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Review

Mechanisms of Long Non-coding RNAs in Mammalian Nervous System Development, Plasticity, Disease, and Evolution

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Only relatively recently has it become clear that mammalian genomes encode tens of thousands of long non-coding RNAs (lncRNAs). A striking 40% of these are expressed specifically in the brain, where they show precisely regulated temporal and spatial expression patterns. This begs the question, what is the functional role of these many lncRNA transcripts in the brain? Here we canvass a growing number of mechanistic studies that have elucidated central roles for lncRNAs in the regulation of nervous system development and function. We also survey studies indicating that neurological and psychiatric disorders may ensue when these mechanisms break down. Finally, we synthesize these insights with evidence from comparative genomics to argue that lncRNAs may have played important roles in brain evolution, by virtue of their abundant sequence innovation in mammals and plausible mechanistic connections to the adaptive processes that occurred recently in the primate and human lineages.

Advances in genome sequencing technologies during the last decade have enabled an unprecedented scale of transcript discovery. One of the key results has been the finding that non-protein-coding transcripts dominate the transcriptional output of mammalian genomes (Birney et al., 2007; Carninci et al., 2005). It is now generally appreciated that at least 80% of the human genome is dynamically transcribed across the developing and adult body (Dunham et al., 2012), to produce a wide range of small non-coding RNAs (sncRNAs; <200 bp) and long non-coding RNAs (lncRNAs; >200 bp). Functional studies have elucidated a diversity of microRNA (miRNA)-mediated mechanisms that influence almost every aspect of metazoan biology (reviewed in Bushati and Cohen, 2007; Esteller, 2011). More recently, lncRNAs have begun to receive similar attention from experimental biologists, resulting in the discovery of a variety of lncRNA regulatory mechanisms with similarly pervasive influence. Here we survey these discoveries as they pertain to the development, plasticity, disease, and evolution of the mammalian brain. We emphasize the abundant opportunities for discovery represented by the wealth of as-yet-uncharacterized nervous system lncRNAs. First, we introduce key results describing the spatiotemporal expression dynamics of lncRNAs in the brain, and the evolutionary conservation of their gene loci.

LncRNAs Are Abundant and Have Precisely Regulated Expression Patterns in Mammalian Nervous Systems

The GENCODE (Harrow et al., 2012) and NONCODE (Xie et al., 2014) consortia have annotated 10,000–50,000 lncRNA genes in the human genome to date. Many are located in regions historically termed gene deserts, between protein-coding genes, while others overlap protein-coding genes in both antisense and sense orientations. They range from small single-exon loci to large multi-exonic transcripts with several alternative splice forms. Remarkably, 40% (equivalent to 4,000–20,000 lncRNA genes) of these are expressed specifically in the brain (Derrien et al., 2012). This number is strikingly large given the human genome contains approximately 20,000–25,000 protein-coding genes in total (Harrow et al., 2012) and around 2,500 miRNAs (Kozomara and Griffiths-Jones, 2014), with only a subset of these that are specific to the nervous system. It begs the question, what is the functional role (if any) of these many lncRNA transcripts in the brain?

Arguing that widespread functional roles may exist for nervous system lncRNAs, their expression is dynamically regulated during development (Aprea et al., 2013; Belgard et al., 2011; Lin et al., 2011; Mercer et al., 2010; Wu et al., 2010) and in response to neuronal activity (Barry et al., 2014; Kim et al., 2010b; Lipovich et al., 2012). It also is often highly restricted to specific brain regions in adult mice, such as the hippocampus or particular cortical domains (Mercer et al., 2008). In fact, it was recently shown that lncRNAs provide more information about cell type identity during mammalian cortical development than protein-coding genes (Molyneaux et al., 2015). These dynamics and region-specific expression patterns are coordinated by cell-type-specific or activity-dependent transcription factors and canonical changes in chromatin state at lncRNA loci (Kim et al., 2010b; Ramos et al., 2013).

While some lncRNAs, such as enhancer RNAs or antisense RNAs (see Box 1 for discussion of lncRNA classes), may have tissue-specific expression as an indirect consequence of some

Box 1. Regulatory Mechanisms of lncRNAs

Since characterization of the first lncRNAs in 1990 (H19, involved in stem cell differentiation [Brannan et al., 1990; Gabory et al., 2010]) and 1991 (Xist, essential for X chromosome inactivation [Brown et al., 1991; Pontier and Gribnau, 2011]), the field has made remarkable progress in determining the mechanisms through which lncRNAs act. Here we summarize some of the key concepts emerging from this fast-moving field to serve as a framework for understanding lncRNA-mediated control of nervous system processes.

MODULAR SCAFFOLDS TO CONTROL PROTEIN COMPLEX FORMATION AND LOCALIZATION

LncRNA transcripts often contain multiple modular functional domains, each with specific affinities for particular RNAs, DNA loci, or protein species (Engreitz et al., 2014; Guttman and Rinn, 2012; Quinn et al., 2014; Tsai et al., 2010). Both sequence-specific and structural features can specify lncRNA interaction partners (He et al., 2011; Johnsson et al., 2014; Rouskin et al., 2014; Smith et al., 2013; Wan et al., 2014). The combination of proteins assembled by a particular lncRNA often determines that transcript's function. Within the nucleus, lncRNA scaffolds influence the genome-wide binding sites and activity of complexes including: PRC1 (Bonasio et al., 2014; Yap et al., 2010); PRC2 (Rinn et al., 2007); DNMT1 (Di Ruscio et al., 2013); the mediator complex; transcription factors such as CBP, NPAS4, CREB, and SRF; and RNA polymerase II (Kaikkonen et al., 2013; Kim et al., 2010b; Lai et al., 2013; Lam et al., 2013; Li et al., 2013; Melo et al., 2013; Mousavi et al., 2013; also see Batista and Chang, 2013b for other examples). Cytoplasmic lncRNA scaffolds have been demonstrated to influence gene expression by controlling the stability (Kretz et al., 2013), degradation (Gong and Maquat, 2011), translational activation (Carrieri et al., 2012), and translational repression (Yoon et al., 2012) of mRNAs containing specific recognition motifs, by assembling appropriate protein machineries on target transcripts.

SEQUESTERING PROTEIN AND RNA (DECOYS)

LncRNAs can influence gene regulation by acting as decoys, which inactivate transcription factors or miRNAs by binding to them and diverting them from their normal sites of action (Hung et al., 2011; Johnsson et al., 2013; Sun et al., 2013; Wang et al., 2013; Willingham et al., 2005). The lncRNA *NEAT1* delays translation of specific mRNAs by sequestering them within nuclear paraspeckles and releasing them for translation upon *NEAT1* downregulation (Chen and Carmichael, 2009). Large intergenic spacer (IGS) ncRNAs are transcribed from rDNA repeats and sequester proteins with specific signal motifs within the nucleolus (Audas et al., 2012).

INTRINSIC CATALYTIC FUNCTIONS (SIGNALS)

Transcripts such as the *CCND1* lncRNAs allosterically modify specific protein targets to activate or deactivate their natural functions via intrinsic catalytic activities (Wang et al., 2008). This mechanism is distinct to scaffolding and decoy functions.

CIS- VERSUS TRANS-ACTING DISTINCTION

A *cis*-regulatory mechanism affects genes that are proximal to a given lncRNAs locus. For example, many protein-coding genes have partially overlapping antisense lncRNAs that regulate their expression (Katayama et al., 2005; Modarresi et al., 2012; Yu et al., 2008), by direct transcriptional interference (Martens et al., 2004) or by recruiting epigenetic machineries such as PRC2 (Kaneko et al., 2013) or SET2 and SET3 (van Werven et al., 2012) as nascent transcripts. In contrast, *trans*-acting lncRNAs bind to and impact the expression of target genes throughout the genome, which are often distant to the lncRNA locus (Chu et al., 2011; Johnsson et al., 2013; Vance et al., 2014).

THE LNCRNAS AND TOPOLOGICAL ORGANIZATION OF THE NUCLEUS

The boundaries between *cis*- and *trans*-acting mechanisms are being blurred by new studies that emphasize the interplay between three-dimensional chromatin organization and RNA function. For example, the lncRNA *XIST* searches for genomic binding sites by spatial proximity (Engreitz et al., 2013). The lncRNA FIRRE is expressed from the X chromosome and drives co-localization of five trans-chromosomal contacts at its site of transcription to control adipogenesis-associated gene expression patterns (Hacisuleyman et al., 2014). Such mechanisms are not easily classified as *cis*- or *trans*-acting by conventional definitions. Super-resolution imaging of lncRNA and protein localization in live cells also is beginning to provide insights into the dynamic features governing lncRNA function within the nucleus (Cerase et al., 2014), further blurring *cis*- versus *trans*-acting distinctions. Some further discussion of principles relevant to this emerging mechanistic paradigm can be found in Batista and Chang (2013a, 2013b).

(Continued on next page)

Box 1. *Continued*

RNA CONFORMATIONAL DYNAMICS

RNA molecules can generally access multiple low free-energy conformations, each of which may have different functional properties depending on the particular structural domains and free sequence motifs associated with it (reviewed in Dethoff et al., 2012). RNA molecules exist within a cell as a distribution of these energetically favorable conformations in numbers proportional to the relative sizes of each accessible local energy minima. Emerging evidence indicates that cells can directly influence this distribution by altering RNA free-energy landscapes downstream of familiar signaling mechanisms. The final effectors driving such changes can be RNA-binding proteins, chaperones, helicases, small molecules, metabolites, or various ions. This theoretically allows the functional repertoire of a given RNA molecule to be modified reliably in space and time, dramatically increasing functional complexity of RNA-mediated cellular mechanisms per RNA molecule, with significant implications for cellular information processing (reviewed in Dethoff et al., 2012). An exciting area of future research might try and connect ionic strength changes during neuronal depolarization to switch-like functions of synaptic lncRNAs.

unrelated mechanism, most large intergenic noncoding RNAs (lincRNAs, >7,000 in the human genome) have their own independently regulated promoters that undergo canonical transcription factor binding and chromatin remodeling events independent of any other known functions performed at that locus (Cabili et al., 2011; Guttman et al., 2009; Ramos et al., 2013). Obviously, regulated expression does not prove a functional role for lncRNAs, but it is consistent with it. The evidence for lncRNA functionality in the nervous system is developed below as the main subject of this review.

Evolutionary Conservation of lncRNA Loci

If functional, we might naturally expect lncRNA loci to show evidence of sequence conservation. Indeed, lncRNAs possess highly conserved promoters whose transcription factor-binding sites correlate with their tissue-specific expression patterns (Derrien et al., 2012; Guttman et al., 2009). They also have highly conserved splice-junction motifs (Nitsche et al., 2015). In contrast, lncRNA gene bodies show relatively low evolutionary conservation, similar to that observed in many *cis*-regulatory sequences and ancient retrotransposons that have been maintained in the mammalian lineages (which may or may not be functional). This observation prompted some to argue that lncRNAs do not have important biological functions, without much consideration of the circularity of conservation indices and the likelihood that regulatory sequences not only have different structure-function constraints but also are the major sites of adaptive radiation (Pheasant and Mattick, 2007). Moreover, experimental studies have now shown that the function of specific lncRNAs can be preserved despite this apparent lack of primary sequence conservation (Pang et al., 2006; Ulitsky et al., 2011). In fact, several human lncRNAs have been shown to phenotypically rescue depletion of their homologs in zebrafish (Ulitsky et al., 2011). Thus, it seems that the low conservation of lncRNA gene bodies relative to protein-coding genes reflects lower sequence constraint, rather than a lack of functional importance, with other studies showing higher conservation of RNA structure (Smith et al., 2013). Further supporting this view, several studies have used mutant mouse model systems to understand the role of lncRNAs in the brain. The results of these studies, notably the *PANTR1*, *VISC2*, and *EVF2* lncRNA loci, indicate that a large fraction is required for mammalian life and development (Sauvageau et al., 2013).

Is lncRNA function truly independent of protein-coding potential? In short, this seems to be the case for the vast majority of lncRNAs. Comparison of RNA sequencing and mass spectrometry data for at least two cell lines indicates that ~92% of annotated lncRNAs produce no detectable peptides (Bánfai et al., 2012; Derrien et al., 2012; also see Gascoigne et al., 2012; Guttman et al., 2013).

Molecular Mechanisms of lncRNAs in Nervous System Development

Nervous system development is a complex and highly stereotyped process that requires precise spatiotemporal regulation of stem/progenitor cell proliferation and differentiation. These developing populations of cells also must form appropriate connections with each other if the brain is to function properly. In this section we introduce emerging mechanistic roles for lncRNAs in controlling both these processes (summarized in Figure 1).

Stem/Progenitor Cell Proliferation and Differentiation

Developmental cell-fate choices are made by the sequential activation of cell-type-specific gene regulatory programs in proliferating embryonic stem/progenitor cells. LncRNAs control this process at various stages along the progression from pluripotent cells, which are found in the early embryo, through to the terminal cell types found in the mature mammalian brain (Figure 1).

Insights In Vitro

The exit from pluripotency and early neural differentiation has been studied extensively using in vitro model systems, such as embryonic stem cells (ESCs). In mouse ESCs, systematic loss-of-function studies have identified dozens of lncRNAs that are necessary for establishing pluripotency or driving neural lineage entry (Guttman et al., 2011; Ng et al., 2012; Sheik Mohamed et al., 2010). These lncRNAs are often directly regulated by canonical pluripotency transcription factors, such as OCT4, SOX2, and NANOG. They in turn exert their regulatory influence by directing transcription factors or chromatin remodeling machineries to specific lineage-specifying genes. For example, the lncRNA *RMST* is regulated by the transcription factor REST, which induces its expression during neural differentiation in vitro. *RMST* then drives the recruitment of the neural transcription factor, SOX2, to key neurogenesis-promoting genes, such as *DLX1*, *ASCL1*, *HEY2*, and *SPS* (Ng et al., 2013a; Figure 1). Loss of *RMST* blocks exit from the ESC state and initiation of neural differentiation; it is required for neural differentiation.

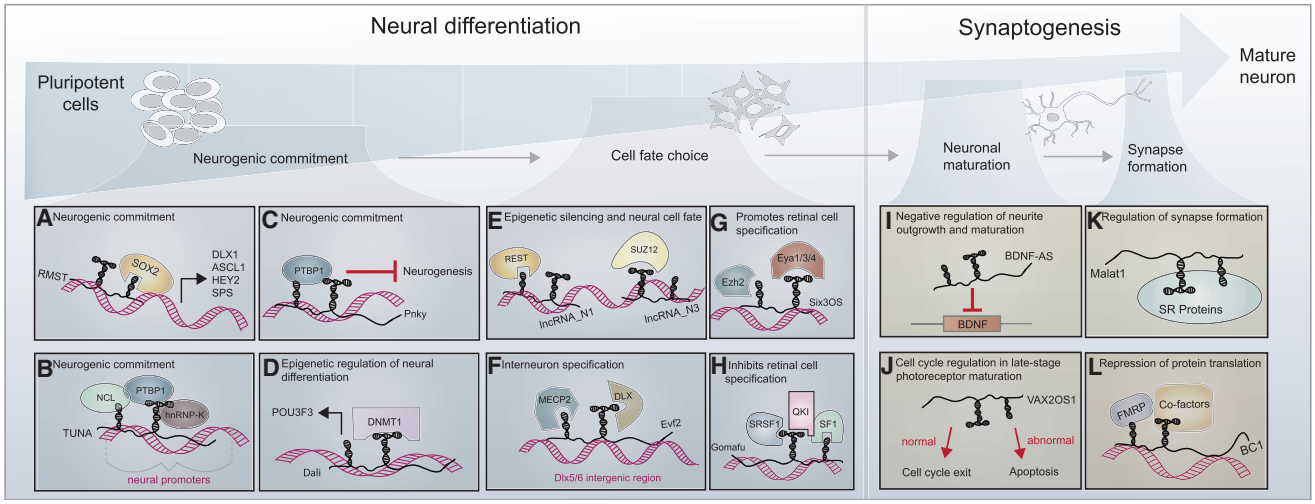

Figure 1. Summary of lncRNA Roles in Neuronal Development and Their Associated Complexes

Emerging functional studies demonstrate that lncRNAs play a major role in neuronal development from early neural differentiation (left) to late-stage synaptogenesis (right).

(A–E) LncRNAs are necessary for early neurogenic commitment, during which they recruit transcriptional machineries to specific neural gene promoters.

(F–H) In dividing neural precursors, lncRNAs also control later cell-fate choices, by similarly scaffolding epigenetic machineries, transcription factors, or splicing regulators, around specific target-gene loci. Examples shown are interneuron specification (F) and retinal cell development (G and H).

(I–L) Late developmental processes, such as neurite outgrowth and synapse maturation (I), as well as regulated cell death in neural circuit establishment (J), are regulated by natural antisense lncRNAs. Additionally, lncRNAs regulate synapse function (K) through their ability to recruit splicing factors to relevant transcripts and through the recruitment of translational repression machineries (L) to specific target mRNAs within local synaptic environments.

Another lncRNA, *TUNA*, regulates neuronal gene expression by a similar mechanism. *TUNA* forms a complex with three RNA-binding proteins, NCL, PTBP1, and hnRNP-K, that together localize to neural gene promoters in differentiating mouse ESCs (Lin et al., 2014). Knockdown of *TUNA*, or any one of the three interacting RNA-binding proteins, is sufficient to inhibit neural differentiation (Lin et al., 2014). Amazingly, experiments in zebrafish showed that this functional role was conserved across relatively distantly related vertebrates, mice and zebrafish, reinforcing the deep evolutionary requirement for lncRNA-driven neural lineage commitment.

TUNA and *RMST* exemplify how lncRNAs can control cell-fate choices by directing transcription factors and chromatin-remodeling machineries to important target loci. There are other examples. The lncRNA *DALI* drives the expression of an essential neuronal differentiation gene expression program in neuroblastoma cells. Genomic target mapping by Capture Hybridization Analysis of RNA Targets (CHART) revealed that this function is mediated through direct interactions with the transcription factor POU3F, the DNA methyltransferase DNMT1, and thousands of target loci across the genome (Chalei et al., 2014). Similarly, the lncRNA *PAUPAR* interacts with the PAX6 transcription factor and localizes to specific promoter loci, including *SOX2*, *NANOG*, and *HES1*, to regulate a transcriptional program that influences the cell-cycle profile and differentiation of neuroblastoma cells (Vance et al., 2014).

The lncRNA-driven transcription factor localization exemplified by *TUNA*, *RMST*, *DALI*, and *PAUPAR* represents an attractive mechanism for regulation of cell-fate choice, because it can allow complex gene expression programs to be controlled by a single lncRNA. It has been suggested that a large fraction of lncRNAs (i.e., hundreds to thousands of other lncRNA genes) also operate this way during differentiation. This is based largely on the observation that more than 30% of lncRNAs in mouse and human ESCs physically interact with particular chromatin-modifying complexes, and they have correlated expression with their targets (Guttman et al., 2011; Khalil et al., 2009; also see Box 1). Although attractive and plausible, the high risk of non-specific interactions between nuclear proteins and large RNA molecules necessitates careful validation to eliminate the possibility of indirect or protein-independent effects misleadingly creating the detected expression correlations. Work in this space is maturing, and will ultimately clarify the biophysical rules that specify which proteins and genomic loci are bound by a given lncRNA.

Examples In Vivo

Regulation of cell-fate choice by lncRNAs has been studied in vivo; it has been shown that lncRNAs control stem cell turnover and the specification of particular lineages in the embryonic mouse brain. *EVF2* was the first nervous system-specific lncRNA to undergo detailed mechanistic characterization in vivo (Bond et al., 2009). It was shown that genetic deletion of *EVF2* in mice disrupts the excitatory to inhibitory neuron balance in the postnatal hippocampus and dentate gyrus. This imbalance was characterized by reduced synaptic inhibition, caused by the failure of GABAergic interneuron specification (Bond et al., 2009). Mechanistically, *EVF2* recruits the transcription factor DLX and methyl-CpG-binding protein MECP2 to regulatory regions controlling the expression of interneuron lineage genes, including as *DLX5*, *DLX6*, and *GAD1*, by both *cis*- and *trans*-acting scaffolding mechanisms (Bond et al., 2009). *EVF2* has also been implicated in controlling the methylation state of the DLX5/6ei enhancer control region, which regulates expression

of the DLX5/6 locus through a chromosomal looping mechanism (Berghoff et al., 2013). How this methylation control by *EVF2* is integrated with its recruitment activities of DLX and MECP2 is unknown. Nonetheless, EVF2 is a clear example of the developmental significance of lncRNA regulatory mechanisms in vivo.

The lncRNA *PNKY* is expressed in the nucleus of dividing neural stem cells (NSCs) in the developing mouse and human brain, where it acts as a regulator of NSC turnover (Ramos et al., 2015). Depletion of *PNKY* leads to loss of the NSC phenotype in ventricular zone cells and expansion of the transit-amplifying neuronal progenitor pool in postnatal mouse brains. Thus, *PNKY* controls the balance between self-renewal and neuronal differentiation in dividing NSCs. *PNKY* appears to execute this function through regulation of an important alternative splicing pathway that involves an interaction with splicing regulator PTBP1 (Ramos et al., 2015), though precise epistatic relationships in this pathway remain to be resolved.

The intergenic lncRNA *linc-BRN1B* controls differentiation of delaminating neural progenitor cells in vivo. Deletion of the *linc-BRN1B* (*Pantr2*) locus results in significant loss of upper cortical layers (II/III–IV), as well as a reduction in barrel number and size within the somatosensory cortex and posteriomedial barrel subfield of developing mouse pups. These reductions originate with loss of basal cortical progenitors, which subsequently drives precocious migration and differentiation of lower layer neurons (Sauvageau et al., 2013). *Linc-BRN1B* appears to regulate the levels of its neighboring BRN1 protein, which may be important for its ability to regulate basal cortical progenitor turnover. Together these studies on *EVF2*, *PNKY*, and *linc-BRN1B* establish the in vivo relevance of lncRNAs in the regulation of nervous system development.

Several other lncRNAs have been associated with cell-fate choice in vivo, in the developing retina, though their precise mechanisms of action are currently less clear. In fact, some of the first lncRNAs to be implicated in the control of cell-fate choice in the nervous system were identified by early studies in the retina (Alfano et al., 2005; Blackshaw et al., 2004; Young et al., 2005). *TUG1*, a nuclear restricted lncRNA, and *VAX2OS1* have been implicated in the control of retinal cell-type specification and proliferation (Meola et al., 2012; Yang et al., 2011; Young et al., 2005). *SIX3OS* controls the specification of photoreceptors, bipolar cells, and Muller glia, possibly through regulation of SIX3 target genes via a mechanism that involves interactions with EZH2 and EYA proteins (Rapicavoli et al., 2011). *GOMAFU* is expressed in the nucleus of dividing NSCs and differentiating neurons. It regulates splicing of several neuronal genes, including *DISC1*, *ERRB4*, and *WNT7B*, likely through interactions with splicing proteins SF1, SRSF1, and QKI (Barry et al., 2014; Sone et al., 2007; Tsuiji et al., 2011). Depletion of GOMAFU in embryonic mice leads to increased amacrine cell and Muller glia differentiation (Rapicavoli et al., 2010), as well as an improperly regulated transition of actively dividing ventricular zone progenitor cells into differentiating neurons as they migrate outward into the cortical plate (Aprea et al., 2013).

Overall, lncRNAs regulate cell-fate choice and stem/progenitor cell turnover during neural development in vitro and in vivo. To do this they execute lineage-specific gene expression programs by organizing epigenetic, transcriptional, or post-transcriptional protein machineries in space and time, in response to differentiation signals (Figure 1; also see Box 1).

Neurite Elaboration and Synaptogenesis

As the nervous system develops, an intricate process of neurite elaboration unfolds, such that populations of neurons faithfully establish the connections required for normal brain function. This is an enormous regulatory task. Examples of how lncRNAs contribute are beginning to emerge and are introduced here.

The first example of a lncRNA that regulates synaptogenesis is *BC1/BC200*, which was one of the earliest studied lncRNAs in any tissue. *BC1/BC200* is expressed in the developing and adult nervous system where it is actively trafficked to dendrites (Muslimov et al., 1997). There it interacts with FMRP and translational machineries, including eIF4a and poly(A)-binding protein (PABP), to control 48S complex formation and repress local translation in synapses (Wang et al., 2002; Zalfa et al., 2003). Through this mechanism, *BC1/BC200* regulates spatially restricted synaptic turnover in vivo (Lewejohann et al., 2004; Skryabin et al., 2003; Zhong et al., 2009). Some of the physiological consequences of this regulation are known; we refer the interested reader to the neural plasticity section below for further discussion.

More recently, it was found that antisense lncRNAs regulate several important proteins that control neurite elaboration, including BDNF, GDNF, and EPHB2 (Modarresi et al., 2012). Inhibition of the lncRNA *BDNF-AS*, which is transcribed antisense to the BDNF growth factor gene, resulted in a 2- to 7-fold increase in BDNF protein levels, which was associated with reduced EZH2 recruitment and altered chromatin state at the *BDNF* locus. The resulting BDNF overexpression then drove elevated neuronal outgrowth, differentiation, survival, and proliferation, both in vitro and in vivo (Modarresi et al., 2012). Thus, antisense lncRNAs control developmental neurite elaboration through regulating local gene expression. Another exciting feature of this work by Modarresi et al. (2012) was their demonstration that antisense lncRNA expression could be easily modulated in vivo by antisense oligonucleotides, to specifically induce expression of their overlapping protein-coding gene, opening the possibility of lncRNA-targeting therapeutics that activate gene expression. Antisense lncRNA transcripts are remarkably pervasive in mammalian genomes (Faghihi and Wahlestedt, 2009; Katayama et al., 2005). Examples such as *BDNF-AS* may thus prelude a form of lncRNA regulation that is of widespread developmental and therapeutic importance.

MALAT1 is another famous lncRNA implicated in regulation of neurite elaboration. It is an interesting case study due to conflicting in vitro and in vivo results. *MALAT1* is abundantly expressed in neurons and is enriched in nuclear speckles in a transcription-dependent manner. In vitro, in cultured hippocampal neurons, *MALAT1* has been shown to actively recruit SR-family splicing proteins to transcription sites to control the expression of synaptogenesis-related genes (Bernard et al., 2010). Moreover, knockdown of *MALAT1* in this system results in a decrease in synaptic density, while overexpression reciprocally increases synaptic density (Bernard et al., 2010). Thus, *MALAT1* appeared to regulate synaptogenesis by modulating synapse formation/

maintenance genes via a splicing-protein-enriched nuclear domain. However, it subsequently was reported that *MALAT1* knockout mice surprisingly showed no overt phenotype, neither in behavior nor in neuropil density (Zhang et al., 2012a).

There has therefore been significant concern about the correspondence of lncRNA behaviors in vitro to their developmental role in vivo. It is possible that closer examination of the neurological and behavioral phenotypes of *MALAT1* knockout mice could reveal more subtle phenotypic defects, or that special redundancies in vivo could potentially compensate for the loss of *MALAT1* function. Nonetheless, such results underscore the importance of completing lncRNA functional analyses with thorough in vivo characterization, although negative results are inconclusive. Note that most of the lncRNAs discussed in this review have indeed been characterized in vivo and so meet this more stringent criteria.

Molecular Mechanisms of lncRNAs in Neuronal Plasticity

Neurons are able to change their set of synaptic connections and the relative strength of each of these connections over time in response to sensory experience and other environmental cues. This so-called plasticity underlies learning, memory, and cognition, as well as the brain's ability to recover from injury or insult. Many molecular pathways that implement plasticity are known (for reviews see Costa-Mattioli et al., 2009 and West and Greenberg, 2011). However, these descriptions are still incomplete. Here we introduce studies that have begun to implicate lncRNAs in the control of neuronal plasticity (summarized in Figure 2). Already two of these lncRNAs, *KCN2AS* and *BC1/200*, have been convincingly tied to concrete behavioral phenotypes. Many others remain exciting candidates for future exploration.

Transcriptional Regulation in Response to Injury and Neuronal Activity

Changes in expression of genes such as ion-channel components or signaling proteins can dramatically alter the excitability and functional properties of a neuron. For example, potassium channels mediate potassium ion influx during neuronal action potential propagation. The shape of an action potential and the sensitivity of a potassium channel to depolarization can be tuned by modifying the stoichiometry of potassium channel protein subunits (Stühmer et al., 1989). Thus, the regulation of these components in response to environmental cues can contribute to neuronal plasticity.

KCNA2 is a core potassium channel subunit, whose expression is regulated by an overlapping antisense RNA in response to peripheral nerve injury and in neuropathic pain (Zhao et al., 2013). In a healthy rat model system, *KCNA2-AS* is expressed only lowly in a subset of ~20% of dorsal root ganglion (DRG) neurons, while KCNA2 is expressed highly in most DRG neurons. However, in response to peripheral nerve injury, *KCNA2-AS* is strongly induced by the MZF1 zinc-finger transcription factor across the DRG neuronal population independently of KCNA2. Elevated *KCNA2-AS* then selectively downregulates KCNA2 mRNA and protein both in vitro and in vivo. The precise mechanism through which *KCNA2-AS* downregulates KCNA2 mRNA and protein awaits future elucidation, though it may involve direct binding between sense and antisense transcripts given their significant sequence overlap, or alternatively, competition for some DNA- or RNA-binding factor that ordinarily drives KCNA2 expression. In any case, this regulation alters the functional properties of DRG neurons in living rats. Specifically, overexpression of *KCNA2-AS* in the DRG was shown to reduce total voltage-gated potassium current and increase neuronal excitability, producing mechanical and pain hypersensitivities that are core neuropathic pain symptoms in the clinic. Remarkably, blocking *KCNA2-AS* induction attenuated these symptoms following peripheral nerve injury. *KCNA2-AS* thus modulates neuronal plasticity in response to peripheral nerve injury, and it represents a potential therapeutic target in the treatment of human neuropathic pain.

There is some evidence that other antisense RNAs also contribute to the regulation of neuronal plasticity through controlling signaling molecule expression, but in response to neuronal activity. BDNF is an important growth factor in the nervous system that controls the decision between synaptic maintenance and elimination in response to sustained versus sparse activity respectively. This regulation underlies the synaptic consolidation hypothesis (Bramham and Messaoudi, 2005). As introduced above, it was recently shown that BDNF expression is regulated by an overlapping antisense RNA, *BDNF-AS*. Interestingly, BDNF-AS expression is activity dependent (Lipovich et al., 2012; Modarresi et al., 2012). It may thus play a mechanistic role in coupling neuronal activity to BDNF expression and synaptic turnover in neuronal plasticity, though this awaits direct empirical support.

Generally speaking, regulated transcription in response to neuronal activity is a central process in long-term neuronal plasticity (reviewed in West and Greenberg, 2011). Such activity-dependent transcription links the transcriptional output and, thus, protein composition of a neuron to its recent firing history and is required for canonical Hebbian learning. In a screen for such activity-dependent transcripts, Kim et al. (2010b) identified thousands of enhancer-associated lncRNAs (eRNAs) that were rapidly induced by >2-fold following depolarization of mouse cortical neurons by potassium chloride in vitro. Though the majority of these transcripts currently have not been subjected to careful functional interrogation, their expression changes correlate strongly with changes in expression of nearby protein-coding genes (Kim et al., 2010b). In other biological systems, an increasing number of mechanistic studies have shown that eRNAs are essential for enhancer function. The eRNAs drive recruitment of the mediator complex, transcription factors such as CBP, NPAS4, CREB, and SRF, and RNA polymerase II, to enhancer loci, and they are required for their transcription promoting activity at target genes (Kaikkonen et al., 2013; Kim et al., 2010b; Lai et al., 2013; Lam et al., 2013; Li et al., 2013; Melo et al., 2013; Mousavi et al., 2013). There is no reason a priori to suspect that a version of this mechanism does not also occur in the nervous system. We suggest that mechanistic exploration of activity-dependent eRNAs in neuronal plasticity is an exciting topic for future exploration.

Similarly to Kim et al. (2010b), others have shown that hundreds of lncRNAs that are not eRNAs are also dynamically regulated by neuronal depolarization in vitro (Barry et al., 2014;

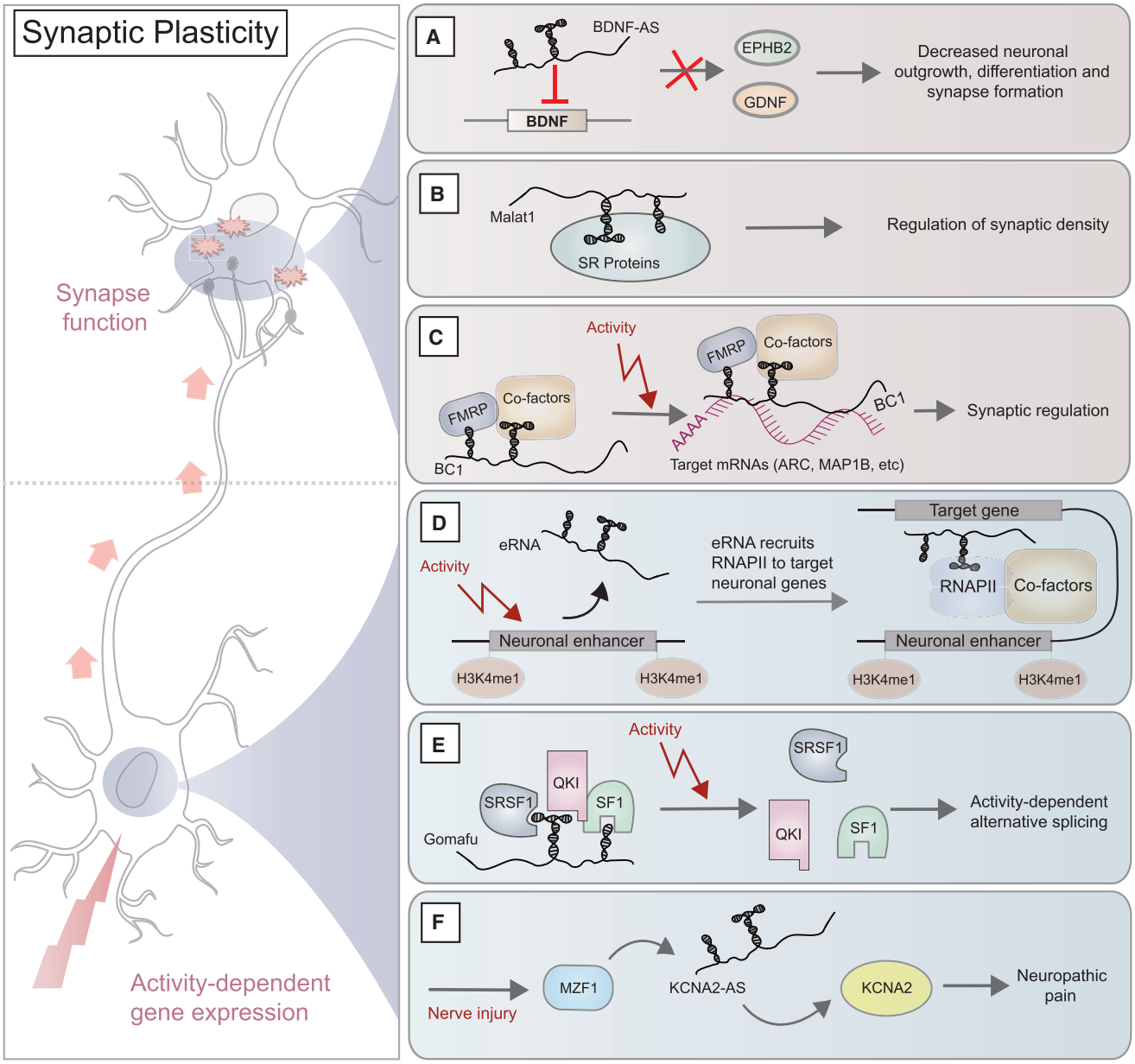

Figure 2. Molecular Mechanisms of lncRNAs in Synaptic Plasticity

LncRNAs respond to neuronal activity or injury and modulate synaptic properties (top) or gene expression (bottom) in neuronal plasticity.

(A) *BDNF-AS* is the natural antisense transcript to BDNF, itself a key contributor to synaptic function. By dynamically repressing BDNF expression in response to neuronal depolarization, *BDNF-AS* modulates synaptic function.

(B) *MALAT1* expression level controls spine maturation and synapse formation, both during development and in response to neural activity, by recruiting splicing factors into a currently poorly understood splicing domain within the nucleus.

(C) *BC1/200* expression is modulated by neuronal activity and controls the translational repression of specific target mRNAs within synapses through a mechanism involving direct recruitment of translational machineries.

(D) Enhancer RNAs are emerging as key activity-dependent regulators of synapse development through their recruitment of multiple proteins required for neuronal gene expression to transcription start sites.

(E) *GOMAFU* controls the activity-dependent release of splicing factors from a nuclear domain to regulate gene expression patterns and splice variant distributions that influence behavior in mice.

(F) In the peripheral nervous system, *KCNA2-AS* is induced in response to nerve injury. It thus dynamically downregulates expression of the KCNA2 potassium channel subunit, which alters neuronal firing properties. This mechanism is a key driver of neuropathic pain symptoms in rats.

Lipovich et al., 2012). Among these, *GOMAFU* and *MALAT1* are potentially interesting examples. Both are abundantly expressed in neurons and form ribonucleoprotein complexes within the nucleus that are enriched in splicing proteins. Though speculative, such transcripts may couple neuronal activity to specific post-transcriptional modifications in neuronal plasticity.

Translational Regulation at Synapses

Translational control of neuronal plasticity plays a key role in regulating long-term changes in neural circuits underlying learning, memory, and behavior (reviewed in Costa-Mattioli et al., 2009).

The *BC1/200* lncRNA regulates translation of specific mRNAs within local synaptic domains in response to neuronal activity. The mechanism through which *BC1/200* controls synaptic translation was introduced in the developmental neurite elaboration section above. Briefly, the *BC1/200* lncRNA is actively trafficked to neuronal dendrites where it acts as a scaffold that interacts with FMRP and translational machineries, including eIF4a and PABP, to control 48S complex formation and repress local translation in synapses. Beyond this previous discussion, here we note that *BC1/200* expression is in fact dynamically upregulated at specific synapses by local neuronal activity (Muslimov et al., 1998). Thus, BC1/200 represents a canonical plasticity gene that modifies the protein composition of synapses in response to neuronal activity. Increased neuronal activity in a particular dendritic region would cause higher local expression of BC1/200, which would then negatively feedback on local translation rates.

Genetic deletion of *BC1/BC200* in mice results in uncontrolled group I metabotropic glutamate receptor-stimulated synaptic translation, neuronal hyperexcitability, convulsive seizures, anxiety, and exploratory behavior defects (Lewejohann et al., 2004; Skryabin et al., 2003; Zhong et al., 2009). Thus, it is clear that translational control by *BC1/200* is an essential neuronal plasticity mechanism; its breakdown leads to abnormal neuronal activity and diverse behavioral defects. This example extends the known influence of lncRNAs in the nervous system outside of the nucleus and into the synaptic environment. It is to our knowledge the only example to date of a lncRNA that regulates translation in neurons.

Implicating lncRNAs in Neural Disease Pathogenesis

Since lncRNAs regulate nervous system development and function, it makes sense that their dysregulation or mutation would cause neurological disorders. Indeed, genome-wide association studies (GWASs) and comparative transcriptomic studies have associated lncRNAs with conditions including schizophrenia, Alzheimer's disease (AD), autism spectrum disorder (ASD), Asperger's syndrome, amyotrophic lateral sclerosis (ALS), bipolar disorder, depression, Parkinson's disease, neurofibromatosis, neuropathic pain, attention deficit hyperactivity disorder, epilepsy, brain cancers, and a range of cognitive performance metrics (Brunner et al., 2012; Cabili et al., 2011; Han et al., 2012; Kerin et al., 2012; Pasmant et al., 2011; Syrbe et al., 2015; Talkowski et al., 2012; Zhang et al., 2012b; Zhao et al., 2013; Zhou et al., 2012). Although the majority of this work has provided association without yet clearly establishing causation, several examples of causal lncRNA disease mechanisms are beginning to emerge. We introduce some of these below. For a broader coverage we refer the interested reader to two comprehensive recent reviews on the topic (see Ng et al., 2013b and Qureshi and Mehler, 2012).

ASD

ASD is a neurodevelopmental condition of complex etiology that is characterized by social and cognitive impairments and repetitive behaviors (De Rubeis and Buxbaum, 2015; McCarroll and Hyman, 2013; Willsey and State, 2015).

GWASs on ASD have identified strongly disease-associated variants in the chromosomal region 5p14.1 (Wang et al., 2009). The lack of protein-coding genes in this region made this association initially difficult to explain. Kerin et al. (2012) later found that these variants reside near a ~4-kb lncRNA that is transcribed antisense to moesin pseudogene 1 (*MSNP1AS*) and that shares 94% sequence identity with the ~4-kb mature MSN mRNA. This finding was immediately interesting because MSN is itself a well-known regulator of synapse development and function (Furutani et al., 2007; Kim et al., 2010a; Paglini et al., 1998; Sanchez et al., 2009). Thus, if *MSNP1AS* locus variants altered the regulation of MSN protein expression or function, it could explain how these variants might underlie the development of synaptic dysfunction in ASD.

Several pieces of evidence support this hypothesis (Kerin et al., 2012). First, all three SNP genotypes in the *MSNP1AS* locus are significantly associated with the expression level of *MSNP1AS*. The expression level of *MSNP1AS* is itself also positively correlated with expression of MSN mRNA and protein in the brain, and both *MSNP1AS* and MSN are overexpressed in ASD patient brains by >10- and ~2-fold, respectively. Second, experiments in vitro indicate that MSNP1AS can directly bind MSN mRNA through its strong sequence homology. Finally, overexpression of *MSNP1AS* directly alters the expression of MSN protein levels, though these results were sometimes conflicting between experiments conducted in different cell lines. Though yet to be carefully validated in vivo, on the basis of these results, Kerin et al. (2012) suggested that *MSNP1AS* may regulate MSN protein by binding to and stabilizing MSN mRNA, and that this mechanism may causally connect SNP variants in the *MSNP1AS* locus to ASD pathogenesis.

Schizophrenia

Schizophrenia is a psychiatric disorder associated with behavioral abnormalities, cognitive and emotional impairment, and psychosis. It commonly manifests between the ages of 10 and 40, has high heritability (estimated at ~80% in twin studies), hundreds of associated risk loci, strong environmental risk factors, and affects ~1% of the population (Rössler et al., 2005; Sullivan et al., 2003; SWGPGC, 2014).

No unique set of abnormalities is currently sufficient for the diagnosis of schizophrenia. Instead, it has been speculated that diverse molecular processes converge in various combinations in the pathophysiology of disease (Harrison and Weinberger, 2005). Though this pathogenic process is complex, several molecular signatures stand out. In particular, changes in the splice-isoform distributions of *ERRB4*, an adhesion molecule and receptor tyrosine kinase (Law et al., 2007), and *DISC1*, a functionally pleiotropic and broadly interacting intracellular molecule (Brandon and Sawa, 2011; Nakata et al., 2009), occur frequently and are thought to directly contribute to pathogenic neural development and function in schizophrenia (James et al., 2004; Veikkolainen et al., 2011). In some cases, direct mutation of *DISC1* or *ERRB4* is thought to underlie their pathological splicing, but for many other patients the root cause of splicing defects is unknown.

We recently produced evidence that the lncRNA *GOMAFU* may be involved in driving this aberrant splicing of *DISC1* and *ERRB4* in schizophrenia (Barry et al., 2014). *GOMAFU* is an interesting candidate gene because multiple independent studies previously have associated its mutation or dysregulated expression with schizophrenia risk (Albertson et al., 2006; Barry et al., 2014; Di Chiara et al., 2004; Michelhaugh et al., 2011; Spadaro et al., 2015; Takahashi et al., 2003). It also forms a ribonucleoprotein complex in the nucleus that is enriched in three splicing proteins, SRSF1, SF-1, and QKI, providing a plausible mechanism that could be dysregulated to generate schizophrenia splicing phenotypes (Barry et al., 2014). In healthy mice, *GOMAFU* controls cell-fate choices in the developing retina, and it is also highly expressed in the CA1 region of the hippocampus and in large excitatory projection neurons of the cortex (Sone et al., 2007). There it is dynamically regulated by neuronal activity (Barry et al., 2014). However, in contrast to these natural and reversible expression level changes, we found that chronic downregulation of *GOMAFU* in human pluripotent-cell-derived neurons in vitro in fact drives splicing defects in *DISC1* and *ERRB4* (Barry et al., 2014). Moreover, these defects exactly quantitatively mimic those seen in schizophrenia patient cortex samples and correspond coherently with the lower *GOMAFU* expression seen in schizophrenia patient brains (Barry et al., 2014). This situation would mirror loss-of-function mutations in the *GOMAFU* locus, suggesting a lncRNA-driven mode of splicing-defect pathogenesis in schizophrenia.

AD

AD is a heritable neurodegenerative disorder that currently affects >1% of the living global population (Brookmeyer et al., 2007; Gatz et al., 2006; also see Karch et al., 2014 for a recent review). AD generally manifests in the elderly and is associated with progressively worsening dementia and memory loss, which eventually leads to the death of patients at an average of 7 years post-diagnosis (Brookmeyer et al., 1998; Mölsä et al., 1986). A core molecular pathway driving progressive neurodegeneration in AD is thought to be the so-called amyloid cascade, in which toxic amyloid peptides accumulate and cause neuronal atrophy (see Musiek and Holtzman, 2015 for a recent review).

The lncRNA *BACE1-AS* has been implicated in a positive feedback loop that drives progression of this amyloid cascade (Faghihi et al., 2008). *BACE1-AS* is transcribed antisense to and overlapping the BACE1 gene, which encodes a trans-membrane beta-secretase protein, whose dysregulation is well known to drive overproduction of pathogenic AB-42 peptides in AD. Functionally, *BACE1-AS* positively regulates BACE1 in vitro and in vivo by binding to and stabilizing *BACE1* mRNA via a 104-nt region of perfect complementarity to exon 6 of the *BACE1* mRNA. By inducing BACE1 expression, overexpression of *BACE1-AS* drives AB-42 production in APP mutant HEK-SW cells (Faghihi et al., 2008). Interestingly, *BACE1-AS* expression is itself induced by elevated AB-42 peptide levels. Together these observations suggest a positive feedback loop, in which *BACE1-AS* drives overproduction of toxic AB-42 peptides, which then feedback to further induce *BACE1-AS* overexpression, accelerating amyloid accumulation (Faghihi et al., 2008). Consistent with this, *BACE1-AS* is expressed at ~2- to 6-fold higher levels in AD patient brains relative to controls. Furthermore, perfusion of *BACE1-AS* targeting small interfering RNAs (siRNAs) into mice brains reduced both *BACE1-AS* and BACE1 expression (Faghihi et al., 2008). Reduction of BACE1 expression has independently been shown to ameliorate disease symptoms in animal models of AD (Singer et al., 2005), making this last result potentially therapeutically relevant. It remains to be seen whether this translates into the human disease context.

The lncRNA *BC1/200* also has been associated with AD disease progression (Mus et al., 2007). Although *BC1/200* expression declines by >60% during normal aging, it was found to be significantly upregulated by up to 2.5-fold specifically in AD-affected brains, in regions including Brodmann area 9 and the hippocampus, relative to healthy age-matched controls. The magnitude of *BC1/200* overexpression also correlated strongly with the clinical dementia score in AD patient brains, and abnormal expression localization (non-somatodendritic) was observed in advanced AD brains. Although it is not yet clear whether these expression changes represent a cause or consequence of AD progression, these observations suggest another interesting candidate lncRNA for further exploration in AD research.

Neuropathic Pain

KCNA2-AS was introduced in the previous section on lncRNAs in neuronal plasticity. We refer the reader to that section for detailed mechanistic discussion of this lncRNA, but mention it again here for its role in neuropathic pain. Briefly, *KCNA2-AS* is induced in the DRG of rats in response to peripheral nerve injury. Specific induction of *KCNA2-AS* negatively regulates potassium channel subunit KCNA2 expression. Remarkably, forced overexpression of *KCNA2-AS* is sufficient to generate symptoms of neuropathic pain. Moreover, blocking *KCNA2-AS* expression with siRNAs attenuates the development of neuropathic pain following peripheral nerve injury, such as spinal nerve ligation or sciatic nerve axotomy (Zhao et al., 2013). Thus, *KCNA2-AS* appears to be a key driver of neuropathic pain symptoms and a potential therapeutic target to prevent human neuropathic pain.

Disease Mechanisms Outlook

All four disease-associated lncRNAs discussed in this section share a mechanism of action that hinges on close collaboration with specific protein partners. *MSNP1AS* regulates levels of MSN protein; *GOMAFU* regulates the activity of splicing proteins QKI, SRSF1, and SF1; *BACE1-AS* regulates beta-secretase expression; and *KCNA2-AS* regulates expression of the potassium channel KCNA2. In cases such as QKI and BACE1, mutation of these interacting proteins also is associated with the same disease state, potentially via disruption of the same pathway. However, the fact that *GOMAFU* and *MSNP1AS* map to independent GWAS risk loci suggests that they can play driving roles in disease progression. Awareness of lncRNA regulatory mechanisms may thus offer useful therapeutic targets, especially since in vivo manipulation of lncRNA expression is becoming possible (Meng et al., 2015; Modarresi et al., 2012). Proof of this concept will necessarily await clinical trials. Nonetheless, the outlook is optimistic, and continued attention to the many hundreds of other lncRNAs with either GWAS or transcriptional ties to neurological or psychiatric disorders will likely uncover additional interesting disease mechanisms.

LncRNAs in Human Brain Evolution

Comparative Genomics Finds a Lack of Evidence for Protein-Driven Human Brain Evolution

The genetic innovations responsible for phenotypic adaptation can be studied by comparing the genome sequences of related species. For example, the FOXP2 transcription factor, which is required for normal development of speech and language in humans (Lai et al., 2001), experienced a very strong selective sweep shortly after the appearance of the *Homo sapiens* (rapid positive selection for specific amino acid substitutions relative to non-human primates, followed by strong purifying selection in human populations), suggesting a role in the evolution of human speech and language (Enard et al., 2002). ASPM and MCPH1 both also show signatures of positive selection in the human lineage, are expressed in dividing neural precursors, and cause microcephaly through loss of outer cortical layers when mutated, suggesting a role in human cortical expansion (Evans et al., 2004). AHI1 is involved in regulating axon pathfinding from the cortex to spinal cord and also experienced positive selection in the human lineage, suggesting a role in regulating human-specific neural connectivity (Ferland et al., 2004).

However, in contrast to these few specific examples of innovations in proteins that are associated with human-specific brain traits, the vast majority of nervous system proteins are in fact nearly perfectly conserved across diverse mammalian phyla (Chimpanzee Sequencing and Analysis Consortium, 2005; Lindblad-Toh et al., 2011; Waterston et al., 2002). Moreover, systematic surveys of positive selection have uncovered a surprising lack of enrichment for accelerated amino acid sequence changes (that imply advantageous variation) in protein-coding genes related to nervous system function in the human lineage relative to primates and rodents (Shi et al., 2006; Wang et al., 2007).

To reconcile this constancy with the obvious and dramatic changes in human brain anatomy and function relative to other mammalian species, it is common to appeal to elaboration of the combinatorial regulatory interactions, which control the spatiotemporal expression of nervous system genes during development, as a causal explanation of human brain adaptations (Levine and Tjian, 2003; Prud'homme et al., 2007). Indeed, accumulating evidence of significant changes in brain-region-specific gene expression across mammalian species implies that regulatory changes are widespread and likely to contribute to phenotypic novelty (reviewed in Somel et al., 2013). Another common explanation of this paradoxical result is that positive selection only needed to act on a small subset of nervous system protein-coding genes, which were themselves sufficient to orchestrate human brain adaptations, while the majority of genes remained under intense negative selection due to their important and highly constrained functional roles (Hill and Walsh, 2005).

Comparative Genomics Implicates lncRNAs in Human Brain Evolution

Independent of protein-driven evolution, there is another attractive and complementary possibility: human brain adaptations were driven by changes in non-protein-coding classes of genes, such as lncRNAs. In support of this and in stark contrast to the highly conserved repertoire of protein-coding genes, recent comparative genomic analyses of mammalian lncRNAs have found that one-third of human lncRNAs appears to be specific to the primate lineage (Derrien et al., 2012), including hundreds of human-specific lncRNAs (Tay et al., 2009). Many lncRNA loci also have experienced positive sequence selection during human evolution. To date, hundreds to thousands of lncRNA loci that are positively selected relative to other mammalian species (Lindblad-Toh et al., 2011), and 48 lncRNA loci that are positively selected within specific human populations (Grossman et al., 2013), have been identified. Hundreds of these are independent of any protein-coding gene.

An interesting example of a positively selected lncRNA is *HARF1*, which is expressed most highly in Cajal-Retzius neurons during gestational weeks 7–19 of human neocortical development, a critical period of neuronal specification and migration, thus consistent with a new functional role in driving human-specific cortical development (Pollard et al., 2006). Another interesting property of HARF1 is that the positively selected regions of its locus are in fact highly conserved in other mammals, which might indicate that they indeed occur in a functional domain of the lncRNA to drive adaption. Overall the vast scale of this novel genetic information uniquely available to the developing human nervous system is difficult to ignore as a potential driver of human brain adaptations (Barry, 2014; Mattick, 2001, 2003, 2004). It motivates us to consider why we might see such a preponderance of genomic innovation in lncRNA genes relative to protein-coding genes, and how this might relate to the emergence of human-specific brain traits.

How Novel lncRNAs Could Contribute to Human-Specific Brain Traits

Increasing Cortical Size and Cellular Diversity. The human brain is approximately three times larger than the chimpanzee brain, from which we diverged 7–8 million years ago, and about twice the size of pre-human hominids that lived approximately 2.5 million years ago (Carroll, 2003). The most dramatically expanded human brain region is the cortex, which serves as the seat of our higher cognitive functions. Relative to lower mammals, the human cortex contains higher overall cell numbers, a relative abundance of cortical interneurons, and some entirely new cell types, such as spindle cells, precocious predecessor cells, and fusiform cells. The human brain also has: higher neuropil density; certain cortical regions that are not present in lower mammals, such as language centers; and a higher degree of asymmetry between hemispheres (reviewed in Carroll, 2003; Kaas, 2013).

Thousands of new lncRNAs have appeared during primate nervous system evolution, where they are expressed in a highly region-specific manner, consistent with a role in the spatiotemporal regulation of cellular identity. In fact, lncRNAs serve as better markers for subpopulations of upper cortical neurons than protein-coding genes (Molyneaux et al., 2015). They can, moreover, directly regulate cell-fate choice in the developing brain through mechanisms analogous to those seen for *EVF2*, *SIX3OS*, and *DLX1AS* (see above), and they can drive cellular identity changes when expressed ectopically (Gupta et al., 2010; Loewer et al., 2010; Rinn et al., 2007). LncRNA genes are, therefore, in principle, ideal genetic substrates to have driven brain expansion and cellular diversification during human evolution. To gain direct evidence of this evolutionary function,

researchers could in the future explore the effects of adding human-specific lncRNAs to ancestrally related mammalian brains or look for human brain diseases associated with mutations in human-specific lncRNAs.

Enhancing Learning through New Regulation of Neural Plasticity. The extent of synaptic interconnectivity, the organization of particular inter- and intra-regional circuit architectures, and the synaptic learning rules governing how specific circuits are formed, lost, strengthened, or weakened are all known to influence the computational properties of the brain (Gardner, 1993). Human cognitive prowess may therefore be related to enhancement of these processes, though this idea awaits empirical support. LncRNAs directly modulate the duration and extent of synaptogenesis during development and mature neural function, opening opportunities for new lncRNAs to influence human-specific circuit architectures. They are also integrated into activity-dependent regulatory circuits, where they dynamically control transcriptional, post-transcriptional, and translational changes, and, therefore, could refine human-specific regulation of neural plasticity. Primate-specific lncRNAs such as *BDNF-AS* (Lipovich et al., 2012), which is activity dependent and regulates dendritic arborization (see above), could be interesting subjects for future evolutionary studies.

Scaffolding Molecular Interactions to Improve Inter-neuronal Communication. Control of signal-to-noise ratios is a major challenge associated with the expansion of highly interconnected neural networks that, if left unchecked, can offset any advantage in the power of growing a larger brain (Koch, 2004; Laughlin and Sejnowski, 2003). Certain molecular mechanisms have evolved that help combat these issues, such as the proteins HOMER and SHANK, which regulate interactions between signaling molecules within synapses to improve the fidelity of neuronal signal transmission (Shcheglovitov et al., 2013). The scaffold-like properties of lncRNAs make them ideal for analogous roles in modulating the biophysical properties of communicating neurons. One known example of a scaffold-like lncRNA that acts at synapses to influence inter-neuronal connectivity is the *BC1/BC200* lncRNA, which as noted above regulates stimulus-dependent translation of key plasticity-related genes and is required for normal brain function in mice (Centonze et al., 2007; Zalfa et al., 2003; Zhong et al., 2009). Other similar examples may be uncovered by future studies.

Acting as New Signaling Molecules at Synapses. Another way to enhance the computational power of a neural network, of given size, is to allow for increasing numbers of independent messages with distinct meaning. Our brains are far more powerful for having multiple classes of neurotransmitters, such as glutamate, serotonin, dopamine, etc., than had they only utilized one such class of signal (Gardner, 1993). Intercellular communication through vesicle-mediated transport of lncRNAs, small ncRNAs, and mRNAs is increasingly being identified as an important physiological and developmental regulatory mechanism (Dinger et al., 2008; Frühbeis et al., 2012; van der Vos et al., 2011). Such mechanisms are known to operate between cells of the nervous system, but the functional consequences of this mechanism remain poorly understood. However, by transmitting lncRNAs across synapses, it would in principle be possible to tune local post-synaptic properties, for example, by regulating post-synaptic translation of specific mRNAs via lncRNAs such as *BC1/200*, or to regulate the global transcriptional state of the post-synaptic neuron by lncRNAs that regulate transcription. If evolution has accessed this communication paradigm, lncRNAs may therefore have contributed to the evolution of human cognition by enriching information encoded in neural communication.

Why Might Evolution Have Extensively Employed lncRNAs as an Adaptive Genetic Substrate?

Modularity is an organizational property that is characteristic of evolvable living systems (Gerhart and Kirschner, 2007; Kirschner and Gerhart, 1998). Theoretical work has shown that modularity of functional RNA molecules arises spontaneously from environmental canalization under constant selective pressures (Ancel and Fontana, 2000). Indeed, metazoan lncRNAs are composed of modular functional domains, which each possess unique sets of DNA, RNA, or protein-binding partners, that form regulatory linkages between pre-existing genetic and biochemical pathways (Box 1). TERC is a good example of such an lncRNA, and it ranges from 200 bp to 5 kb in size depending on the set of modules spliced into the mature lncRNA from the multiexon gene locus (Lingner et al., 1997).

The modular organization of lncRNAs is highly evolvable for at least three reasons. First, it allows each domain to explore new functional properties, such as new or altered binding partners, somewhat independently of the other functional domains. This is different to the case of proteins, where the overall structure and function of the protein macromolecule often depends more heavily on a large number of the constituent residues. Second, it allows parts of the lncRNA gene body that do not already contain functional domains to freely explore sequence space and potentially evolve new domains. There may be some selective pressures on maintaining particular secondary structures, but this is dramatically lower than in protein-coding genes, as evidenced by the much lower sequence conservation in lncRNA gene bodies relative to coding sequences. Finally, it allows new combinations of modules to be generated readily by transposition or recombination events (Johnson and Guigó, 2014; Kelley and Rinn, 2012).

Here we suggest that the evolvable nature of lncRNA molecules helps to explain why lncRNA genes appeared and then rapidly expanded in modern metazoan species, and it furthermore supports a role for lncRNAs in driving the adaptive processes underlying human brain evolution.

Conclusions

The initial discovery of tens of thousands of lncRNAs that show exquisitely spatiotemporally specific expression patterns in the mammalian brain raises the obvious prospect that they are biologically meaningful and begs the question as to what their functional roles (if any) may be. Here we have reviewed emerging studies that have begun to address this question and uncovered essential roles for lncRNAs in the development, plasticity, and disease of mammalian nervous systems. The basic and biomedical implications of these findings, especially with many thousands of other lncRNA transcripts that remain poorly understood, are abundantly clear. We have further synthesized these results within a comparative genomics framework to argue that

lncRNAs may furthermore represent a previously poorly appreciated driver of human brain adaptations responsible for the evolution of human cognition (Koziol and Rinn, 2010; Mattick et al., 2009; Mercer and Mattick, 2013). We hope that other researchers share our enthusiasm moving forward, and consider the potential underlying roles of lncRNAs in their neural system of study. Looking further forward, we anticipate that expanding field of lncRNA research in the nervous system will merge with the emerging field of epitranscriptomics (Saletore et al., 2012), as the molecular basis of the plasticity of the nervous system appears to be intimately linked to the expansion of RNA editing and RNA modification, which have expanded during cognitive evolution, as well as DNA remodeling (by reverse transcriptase-linked DNA repair) and retrotransposon mobilization, which both occur in the human brain (Baillie et al., 2011; Cantara et al., 2011; Mattick, 2010; Mattick and Mehler, 2008; Saletore et al., 2012).

AUTHOR CONTRIBUTIONS

All authors were involved in conception, supervision, and editing of the manuscript. J.A.B. wrote the manuscript. G.B. prepared the figures, and assisted with writing.

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