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EXPERT REVIEW

Integrating the roles of long and small non-coding RNA in brain function and disease

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Regulatory RNA is emerging as the major architect of cognitive evolution and innovation in the mammalian brain. While the protein machinery has remained largely constant throughout animal evolution, the non protein-coding transcriptome has expanded considerably to provide essential and widespread cellular regulation, partly through directing generic protein function. Both long (long non-coding RNA) and small non-coding RNAs (for example, microRNA) have been demonstrated to be essential for brain development and higher cognitive abilities, and to be involved in psychiatric disease. Long non-coding RNAs, highly expressed in the brain and expanded in mammalian genomes, provide tissue- and activity-specific epigenetic and transcriptional regulation, partly through functional control of evolutionary conserved effector small RNA activity. However, increased cognitive sophistication has likely introduced concomitant psychiatric vulnerabilities, predisposing to conditions such as autism and schizophrenia, and cooperation between regulatory and effector RNAs may underlie neural complexity and concomitant fragility in the human brain.

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INTRODUCTION

Our understanding of mechanisms underlying the complex functioning of the human brain is experiencing a transformation. Less than 2% of the transcribed human genome (currently estimated to be around 80%) consists of protein-coding transcripts while the overwhelming proportion is non-protein-coding.[1] Furthermore, while the protein-coding genome has undergone only modest alterations throughout evolution, the non-coding genome has scaled with biodiversity and organismal complexity[2] (Figure 1). Importantly, evidence is now rapidly accumulating to suggest that most cellular transcription is functional thereby exposing our current lack of knowledge regarding the protein regulatory system that governs cellular processes. We have hypothesized that the evolution of a more sophisticated regulatory system, afforded by the expansion of non-coding RNA, has allowed the emergence of cellular complexity and abilities such as higher-order cognition as is illustrated through the striking and rapid expansion of the primate brain[3] (Figure 1). For such precipitous changes to occur in a relatively abrupt evolutionary window there must exist mechanisms that permit, but tightly regulate, heritable genetic adaptations. To this end, non-coding RNA could significantly contribute by encompassing (1) evolutionarily conserved classes of genomic controllers, such as families of small RNA (sRNA) (for example, microRNA (miRNA), small interfering RNA (siRNA) and small nucleolar RNA (snoRNA)) and (2) newly emerged families, such as long non-coding RNA (lncRNA). The picture that is forming is one of evolving systems that increase genetic complexity through unique temporal and spatial regulation of existing processes. Here I examine two such systems; one present in all life forms (sRNA) and one newly evolved (lncRNA). Recent evidence suggests lncRNAs may provide an additional layer of regulation for the refinement of sRNA function, particularly in the brain.

SMALL NON-CODING RNA: IT IS A SMALL WORLD (OF GENE REGULATION) AFTER ALL

Throughout evolution of life on earth, small non-coding RNAs have been present (Figure 1). For example, snoRNAs are present in archae, bacteria and eukaryotes where they direct chemical modifications such as methylation and pseudouridylation of other RNA classes including transfer and ribosomal RNA.[4] Plants possess miRNAs and siRNAs and these sRNAs have evolved to play vital roles in diverse functional capacities such as development, defense against transgenes and viruses and long-range signaling.[5] All eukaryotes possess RNA interference systems capable of repressing gene expression post-transcriptionally. The earliest evolved of these classes, siRNAs, are transcribed from repeat-containing elements in the genome or generated from double-stranded RNA, and repress gene expression through exact complementary binding of target sequences.[6] miRNAs emerged during early metazoan evolution[7] and function to repress gene expression via binding to sequences with imperfect complementarity[8] (that is likely to increase target range) and interfere with protein expression or induce transcript degradation. Strong evolutionary pressure has resulted in an overall expansion in miRNA numbers, especially in mammals, with a high rate of turnover[9] suggesting a progressively complex and adaptable set of genomic regulators. A less well understood family of sRNAs is the piwi-interacting RNAs (piRNAs) that were initially reported to only function in the germ-line as a defense against retrotransposon activity.[10,11] However, this view has been recently overturned with piRNAs detected in functional

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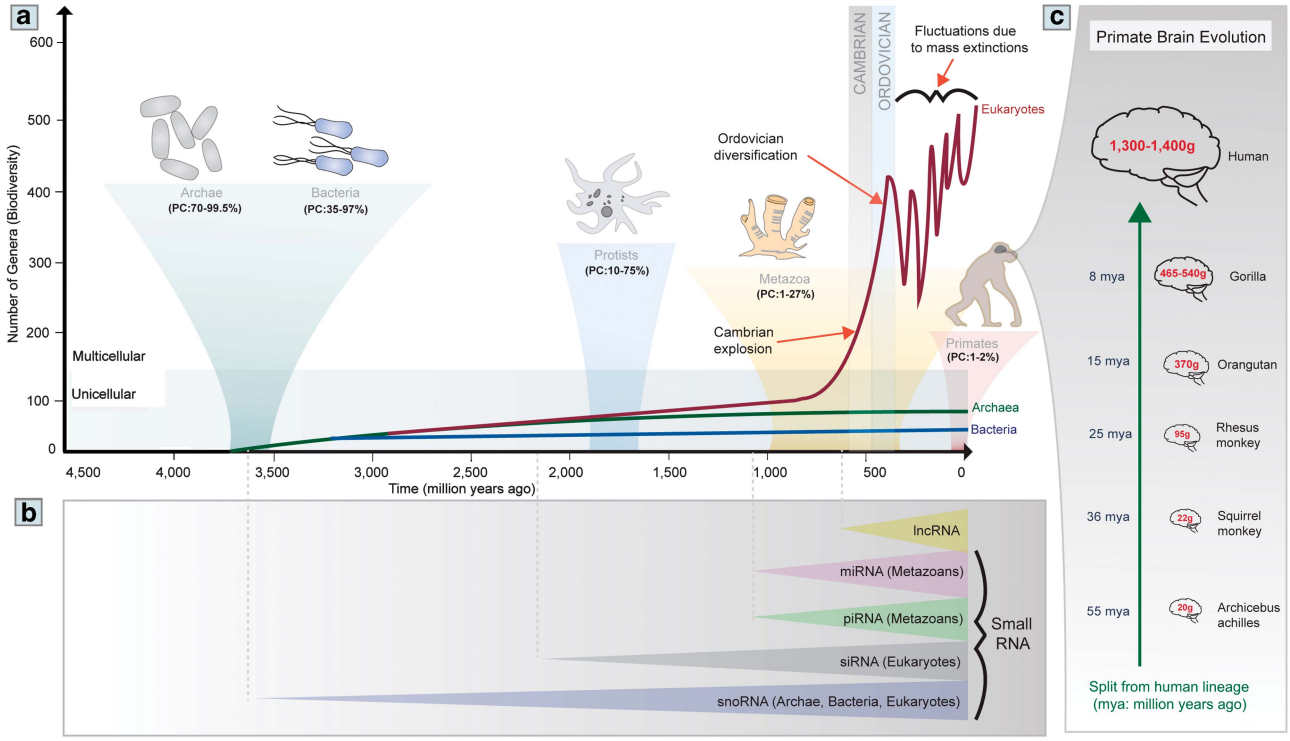

Figure 1. Evolution of organismal complexity scales with the increase in non-protein-coding size of the genome. (**a**) Life originated on earth between 3.5 and 4 billion years ago with the appearance of archae and bacteria. Their respective genomes consisted of a high percentage of protein-coding (PC) transcripts (archae 70–99.5%; bacteria 35–97%).[2] The emergence of protists between 1.5 and 2 billion years ago saw a significant reduction in PC percentage of the genome (10–75%) whereas metazoans first emerging around 500 million years later showed an even more striking decrease (1–27%). Primates possess only around 1–2% PC capacity in their genome. (**b**) The increase in non-protein-coding regions of the genome has seen an expansion in classes on non-coding RNA, including both small (for example, piRNA, miRNA) and long (for example, lncRNA) families. (**c**) The development of protein regulatory systems via non-coding RNA may have contributed to the considerable and rapid growth during primate brain evolution and the acquisition of higher-order cognition.[3]

capacities in the brain.[12–14] Although the ancient RNAi system initially functioned mainly, or exclusively, as protectors of the genome against endogenous and exogenous threats, recent evolution has co-opted sRNA for a wide range of additional functions, notably in the brain.

SRNAs in brain development

Globally, many sRNAs are dynamically regulated during embryonic stem cell neural differentiation[15] and nervous system development.[16] Furthermore, disruption of common sRNA biogenesis machinery significantly impacts brain development. For example, a general loss of Dicer (resulting in absence of mature miRNA and siRNA) produces widespread neuronal alterations during development including cortical deficits and diminished differentiation,[17,18] while conditional loss in specific cellular populations has demonstrated mechanistic deficits particular to neuronal subtypes.[19–21] These effects may be commonly due to a critical requirement of sRNAs early in neural stem cell maintenance and expansion.[22] There are many brain-specific sRNAs that clearly indicate roles in neural development and function.[23] Furthermore, distinct miRNAs have been demonstrated to influence neuronal differentiation such as brain-specific miR-9/9* and miR-124 that, when expressed in human fibroblasts, induce a neuronal fate through compositional changes of chromatin-modifying complexes.[24] Many additional studies supporting particular miRNAs involved in neuronal development have been documented affecting a wide variety of cellular pathways.[25] Prader–Willi syndrome offers insight into the importance of small interfering RNAs for early neuronal development with both SNORD115[26,27] and SNORD116[28] potentially important factors for clinical phenotypes, including neural abnormalities, observed in these patients.

SRNAs in neuronal function and dysfunction

There is an extensive body of work investigating the connection between miRNAs and neuronal activity that has been recently reviewed.[29] Neuronal activity reduces the expression of most neural miRNAs[30] emphasizing their role in fine-tuning neuronal plasticity. Indeed, Dicer gene inactivation abolishes mature miRNA formation in adult mouse forebrain and results in an enhancement of learning and memory[31] as reduced miRNA activity permits local translation of mRNAs encoding synaptic proteins at dendritic spines and postsynaptic densities necessary for synaptic function.[32,33] Intriguingly, a group of miRNAs, including miR-132 and miR-134, is increased in response to activity and may combine to ultimately enhance expression of proteins such as BDNF and CREB resulting in increased dendritic spine formation and maturation.[34] Following the recent discovery of piRNAs in the brain[13,35] it has now been shown that this class of sRNA may have important functions in neuronal activity.[14] As piRNAs essentially function through epigenetic rearrangements to block transcription of inhibitors of synaptic facilitation, and miRNAs repress translation of synaptic enhancer genes, these two classes of sRNAs when oppositely regulated by neuronal activation may synergistically combine to increase synaptic strength.[12] piRNA-mediated epigenetic changes are enduring, across many generations, and may underlie mechanisms of activity-dependent, adaptive 'memory storage'.[12] SnoRNAs have been demonstrated to also have potential involvement in learning and memory. For example,

expression pattern studies of the brain-specific snoRNA AF357425 and Snord115 showed dynamic regulation in mouse hippocampus following contextual fear conditioning.[36] Additionally, an increase in the expression of Snord115, that contains complementary sequences to the serotonin 2c receptor, correlated with altered neuronal activity and autistic phenotypes *in vivo*.[37]

The brain is highly susceptible to sRNA disturbances highlighting their critical role for correct neural function. In particular, brain disorders involving aberrant miRNA activity are extensive and well documented. For example, miRNAs are implicated in neurodevelopmental disorders (for example, Fragile X[34,38] and Rett syndromes[39,40]), neurodegenerative disorders (for example, Alzheimer's and Parkinson's disease[41–43]), neuropsychiatric disorders (for example, schizophrenia[44,45] autism[45] and drug addiction[34]) and brain cancers.[46,47] Disturbances in snoRNAs may also result in deleterious neural phenotypes such as autism.[37,48] Moreover, preliminary data suggest snoRNAs are involved in stem cell differentiation in the brain[15] implying that altered activity may lead to a cancerous state and, although not yet linked to brain cancers per se, snoRNAs are emerging as potential contributors to tumorigenesis.[49] Vault RNAs are components of the vault ribonucleoprotein complex[50] and these small non-coding RNAs, although their exact functions are not yet clear, have recently been linked with brain cancers,[51] intellectual disability[52] and neurodegeneration.[53] The current escalation of research focusing on investigating sRNAs (especially those in addition to miRNA), coupled with techniques such as transcriptome-wide deep sequencing, will undoubtedly result in rapid progress to determine the full impact of their involvement in brain function and disease.

lncRNA: FINE TUNING FOR FUNCTIONAL SOPHISTICATION

In contrast to the more ancient sRNAs discussed above, lncRNAs appear later in evolution (Figure 1), where they are present in invertebrates,[54] vertebrates[55] and plants,[56] and about one-third are primate-specific.[57] LncRNAs maintain features common to protein-coding genes such as promoter regions, intron–exon boundaries and alternative splicing; however in contrast, they are mainly nuclear localized, less polyadenylated and far more tissue-specific than protein-coding genes.[57] These differences are significant as they imply that the functions of lncRNAs lie in the refinement of regulatory circuits specific to particular cells and activities, especially in the brain.[57] It is well documented that lncRNAs display strikingly unique spatial and temporal expression and are highly expressed in the specific cells where they are active.[58,59] However, although some lncRNAs are highly expressed throughout multiple tissues, their function may not always be obvious when using a traditional knockout strategy if functional analyses are not carried out that would specifically uncover their precise, and sometimes subtle, role within the cell. For example, while mouse knockouts of the lncRNAs Hotair[60] and Xist[61] result in severe phenotypes, mice with a knockout of the ubiquitously and highly expressed lncRNA Malat1,[62,63] displayed no obvious phenotype. While indications of function, such as regulation of synaptogenesis,[64] alternative splicing,[65] cell cycle control[66] and cancer[67] have been reported for Malat1 it is still unknown what the precise role is of this abundant and broadly expressed lncRNA.

lncRNAs in brain function and dysfunction

Research investigating lncRNAs is still in its relative infancy; however, great strides are rapidly being made in their functional annotation. In 2011, Guttman and colleagues used a loss-of-function strategy to extensively investigate many lncRNAs in mice and their roles in embryonic stem cell biology and differentiation.[58] Their results, and others,[68–70] have reinforced the importance of lncRNAs in the regulation of cellular maintenance and cell fate, especially in the brain and particularly through governing epigenetic processes. In fact, an emerging theme for lncRNAs is control of epigenetic targeting via three-dimensional modularity through their ability to bind RNA, DNA and protein.[71–73]

Functional domains contained within lncRNAs allow fine-tuning of activity-dependent cellular regulation by way of scaffolds or molecular 'sponges'.[65,72–74] For example, Malat1 has been shown to be involved in synapse formation[64] and act as a splicing factor 'sponge' suggesting a role in alternative splicing in neural cells.[75] Recently, the first mechanistic illustration of an lncRNA involved in neuronal activity-dependent alternative splicing was demonstrated for Gomafu.[74] This study also showed Malat1 expression was unchanged in induced pluripotent stem cell-derived neurons upon stimulation, indicating that Malat1 may either play a subtly different role in human neuronal function, or an equivalent role in different subsets of neural cells. The former hypothesis may be more likely as both Malat1 and Gomafu are present in non-overlapping nuclear-retained speckle-like structures in neurons[76] suggesting discrete nuclear speckle bodies are independently regulated. In addition to epigenetic targeting and alternative splicing, lncRNAs are involved in mRNA processing, stability, translation and decay[66,72,77] and, taken together, places the modular functions of lncRNAs at a cellular crossroad with enormous regulatory potential.

As with sRNAs, disruptions to widespread lncRNA-mediated functions would have adverse consequences. This is especially important in the brain where most tissue-specific lncRNAs are expressed.[57] Indeed, it is emerging that lncRNAs are associated with a wide range of neurodevelopmental, neurodegenerative and psychiatric diseases[74,78–84] and brain cancer.[85,86] With the advent of induced pluripotent stem cell technology, coupled with next generation sequencing, it is now possible to generate tissue- and subtype-specific human neural cells from disease-affected and unaffected subjects, and comprehensively analyze the transcriptome and proteome to determine causality.[87] These techniques, focusing on distinct cell populations, will undoubtedly reveal many more long non-coding RNAs as critical regulators of normal human brain activity and associated disorders.

LONG AND sRNAs: REGULATING THE REGULATOR

The discussion to this point has revealed many overlapping functional themes between long and sRNAs in the brain with common links to neurogenesis and neuronal activity and, in dysfunction, to neurodevelopmental, psychiatric and neurodegenerative disease. Therefore, it should come as no surprise that these two classes of non-coding RNA would be intimately linked in cellular function. Furthermore, evolutionary data suggest that lncRNA, appearing after sRNAs, may have arisen as an upstream regulatory tier of sRNA-directed transcriptional control. This seems to be the case, especially for widely studied miRNAs, although feedback mechanisms may also be employed[88,89] (Figure 2). First, lncRNAs can act as precursors from which miRNAs are generated,[90] such as in the case of miR-675 that is contained within exon 1 of the lncRNA H19.[91] The role of H19, therefore, may function to regulate the release of miR-675 and its potent anti-proliferative and anti-Igf signaling properties.[92] The process of sRNA regulation by lncRNA precursors also extends to snoRNAs[93] and piRNAs,[11] and may well exist as a common checkpoint for sRNA production. Second, lncRNAs and miRNAs may bind directly to affect the transcriptional landscape. A recent report examined deep sequencing data sets produced by photoactivatable ribonucleoside-enhanced cross-linking and immunoprecipitation (PAR-CLIP) for Argonaute proteins in HEK293 cells to elucidate direct miRNA-binding sites in the human genome.[94] The authors report finding expected protein-coding gene targets but,

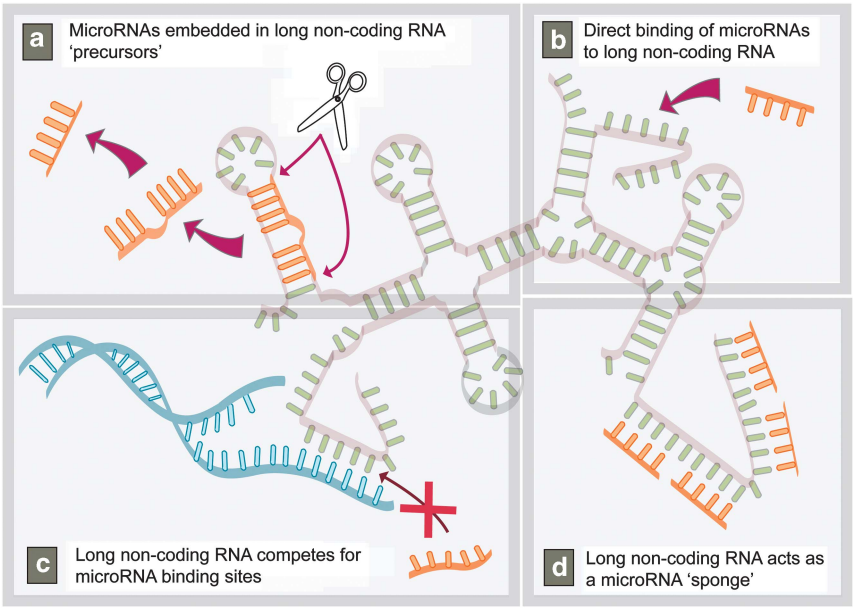

Figure 2. Regulatory interactions between long non-coding RNA (lncRNA), and microRNA (miRNA). (**a**) miRNAs may be embedded in lncRNA precursors from which they are generated;[90] (**b**) miRNAs may bind broadly to lncRNAs resulting in as yet undefined cellular effects;[94] (**c**) lncRNAs compete for miRNA binding sites[96] and (**d**) lncRNA act as miRNA 'sponges' to regulate suppressor activity.[97]

surprisingly, many lncRNAs were additionally targeted by miRNAs inferring an as yet unexplored feature of small and long RNA interaction. This is illustrated in a separate study that assembled all known miRNA–lncRNA interactions and computationally predicted that the majority of interactions are yet to be discovered.[95] Third, lncRNAs can alter miRNA binding through direct competition for binding sites. For example, the lncRNA BACE1-antisense, that is upregulated in Alzheimer's disease pathology, physically blocks the binding of miR-485-5p to BACE1 target sites resulting in a reduction of miR-485-5p-induced repression of endogenous BACE1 protein translation.[96] Finally, lncRNAs may also function as miRNA 'sponges' thereby decreasing their suppressor functions. This is illustrated by an lncRNA expressed antisense to the PTEN pseudogene, PTENpg1 that regulates PTEN expression partly through its miRNA sponge activity.[97] The lncRNA lincRNA-RoR also binds miRNAs resulting in the upregulation of principal transcription factors involved in embryonic stem cell maintenance and differentiation.[98] Collectively, regulatory interactions between long and sRNAs represent an advanced layer of genome modulation, combining the widespread effects of sRNAs with the specificity of lncRNAs.

TARGETING NON-CODING RNAs FOR THERAPEUTIC INTERVENTION

Genome-wide association studies of psychiatric disease have uncovered only a small amount of genetic links to protein-coding regions.[99] It has been observed that most disease-associated haplotype blocks from genome-wide association studies occur in non-coding regions of the genome[100–103] and combined with strong experimental evidence for lncRNAs and sRNAs underpinning neural processes (for example, lncRNAs;[3,74,104,105] sRNAs[14,25,34,106]) and brain disease (lncRNAs;[74,104] sRNAs[42,45,46]) suggest that a major proportion of disease-causing variants reside in regulatory non-coding genes.

Clearly, regulatory RNAs provide excellent opportunities for targeted therapeutic interventions in brain disease given their functional and regional specificity and an implied reduction in potential adverse side effects. The challenges are (1) uncovering the disease-relevant signaling pathways and related druggable targets, (2) an efficient method of genetic manipulation and (3) effective delivery systems. Overcoming the first challenge is underway with specific small[23,28,29,34,42,44–47,106–109] and long[74,78,83,85,104,110] RNA targets, and their related signaling pathways, being discovered that may alleviate brain disorders if effectively targeted. The second challenge requires strategies for genetic manipulation and assessment and viability of multiple methods for targeted transcript knockdown. This includes sRNA mimics and antagonist oligonucleotides (for example, AntagomiRs), locked nucleic acid antisense oligonucleotides and synthetic miRNA sponges.[111–113] An effective delivery method is the final challenge and positive progress is being made exploiting a number of different systems such as exosomes[114–116] and nanoparticles,[117,118] while increasing our understanding of the optimal formulation for non-viral siRNA delivery.[119]

FUTURE PERSPECTIVES

This article aims to elaborate on the significance of non-coding RNA in brain function and to unify the independent fields of small and long non-coding research to better understand protein regulatory systems that underpin evolved neurological processes. An appealing picture emerges where the ancient sRNA system, a widespread post-transcriptional genomic supervisor, is itself governed by a newly evolved layer of regulation imparted by the lncRNAs. Our knowledge of brain function will undoubtedly improve through research into all classes of sRNAs and uncovering the functional repertoire of lncRNAs, to complement the more comprehensively investigated miRNAs. Moreover, a deeper appreciation of the interactions between DNA, proteins and regulatory RNA within a three dimensional cellular architecture (or four dimensional when including time or activity) will allow deciphering of the exquisite cellular control of human neural cells and how dysregulation at multiple levels may result in similar adverse neurodevelopmental, psychiatric and neurodegenerative phenotypes.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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