 2006; Tuorto et al., 2012), DNMT2 loss-of-function mutant flies show increased sensitivity to oxidative stress, and DNMT2 loss in zebrafish affects liver, retina, and brain development (Rai et al., 2007; Schaefer et al., 2010). Studies performed in NSUN2-deficient mice, flies, and cell lines, suggest roles for m^5C RNA modification in cellular signaling, stem cell biology, tissue development, differentiation, and cancer (Frye and Watt, 2006; Sakita-Suto et al., 2007; Hussain et al., 2009; Frye et al., 2010; Blanco et al., 2011; Abbasi-Moheb et al., 2012; Tuorto et al., 2012; Hussain et al., 2013c). NSUN2 is highly expressed during mouse embryogenesis and is specifically enriched in the brain (Blanco et al., 2011). Most remarkably, human whole exome sequencing studies recently have correlated NSUN2 gene mutations with a syndromic form of autosomal-recessive intellectual disability, as well as a Dubowitz-like syndrome, and a Noonan-like syndrome (Abbasi-Moheb et al., 2012; Khan et al., 2012; Martinez et al., 2012; Fahiminiya et al., 2014). Dubowitz-like syndrome includes an intellectual disability phenotype as well as microcephaly, and facial dysmorphism, whereas individuals affected by Noonan-like syndrome present developmental delay as well as facial dysmorphism. The described substitutions result in truncation and degradation of NSUN2 transcript leading to complete loss or mislocalization of NSUN2 protein into the cytosol. Similar to patients, NSUN2 knock-out mice are smaller than their littermates and have microcephaly and behavioral and memory deficits (Blanco et al., 2014).

Together, these data suggest that NSUN2-mediated RNA methylation plays an essential role in brain development. But how loss of this methylation causes the disease symptoms described above is not yet fully understood. Loss of tRNA methylation could be the main defect leading to these complex intellectual disorders because the vast majority of NSUN2 targets are tRNAs (Squires et al., 2012; Hussain et al., 2013b; Khoddami and Cairns, 2013). It is known that loss of DNMT2-mediated m^5C methylation increases tRNA stress-induced cleavage in flies, and cleavage of tRNAs and repression of protein translation is a conserved response to several stress stimuli in eukaryotes (Thompson et al., 2008; Fu et al., 2009; Yamasaki et al., 2009; Emara et al., 2010; Schaefer et al., 2010; Spriggs et al., 2010; Ivanov et al., 2011; Gebetsberger et al., 2012; Sobala and Hutvagner, 2013). Furthermore, neurodevelopmental disorders are commonly associated with oxidative stress (De Felice et al., 2012; Lintas et al., 2012) and increased tRNA cleavage has been recently directly linked to neurodevelopmental and neurodegenerative conditions (Karaca et al., 2014; Schaffer et al., 2014). Additionally, in recent studies performed by Sandra Blanco and colleagues

in Dr. Michaela Frye's laboratory, loss of NSUN2-mediated tRNA methylation-induced angiogenin-mediated tRNA cleavage and led to accumulation of 5′ tRNA fragments. These tRNA fragments activate stress response pathways leading to reduced rates of protein translation, decreased cell size, decreased synaptogenesis, and increased cell death. These phenotypes can be rescued by inhibition of angiogenin and stress pathways during mouse embryogenesis (Blanco et al., 2014). This study shows the first association between m^5C regulation during cellular stress responses and noncanonical functions of tRNAs in neurodevelopment and in human diseases.

Identifying novel RNA modifications in the nervous system

We have just described the known functions of two of the most well-characterized mammalian RNA modifications; however, many others exist and some have recently been found in ncRNAs (Storz, 2002; Matera et al., 2007; Yu and Chen, 2010; Squires et al., 2012). Current technologies, such as immunoprecipitation followed by RNA-sequencing, have allowed us to monitor a select number of modifications, including m^5C, m^6A, and inosine (I). This advance has enabled researchers to test whether specific RNA modifications are associated with genes related to brain function and the development of neurological disorders (Meyer et al., 2012; Squires et al., 2012; Li and Church, 2013). However, many of these post-transcriptional modifications are not present on RNA at high levels and consequently little is known about the extent to which they are found in individual RNAs, classes of RNAs (e.g., ncRNAs or mRNAs), or cell types. Thus, highly sensitive and accurate technologies are needed to monitor and quantify RNA modifications that occur in low abundance RNA species, such as certain mRNAs, snoRNAs, miRNAs, siRNAs, and lncRNAs. Mass spectrometry (MS) in combination with high resolution separations, such as ultra-high performance liquid chromatography, can provide these sensitive, accurate, and robust measurements.

ncRNAs are highly expressed in the brain and play an essential role in neural functions, brain development, and evolution (Satterlee et al., 2007; Im and Kenny, 2012; Qureshi and Mehler, 2012; Ng et al., 2013; Petri et al., 2014; Roberts et al., 2014). Recently, robust methods have been developed to produce medial frontal cortex cells from human pluripotent stem cells in a highly efficient and reproducible manner (van de Leemput et al., 2014). This system enables the monitoring of modifications of ncRNAs and mRNAs during the different stages of brain development that may be important for differentiation. The chemical composition and physical properties of the modified nucleosides allow for individual characterization using chromatography in combination with collision-induced fragmentation and tandem MS (Quinn et al., 2013; Su et al., 2014). Ultra-high performance liquid chromatography-MS analysis of total RNA extracted from medial frontal cortex cells reveals low femtomole to attomole levels of 33 of 112 currently known RNA modifications (unpublished data). Increases in the levels of certain modifications, such as m^5C, m^7G, m^1A, and m^6A, are highly indicative of the level of transcription during cortical differentiation. Less common modifications, not as directly related to transcription (e.g., 2-thiocytidine (s^2C) and I), were also found to increase during cortical differentiation, whereas pseudouridine (Ψ) and 2′-O-methylcytidine (Cm) remained fairly constant. Ongoing efforts are focused on the separation of individual RNAs types, such ncRNAs and mRNAs, to investigate the level of modifications more accurately. Complementary RNA-seq data will be used to determine the existence of the corresponding modifying enzymes and to work towards understanding the biological pathways involved. Ultimately, these new methods will yield insights into which RNA modifications are present in brain ncRNAs and mRNAs and how they change during cortical differentiation. These studies will be the first step toward better understanding the functions of messenger and regulatory RNAs and their modifications in human brain disorders.

A-to-I RNA editing in the nervous system

Adenosine-to-inosine (A-to-I) editing is a cotranscriptional phenomenon that occurs at the pre-mRNA level. It is catalyzed by adenosine deaminases acting on RNA (ADARs), which bind double-stranded RNA and deaminate adenosine to form inosine, which is recognized as guanosine during translation (Nishikura, 2010; Rosenthal and Seeburg, 2012; Li and Church, 2013). Thus, RNA editing can contribute to the diversity of the transcriptome by changing the amino acid sequences of proteins, altering the locations of start or stop codons, influencing alternative splicing patterns, and affecting the ability of miRNAs to bind to their target sites (Rueter et al., 1999; Kawahara et al., 2007; Nishikura, 2010). Dysregulation of A-to-I RNA editing can lead to severe consequences. For example, ablation of editing in the glutamate receptor gene GluA2 Q/R site results in excess influx of calcium into neurons leading to postnatal death in mice (for further details, see below) (Brusa et al., 1995). Importantly, abnormal editing levels have been observed in a variety of diseases, such as depression and suicide, epilepsy, amyotrophic lateral sclerosis, and several cancers (Tariq and Jantsch, 2012; Slotkin and Nishikura, 2013).

Although previous work has shown that ADAR expression levels are generally higher in brain than other tissues, we lack comprehensive studies examining how RNA editing is spatiotemporally regulated in mammals. With the recent expansion of RNA editing sites in mouse and human, there is an immediate need to comprehensively characterize the extent of editing at individual sites in different biological contexts (Geiger et al., 1995; Melcher et al., 1996; Chen et al., 2000; Li et al., 2009; Bahn et al., 2012; Danecek et al., 2012; Peng et al., 2012; Ramaswami et al., 2012, 2013; Ramaswami and Li, 2014).

Transcriptome-wide profiling of A-to-I RNA editing in a large number of human and mouse samples has been performed using a recently developed targeted RNA sequencing method (Zhang et al., 2014) as well as publicly available data. Differences in RNA editing levels between tissue types, developmental stages, and species were observed, leading to findings that agree with previous, small-scale studies (Wahlstedt et al., 2009), as well as findings that are novel and unexpected. At an unprecedented scale, this study underscores the unexpected complexities of A-to-I RNA editing and paves the way for future studies aimed at understanding this important gene regulatory mechanism.

A-to-I RNA editing: glutamate receptors and addiction

Glutamate receptors are among the most well-studied edited mRNAs. Glutamate is the major excitatory neurotransmitter in the nervous system, and it mediates its fast synaptic action through the activation of three types of ionotropic glutamate receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors (Choi, 1995; Dingledine et al., 1999). AMPA receptors (AMPARs) are tetrameric protein complexes comprised of GluA1-GluA4 subunits. Although all AMPARs are permeable to sodium and potassium (Song and Huganir, 2002), some AMPARs are also calcium permeable (CP). The synaptic incorporation of CP-AMPAR is highly regulated

Table 1. Selected RNA modifications and their potential nervous system functions

Modified RNA	Enzyme(s)	Potential nervous system function
m^6A	Methyltransferase:	RNA splicing (Dominissini et al., 2012)
	METTL3/METTL14/WTAP (Liu et al., 2014; Ping et al., 2014)	RNA stability (Wang et al., 2014b)
	Demethylase:	Nuclear export (Dominissini et al., 2012)
	FTO (Jia et al., 2011)	Activity of dopaminergic neurons (Hess et al., 2013)
	ALKBH5 (Zheng et al., 2013)	Hypothalamic response to nutrient status (Boender et al., 2012; Vujovic et al., 2013)
m^5C	Methyltransferase:	tRNA stability (Tuorto et al., 2012)
	DNMT2 (Goll et al., 2006)	tRNA and ncRNA processing and cleavage (Schaefer et al., 2010; Hussain et al., 2013b; Blanco et al, 2014)
	NSUN2 (Brzezicha et al., 2006; Frye and Watt, 2006)	Gene silencing (Hussain et al., 2013b)
	NSUN4 (Metodiev et al.,. 2014)	Protein translation (Tuorto et al., 2012; Blanco et al., 2014)
	Demethylase:	Stress response (Schaefer et al., 2010; Blanco et al., 2014)
	unknown	Differentiation and development (Blanco et al., 2011; Tuorto et al., 2012; Hussain et al., 2013c)
Inosine	ADARs (Nishikura, 2010)	RNA editing (Slotkin and Nishikura, 2013)
		Generation of nongenomically encoded proteins (Rueter et al., 1995; Wahlstedt et al., 2009)
circRNAs	Unknown	MicroRNA sponge (Hansen et al., 2013; Memczak et al., 2013)
		Modulation of gene expression (Zhang et al., 2013)

and important for the enhanced synaptic strength associated with neuronal plasticity (Isaac et al., 2007; Liu and Zukin, 2007). CP-AMPARs are altered by different pharmacological agents, demonstrate greater single-channel conductance, and demonstrate inward rectification due to voltage-dependent blockade by endogenous polyamines. Calcium-impermeable AMPARs are comprised of GluA2 subunits that have undergone RNA editing, which involves the enzymatic deamination of an adenosine residue in GluA2 pre-mRNA before splicing by the enzyme ADAR2 (Rueter et al., 1995; Melcher et al., 1996; Bass, 2002). ADAR2 specifically targets the glutamine (Q) codon, deaminating an adenosine residue to an inosine that is read as guanosine (C***A***G → C***G***G), by reverse transcriptase. Thus, ADAR2 converts the glutamine (Q) to arginine (R) at amino acid 607 changing a critical residue within the ion channel and thus generating AMPA receptors comprised of calcium-impermeable AMPARs with GluA2(R) subunits (Sommer et al., 1991; Geiger et al., 1995; Wright and Vissel, 2012). Although there is strong evidence that AMPARs lacking GluA2 contribute to normal brain function as well as disease, the functional significance of unedited GluA2(Q) containing AMPA receptors in the brain is unclear (Isaac et al., 2007; Wiltgen et al., 2010; Wright and Vissel, 2012). Recent studies demonstrate that unedited GluA2(Q) can play a role in both neurologic as well as psychiatric disorders (Morabito and Emeson, 2009). Unedited GluA2(Q) has been shown to regulate excitotoxic neuronal cell death in ischemia and neurodegenerative disease (Akbarian et al., 1995; Kawahara et al., 2004; Kwak and Weiss, 2006; Peng et al., 2006; Aizawa et al., 2010; Hideyama et al., 2010). Additionally, ADAR2 levels and GluA2 Q/R editing are decreased in the brains of patients with major depressive disorder and schizophrenia (Akbarian et al., 1995; Silberberg et al., 2012; Kubota-Sakashita et al., 2014).

Although a role for GluA2 Q/R site editing in excitotoxicity and neuronal death is becoming clear, its role in normal and aberrant behavioral phenotypes is largely unknown. Specifically, there are no studies that have yet examined a potential role for GluA2 editing in animal models of addiction. Recently, the Sadri-Vakili group, together with Drs. Christopher Pierce and Heath Schmidt (University of Pennsylvania), has begun to elucidate the role of ADAR2-mediated GluA2 Q/R site editing in the nucleus accumbens (NAc) of rats following cocaine self-administration. It is now clear that AMPAR activation in the NAc promotes the reinstatement of cocaine-seeking behavior (Schmidt and Pierce, 2010). Although administration of an AMPAR agonist directly into the NAc reinstates cocaine seeking, intra-accumbal administration of an AMPAR antagonist decreases the reinstatement of drug seeking (Cornish and Kalivas, 2000; Di Ciano and Everitt, 2001; Backstrom and Hyytia, 2007; Conrad et al., 2008; Famous et al., 2008). Additionally, cocaine seeking is associated with increased synaptic expression of CP-AMPARs in the accumbens (Anderson et al., 2008; Conrad et al., 2008; Famous et al., 2008). Given that the majority of GluA2 subunits in the adult brain are edited GluA2(R), it has been speculated that cocaine-induced increases in NAc CP-AMPARs may reflect decreased expression of GluA2-containing AMPARs (Burnashev et al., 1992; Kawahara et al., 2003; Schmidt and Pierce, 2010; Pierce and Wolf, 2013). Alternatively, CP-AMPARs containing unedited GluA2(Q) subunits also could contribute to this process. The Sadri-Vakili laboratory is focused on addressing this issue by determining the effects of cocaine on ADAR2-mediated GluA2 Q/R site editing in a rat self-administration model.

Circular RNAs (circRNAs)

The final class of modified RNA we will discuss are the circular RNAs. circRNAs are a newly discovered class of stable, naturally occurring noncoding RNAs, with widespread expression in eukaryotic cells. Their extraordinary stability, due to their resistance to exonucleolitic RNA decay, offers the ability to efficiently sequester miRNAs or RNA-binding proteins and thereby affect their function. Although thousands of circular RNAs have been identified and many of these were shown to be the predominant transcript isoforms, little is known about their biogenesis, degradation, or function (Salzman et al., 2012, 2013; Jeck et al., 2013; Memczak et al., 2013; Wang et al., 2014a).

The Rajewsky laboratory has obtained evidence for numerous circRNAs with high expression in mammalian brain. Many of these circRNAs map to the exons of genes crucial for neuronal function. The best-characterized circRNA transcript CDR1as, antisense to cerebellar degeneration-related protein 1 (CDR1as/ciRS-7), is densely bound by miRNA effector complex and harbors 63 conserved miR-7 binding sites (Hansen et al., 2013; Memczak et al., 2013). Gain-of-function experiments demonstrate that CDR1as acts as a natural miR-7 antagonist in neuronal tissues. Expression of CDR1as in zebrafish results in severe impairment of midbrain development, similar to miR-7 depletion, which indicates the ability of CDR1as to regulate miRNA-7

levels (Memczak et al., 2013). Interestingly, miR-7 targets have been previously linked to Parkinson's disease etiology, and circCDR1as was shown to be strongly downregulated in the hippocampi of patients with Alzheimer's disease (Junn et al., 2009; Lukiw, 2013). This work suggests that maintenance of balance between CDR1as and miR-7 may be crucial for the prevention of neurodegenerative disease. The function of circRNAs is not necessarily limited to miRNA regulation. The biochemical heterogeneity and wide expression range of circRNAs suggest potential functions, such as delivery vehicles, RNA-binding protein sponges, assembly of RNA-binding protein factories, or as potential templates for translation (Hentze and Preiss, 2013; Memczak et al., 2013). Dr. Rybak-Wolf and her colleagues in the Rajewsky laboratory have investigated changes in circRNA expression during embryonic stem cell differentiation into neurons and have characterized some neuron-specific circRNAs with possible functions in the control of neuronal identity and development.

Future exploration

Research into RNA modifications is undergoing the beginnings of a renaissance thanks to improved tools and technologies for detecting these modifications. Table 1 summarizes the RNA modifications discussed in this mini-review and provides a brief description of their current known functions in the nervous system. These exciting discoveries will spur further investigations of the role of modified RNAs in a myriad of nervous system processes. Moving forward, it is evident that a few key obstacles must be overcome to achieve maximal progress in this area. These include the generation of improved affinity reagents to monitor specific modified RNAs, as well as improved assays that enable determination of specific RNA modifications at single base resolution. Additionally, a global survey of the RNA modifications in diverse neuronal and glial subtypes would be of great value in understanding the extent to which these modifications permeate the nervous system. It would also be important to identify and characterize the proteins that write, erase, or interact with these RNA modifications. If research in this field flourishes, an Epitranscriptome Catalog of RNA species and their modifications from a variety of key mammalian nervous system cell types or tissues will be of great use, as would computational approaches for predicting the presence of modifications in a given RNA. Certainly, additional mechanistic studies will be required for a more in-depth investigation of the mechanisms by which modified RNAs are generated and how these impact neurobiological and disease processes. Finally, genetic and pharmacological tools will need to be developed to enable temporal and cell-type-specific manipulation of RNA modifications and the proteins involved in their functions.

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