Noncoding RNAs and RNA Editing in Brain Development, Functional Diversification, and Neurological Disease

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Mehler MF, Mattick JS. Noncoding RNAs and RNA Editing in Brain Development, Functional Diversification, and Neurological Disease. Physiol Rev 87: 799–823, 2007; doi:10.1152/physrev.00036.2006.—The progressive maturation and functional plasticity of the nervous system in health and disease involve a dynamic interplay between the transcriptome and the environment. There is a growing awareness that the previously unexplored molecular and functional interface mediating these complex gene-environmental interactions, particularly in brain, may encompass a sophisticated RNA regulatory network involving the twin processes of RNA editing and multifaceted actions of numerous subclasses of non-protein-coding RNAs. The mature nervous system encompasses a wide range of cell types and interconnections. Long-term changes in the strength of synaptic connections are thought to underlie memory retrieval, formation, stabilization, and effector functions. The evolving nervous system involves numerous developmental transitions, such as neurulation, neural tube patterning, neural stem cell expansion and maintenance, lineage elaboration, differentiation, axonal path finding, and synaptogenesis. Although the molecular bases for these processes are largely unknown, RNA-based epigenetic mechanisms appear to be essential for orchestrating these precise and versatile biological phenomena and in defining the etiology of a spectrum of neurological diseases. The concerted modulation of RNA editing and the selective expression of non-protein-coding RNAs during seminal as well as continuous state transitions may comprise the plastic molecular code needed to couple the intrinsic malleability of neural network connections to evolving environmental influences to establish diverse forms of short- and long-term memory, context-specific behavioral responses, and sophisticated cognitive capacities.
I. INTRODUCTION

The mammalian and in particular primate nervous systems are characterized by an unprecedented degree of cellular diversity, anatomical asymmetries (laterality) and specialized regional microdomains, regional interconnections, structural and functional plasticity, and exquisite environmental responsiveness. These dual attributes of modularity and adaptation are mediated by sophisticated forms of cellular specialization and polarity, unique intracellular transport mechanisms, long-term adult neuronal survival and maintenance of cellular traits, elaborate membrane electrical and chemical properties, rapid and robust homeostatic responses to environmental perturbations, elaborate afferent and efferent connections between mature nerve cells (i.e., synapses), and dynamic activity-dependent alterations in synaptic strength underlying sophisticated cognitive functions such as higher order intelligence, language, working memory, symbolic representations, social organization, and adaptive behavioral repertoires (3).

These adult nervous system functional properties are progressively elaborated in response to evolving developmental blueprints that include distinct sets of critical periods designed to ensure that the fidelity of neural network connections, propagated informational cues, synaptic plasticity, and environmental responsiveness is preserved throughout life (206, 207). Neural induction from embryonic ectoderm, establishment of laterality and axis formation within the evolving neural plate, three-dimensional patterning of the neural tube, elaboration of regional stem cell generative zones, and the development of interconnections between specialized regional neuronal and glial cell types proceed through dynamic temporally and spatially mediated interactions between inductive signals from nonneural embryonic and extraembryonic cells and tissues and by intersecting axes of soluble gradient morphogens. Thereafter, regional neural stem cell subpopulations undergo self-renewal, expansion, cellular migration, progressive lineage restriction, progenitor cell cycle progression and exit, neuronal and glial subtype specification, progressive stages of neural cell differentiation, and mature neural trait elaboration including dendritogenesis, axonal path finding, synaptogenesis, and neural network integration through the complex interplay of gene-environmental interactions within context-dependent developmental settings.

A complex array of neurodevelopmental syndromes and an interrelated spectrum of neurodegenerative diseases, neuropsychiatric disorders, and brain cancers result from aberrations of individual molecular and cellular processes embedded within these progressive developmental stages and nested maturational events. The etiology of these diverse nervous system disorders is poorly understood but likely involves mechanisms selective to the nervous system such as those involved in regional neural stem cell diversification, neuronal and glial subtype specification, long-term neuronal viability, stress responses and homeostasis, cellular polarity and intracellular transport, axodendritic process outgrowth, synaptic specialization, stabilization and plasticity, and adaptations to environmental cues and insults.

It is known that the extent of non-protein-coding sequences in eukaryotic genomes rises as a function of developmental complexity (201), although the numbers of genes and the extent of sequences encoding proteins (the analog components of the system) remains relatively static (86, 280). Humans appear to have barely more protein-coding genes (55, 100) than the nematode worm Caenorhabditis elegans (56), which has just 1,000 cells. While some of this anomaly can be explained by increased levels of alternative splicing in more complex organisms, which increases the range and diversity of protein isoforms, it is also becoming apparent that the expanded intronic and intergenic non-protein-coding sequences themselves harbor large amounts of regulatory information, much of which may be transacted by RNA (280). Although <1.5% of the mammalian genome encodes proteins, at least 5% is apparently conserved (299), and recent large-scale cDNA and genome tiling array transcriptomic analyses have shown that most of the genome is transcribed, in complex patterns of nested and overlapping transcripts with interlaced intron-exon structure, often from both strands (36, 46, 86, 136, 137, 203). A significant proportion of these transcripts are processed to form large and small regulatory RNAs (reviewed in Refs. 203, 204) that act through sequence-specific recognition of other RNAs and DNA to promote diverse developmental programs through modulation of RNA structure, splicing and stability, transcription and translation, as well as chromatin architecture (82, 199, 200, 202, 255) and other mechanisms (304).

Several distinct classes of non-protein-coding RNAs (usually referred to simply as noncoding RNAs or ncRNAs) are overly represented in the central and peripheral nervous systems (35, 47, 67, 119, 146, 154, 245, 249, 250). It is also clear that epigenetic mechanisms including RNA editing (18, 288, 300) and the modulation of chromatin architecture, which is almost certainly RNA-directed (25, 204, 255), are central to nervous system development as well as mature function (35, 53, 119), adaptive responses (4, 176), and dysfunction (26, 266, 282). In addition, genes with large introns are strongly associated with neural functions and processes and are significantly overrepresented among highly expressed genes in nervous system tissues (271, 272, 280, 290). These observations suggest that nervous system development, adult function, plasticity, and vulnerability to neurological disease states are inextricably linked to, and may be fundamentally based
or the actions and potential perturbations of complex and nuanced RNA regulatory networks.

II. RNA EDITING

RNA editing is a dynamic and versatile posttranscriptional mechanism of base recoding catalyzed by specific classes of editing enzymes that can significantly modify the functional properties and levels of expression of a spectrum of protein-coding mRNAs as well as influence a potentially broad array of ncRNAs (18, 140, 222, 288). Although various forms of RNA editing are known to occur throughout the metazoa, a dramatic increase of RNA editing has occurred during mammalian evolution, with the hominid lineage exhibiting the highest levels and the most complex forms of editing of individual transcripts. Moreover, adenosine to inosine (A-I) RNA editing catalyzed by adenosine deaminases acting on RNAs (ADARs) is particularly active in the brain (18, 288).

A. Targets of A-I RNA Editing in the Nervous System

It has been known for some time that transcripts encoding proteins involved in fast neurotransmission, including voltage-gated ion channels and neurotransmitter receptors, are subject to A-I editing (18, 288). However, recent studies show that protein-coding genes involved in neural signal propagation represent only a small subset of edited transcripts in brain, which also include those from loci encoding proteins involved in patterning of the evolving neural tube, neural stem cell self-renewal and progressive lineage restriction, neurogenesis and gliogenesis, neural subtype specification, neuroblast migration, dendritogenesis and spine morphogenesis, axonal myelination and thermoregulation, synaptogenesis, neural network integration, DNA surveillance, repair and cell cycle checkpoint control, cellular stress and senescence pathways, pro- and antiapoptotic signaling cascades, ubiquitin-proteasomal and autophagy protein turnover pathways, endosomal trafficking/vesicular transport, nuclear envelope and matrix organization and nucleocytoplasmic shuttling, epigenetic and RNA regulatory circuity, energy metabolism and cellular homeostasis. They also include transcripts from other loci involved in neurodevelopmental, neurodegenerative, neuropsychiatric disorders as well as protooncogenes and tumor suppressor genes involved in central nervous system (CNS) neoplasia (13, 27, 117, 175, 191, 215, 288). This extraordinary repertoire of targets suggests that RNA editing in the nervous system is involved in all aspects of neural development, mature steady-state functions, synaptic and neural network plasticity, as well as preservation of genomic as well as cellular integrity against a wide variety of intracellular and environmental stressors that predispose to neurodevelopmental, neurodegenerative and neuropsychiatric diseases, and nervous system neoplasia.

A-I RNA editing of targeted substrates in the nervous system can alter the functional properties of proteins, silence constitutive activity, and modulate RNA translation, localization, and stability (18). In addition to changing codons in mRNA (18), A-I editing also has the capacity to modulate splice site choice (171), small nuclear RNA precursors (191), endogenous antisense RNAs (140) and miRNA target diversity (28), miRNA processing (310), and other ncRNA and ribonucleoprotein complex targets (175), further attesting to the functional complexity of RNA regulatory networks. Recent evidence implicates ADARs and A-I editing in chromatin modification and possibly in genomic imprinting and X-chromosome inactivation, indicating the interplay between these processes and RNA-based silencing mechanisms (83, 288). Moreover, ADAR2-mediated editing of RNA substrates in the nucleolus may be inhibited by snoRNAs (291); ADAR2 activity can be modulated by sequestration in the nucleolus and nucleolus-nucleoplasm shuffling (256), and ADAR mRNA itself is subject to editing which restricts its function in the adult, at least in Drosophila (142).

B. Enzymes Involved in A-I RNA Editing

There are three A-to-I RNA editing enzymes (ADAR1–3) in mammals, all differentially expressed during organogenesis with ADAR3 restricted to brain and ADAR1 and ADAR2 preferentially expressed in the nervous system (18, 45). RNA editing also displays complex and dynamic profiles of subcellular localization and spatiotemporal expression during progressive stages of nervous system maturation (24, 157, 169, 234). Furthermore, RNA editing is modified by behavioral state and modulated by genetic background (79). Moreover, the activity profiles and molecular properties of ADARs can be orchestrated by changing environmental signals including inflammation and by feedback regulation (18, 288, 309). All ADARs normally exist as functional homodimers, although ADAR3 requires additional CNS environmental cues to undergo dimerization and ADAR1 isoforms can also exist as “internal” heterodimers (49). Multiple isoforms of ADAR1 and ADAR2 are observed, and ADAR1 displays preferential tissue-specific promoter utilization whereas ADAR2 exhibits more pronounced alternate splicing of multiple exons to each generate a broad array of protein species with unique enzymatic properties and remarkable degrees of molecular diversity (94, 139). Different ADARs can also edit multiple different sites on the same RNA species with diverse functional outcomes (288).

The potential biological roles of ADAR3 in the nervous system are particularly intriguing because of its
broad substrate specificity (binding single-stranded as well as double-stranded RNA), its selective presence in restricted brain regions and postmitotic neurons, and its ability to act as a dominant negative for both ADAR1 and ADAR2 (45), strongly implying that it can form heterodimers with ADAR1 and ADAR2, further adding to the functional complexity of these enzymes in the brain. Environmentally responsive forms of ADARs (the interferon-inducible p150 long cytoplasmic isomor of ADAR1 that selectively targets endogenous antisense RNA pathways) and those species with unique ADAR modulatory roles and substrate specificity (ADAR3) exhibit selective regional developmental and mature nervous system expression. Moreover, the crystal structure of the catalytic domain of human ADAR2 reveals that inositol hexakisphosphate (IP$_6$) is buried within the enzyme core, implying a link to cell signaling (192). Moreover, amino acids that coordinate IP$_6$ in ADAR2 are also conserved in adenosine deaminases that act on transfer RNAs (ADATS; see below), and there is evidence that IP$_6$ is required for ADAT activity (192). These observations highlight the potentially central roles of RNA editing in brain evolution (see below), gene-environmental interactions during nervous system ontogeny, and functional specialization during neural maturation.

C. Adenosine Deaminases That Act on Transfer RNAs

An additional level of regulatory control involving RNA editing is suggested by the ability of ADARs to act in concert with ADATS that modify tRNAs to change codon recognition during mRNA decoding (288). All eukaryotes possess ADAT1–3, and ADAT heterodimers are common (49, 144). ADATS are present in the embryonic and adult nervous system and are thought to influence the stability and structural properties of tRNAs to enhance the fidelity and efficiency of tRNAs in decoding mRNAs and in generating protein diversity (95, 143, 258). Interestingly, a mutation in the “editing” domain of a specific aminoacyl-tRNA synthetase results in mischarged tRNAs, intracellular accumulation of misfolded proteins in neurons, and induction of the endoplasmic reticulum-mediated unfolded protein stress response with associated neurodegeneration (172). Mischarged tRNAs are normally cleaved by the editing function of aminoacyl-tRNA synthetases through the actions of a domain distinct from the aminoacylation domain (172). Genomic clustering, structural similarities, and environmental responsiveness between aminoacyl-tRNA synthetases and ADATS suggest that these enzymes may play complementary roles in enhancing the fidelity of protein translation and in promoting structural and functional diversification (185, 190, 259).

D. RNA Editing in Brain Evolution and Disease

These general observations suggest that RNA editing may promote molecular and informational diversity by modulating both RNA and protein regulatory networks. Analysis of ADAR mutants in C. elegans, Drosophila, and mice demonstrate that the process of RNA editing is essential for nervous system cognitive and behavioral functions and has been further coopted for essential roles in the mammalian brain (246, 286, 296). Finally, deregulation of RNA editing resulting in uncorrected forms of hyper- or hypoediting has been implicated in a spectrum of neurodevelopmental, neurodegenerative, and neuropsychiatric disorders (reviewed in Ref. 288).

In humans, A-I editing occurs far more frequently than has been previously appreciated, with the vast majority of the editing occurring in a subclass of primate-specific short interspersed nuclear elements (SINEs) consisting of inverted Alu repeats predicted to form intramolecular duplexes in noncoding RNA sequences in introns, intergenic transcripts, untranslated regions (UTRs) and nontranslated exons, but not in translated exons or in the mitochondrial genome (13, 27, 175). The predominance of human A-I RNA editing over similar forms of editing in lower organisms has recently been shown to be due to unique properties of the single SINE (Alu) element active in the primate lineages as opposed to those SINE elements present in lower mammals (B1, B2, ID, B4): long repeat length, prevalence and lack of evolutionary divergence (223), the latter of which is most easily explained by functional selection mitigating against mutational drift. In addition, through a wide spectrum of molecular mechanisms, Alu elements appear to exert their effects at all levels of gene regulation with profound effects on specification, gene-environmental interactions, ontogeny, homeostasis, and stress modulation and susceptibility to disease (105) and may therefore represent an important driving force in primate evolution. Indeed, these observations strongly suggest that the predominance of Alu elements in the human genome may not represent an idiosyncratic but otherwise evolutionary neutral invasion of the primate lineage by this class of SINEs, but rather be the result of positive selection for these sequences as a modular substrate for A-I editing, in turn driven by positive selection for increased brain capacity and cognitive complexity in the primate lineage.

E. Cytidine Deaminases That Act on RNA and DNA

An additional class of editing enzymes is represented by the cytidine deaminases that can act on both RNA and DNA by changing cytidine (C) and deoxyC (dC) to uridine (U) and deoxyU (dU), respectively (222). The apolipoprotein B editing catalytic subunit (APOBEC) editing family
consists of several members including APOBEC1, -2 CEM15, and activation-induced cytidine deaminase (AID) in mice and APOBEC1, 2, 3A-H, and AID in humans. Each APOBEC-related protein (ARP) possesses intrinsic substrate specificity through recognition of unique cis-acting sequences/structures or through the actions of trans-acting factors that confer site preferences (300). C-to-U editing of apolipoprotein mRNA by APOBEC1 is thought to reduce the incidence of nonsense mediated RNA decay (300). Overexpression of APOBEC1 results in hyperplastic and neoplastic diseases due to loss of editing fidelity and stabilization of cytoplasmic mRNAs encoding growth-promoting proteins (300). Similarly, inactivation of APOBEC1 or overexpression of APOBEC1 resulting in formation of a stop codon in neural tumors from patients with neurofibromatosis type 1 prevents the GTPase activating protein neurofibromin from properly modulating the Ras signaling pathway (216, 273, 300). There are eight human APOBEC3 enzymes (APOBEC3A-H) that probably arose from a primordial cytidine deaminase through a series of tandem gene duplications followed by rapid divergent evolution (300). The mouse CEM15 gene exhibits structural homology to APOBEC3G and resides on chromosome 15, which is syntenic to human chromosome 22, the location of the APOBEC3 gene family (300). APOBEC3 is overexpressed in various cancers, suggesting that it functions as a protooncogene (222). APOBEC3 family members exhibit distinct editase activities, significant alternate splicing, presence or absence of pseudogenes, and DNA mutator functions (300). APOBEC3 isoforms play essential roles in restriction of transposition of endogenous retroelements and in antiretroviral events (210, 300). APOBEC3G is selectively present in primate pyramidal neurons but not glial cells within the cerebral and cerebellar cortices in a nuclear pattern that is distinct from its cytoplasmic subcellular localization in peripheral tissues (113). Interestingly, APOBEC3G displays special roles in cell growth and cell cycle regulation and functions as a cytidine deaminase for both RNA as well as DNA (300). APOBEC3G protects against retroviral infection by deaminating first-strand cDNA synthesis catalyzed by reverse transcriptase, thus establishing links between recoding of RNA and DNA (113, 300).

Perhaps the most interesting ARP is AID, a cytidine deaminase acting preferentially on DNA but also on RNA, which is present almost exclusively in maturing B cells in the germinal centers of secondary lymphoid organs and at lower levels in human brain (220, 300). AID acts on single-stranded DNA of actively transcribed genes rather than double-stranded DNA or DNA/RNA hybrids by converting dC to dU (78, 170, 186). During later stages of B-cell affinity maturation, AID promotes class switch recombination and somatic hypermutation as well as gene conversion in certain contexts (78, 218, 300). Inappropriate actions of AID result in hematolymphopoietic malignancies, solid tumors, and chromosomal translocations (236). To effectively carry out its myriad of enzymatic roles required to generate unusual degrees of molecular and informational diversity, a spectrum of DNA repair enzymes is coopted to assist AID in repairing DNA nicks and double-strand breaks necessary to maintain genomic integrity during active transcription and also in generating additional surrounding mutations to further enhance AID-mediated molecular diversity and plasticity (37, 73, 186, 236). The use of different classes of DNA repair enzymes (base excision repair, mismatch repair, nonhomologous end-joining, and translesional synthesis error-prone Y-family DNA polymerases) for diverse AID-dependent biological processes, substrates and stages of enzymatic process elaboration ensure that transcription-coupled repair is efficiently carried out and is coupled to ongoing mutational activities of specific DNA sequences for the generation of additional dynamic forms of informational processing (37, 73, 170, 186, 236). Recent studies suggest that postmitotic neurons present unique challenges to genomic integrity because of the presence of high transcriptional activity in the absence of active DNA replication, unusual degrees of oxidative stress, and selective reductions in key classes of DNA repair enzyme pathways with the exception of transcription-coupled repair (205, 233). Additional reports suggest that dynamic changes to the genome, the transcriptome, and the machinery of protein translation and function may play previously unimagined roles in neuronal network functions, information processing, and environmental communications (16, 52, 149, 247, 269, 270).

F. Recoding of the Genome, Transcriptome, and Proteome in Brain Development, Learning, and Cognition

These overall observations suggest the novel hypothesis that the global sphere of “RNA editing” may provide the molecular foundations for brain development and function, i.e., for the transfer, encoding, consolidation, retrieval, and long-term integration of sophisticated environmental inputs into both infinitely malleable as well as stable informational traces through neural network activities mediated by dynamic and interactive RNA, DNA, and protein regulatory circuits operating in real-time spatiotemporal synchrony. Indeed, the fact that mRNAs, tRNAs, and rRNAs (rather than synthesized proteins) are trafficked to synaptic termini (148, 153, 285) may be mandated by the requirement for RNA editing to occur specifically in these places to modulate synaptic activity and synaptic connectivity in response to local environmental inputs, and that these changes are communicated back to the nucleus by the retrograde trafficking of the
RNA modification processes of 2'-ribose methylation and pseudouridylation of nucleotides in target RNAs through the twin cleotides in length and orchestrate the site-specific modification of RNAs (snoRNAs). These RNAs range from 60 to 300 nucleotides, different from the parental genotype, which can be tested by sequencing. Indeed, this may be the source of many variant transcripts currently thought to be the immediate (as opposed to historical) result of RNA editing. It also predicts that memory consolidation should be inhibited by short-term knockdown of the relevant enzymes (e.g., by RNA interference).

III. SMALL NUCLEOLAR RNA

Another important and previously underappreciated form of posttranscriptional RNA processing is mediated by a class of noncoding RNAs termed small nucleolar RNAs (snoRNAs). These RNAs range from 60 to 300 nucleotides in length and orchestrate the site-specific modification of nucleotides in target RNAs through the twin processes of 2'-O-ribose methylation and pseudouridylation, modulated by the box C/D and box H/ACA snoRNAs, respectively (15, 174, 208). Most of the known snoRNAs are involved in rRNA modifications during ribosomal biogenesis and are localized in the nucleolus. A subset of related RNAs (scaRNAs) guide modifications of spliceosomal RNAs and reside within cytoplasmic Cajal bodies (208). Members of the greater snoRNA superfamily have been implicated in a broad array of biological processes including alternate splicing, transcription, chromosome maintenance and segregation, genomic imprinting, and cell cycle regulation (121, 250, 253) and have also been shown to be subject to imprinting (263). Thus it appears likely that RNA modifications are utilized as a global mechanism of gene regulation and that many additional snoRNA species and subtypes remain to be identified and interrogated.

Multiple brain-specific snoRNAs have been identified in mice (MBI-36, HBII-13, HBII-48, HBII-49, HBII-52, MBII-78, and MBII-85), all representatives of the C/D class with the exception of MBII-13, a constituent of the H/ACA class (39, 122, 250). Human homologs of these snoRNAs also display high levels of expression in the nervous system (39). Interestingly, individual snoRNAs (RBI-36) exhibit genus-specific (rat) brain functions. These overall observations further suggest that snoRNAs exhibit a broad range of nonhousekeeping regulatory functions in brain (40).

In situ hybridization analysis in adult mouse brain has shown that several snoRNAs (MBI-36, HBII-48, HBII-52, MBII-85) have greatest expression in the hippocampus and amygdala, areas associated with learning and memory (251). Within these specialized brain regions there is an additional preferential stratification of expression between the hippocampal CA1 region (MBII-48) and the dentate gyrus (MBII-85). Moreover, MBII-36, MBII-48, and HBII-52 display greater ventral than dorsal hippocampal expression profiles.

The ventral hippocampus is known to be involved in contextual conditioning, and therefore, it is interesting to note that the expression of MBII-48 and MBII-52 but not MBI-36 and MBII-85 are transiently modulated during contextual memory consolidation (fear conditioning) (251). In addition, during the time scale associated with immediate early gene modulation (1.5 vs. 25 h), MBII-48 displays transient downregulation, whereas MBII-52 displays upregulation. These observations suggest that the stimulus association regulates specific snoRNA expression during the process of storage of learned memories.

HBII-52 modifies the expression of the serotonin 5-HT (2C) receptor subunit, involved in memory consolidation, including alternate splicing and A-I RNA editing (151). In Prader-Willi syndrome, HBII-52 is not expressed and serotonin 5-HT (2C) receptor isoforms distinct from those that characterize normal expression patterns are elaborated (151). This suggests that anomalous splicing may contribute to the pathogenesis of this developmental syndrome associated with genomic imprinting. HBII-13, HBII-52, and HBII-85 map to the Prader-Willi syndrome locus, thereby suggesting that snoRNAs may actively participate in genomic imprinting (250). A significant array of other classes of noncoding RNAs are also intimately associated with the process of genomic imprinting, a still poorly understood mechanism of allele-specific gene silencing that clearly affects brain development and function in a variety of ways (see below). It is interesting to note that the small nucleolar ribonucleoprotein particles that contain H/ACA box snoRNA also contain the protein NAP57/dyskerin that is highly expressed in embryonic...
neural tissues including cerebellar Purkinje cells and mitral cells of the adult olfactory bulb (112).

IV. MICRORNA

MicroRNAs (miRNAs) are short (~22 nucleotide) regulatory molecules that modulate the translation or stability of target RNAs (reviewed in Refs. 204, 316). Despite their relatively recent discovery, it is already clear that miRNAs play important roles in nervous system development including neurulation, neural tube patterning, segmental morphogenesis, laterality, neural stem cell self-renewal, proliferation, lineage restriction, neuronal and glial lineage specification, differentiation, polarity, dendritogenesis, axonal path finding, apoptosis, protein turnover, cell positioning, and maintenance of mature neural traits. They also play key roles in a range of neurodevelopmental, neurodegenerative, and neuropsychiatric diseases as well as in brain cancer.

A. MicroRNAs in Neural Development

Zebrafish dicer mutants that are defective in miRNA processing exhibit defects in neural induction, neural plate and neural tube formation and three-dimensional patterning, segmental morphogenesis, neural stem cell self-renewal, proliferation, progressive lineage restriction, and axonal path finding (97, 98). These defects are largely, although not completely, rescued by complementation with the miRNA mir-430 (97, 98). These findings suggest that individual miRNAs can induce large-scale spatiotemporally mediated changes in the transcriptome. mir-430 shares origins with mir-302 and mir-372, which are both present in embryonic stem (ES) cells (118). Multiple miRNAs also exhibit both overlapping and inverse expression patterns to those of Hox segmentation genes, thus suggesting additional levels of complexity in the mechanisms of neural gene regulation (195, 232).

The presence of minor neuronal defects in later developmental stages in mir-430 rescue experiments of dicer mutants demonstrates that other miRNAs and/or short interfering RNAs (siRNAs) are involved in later stages of neural development and maintenance of mature neural cell fates (97, 98). A large group of miRNAs is involved in human ES cell maintenance (279), a subset of which affect subsequent neural development. During ES cell-derived neurogenesis in vitro and in vivo, miR-124a and miR-9 differentially enhance neural lineage elaboration by positively promoting neurogenesis while actively inhibiting gliogenesis, respectively, through STAT3-mediated developmental signaling pathways (167). Overexpression or inhibition of brain-specific miRNAs disrupts the balance between neurogenesis and gliogenesis and the normal temporal transition from neurogenesis to gliogenesis (35, 47, 154, 164), thereby demonstrating the intricate combinatorial profiles of miRNA-mediated sensor and effector state transitions.

In mice, there are numerous brain-specific miRNAs, with miR-124 being the most abundant (47, 166, 180, 306). Approximately 20% of these miRNAs exhibit regulated expression during neural development with a subset upregulated during retinoic acid-induced neural cell differentiation from embryonic carcinoma cells (141, 275). Conversely, experimental downregulation of miR-23 significantly enhanced the Notch pathway-associated transcriptional repression mediated by the downstream effector Hes1, with concurrent preservation of the self-renewing neural stem cell basal state (141). Several predicted targets of miRNAs, such as Notch, PTEN, and IDs (inhibitors of differentiation), are important mediators of neural stem cell self-renewal and exhibit coordinate regulation (47, 104, 252). Components of the pri-miRNA processing complex including DGCR8 and Drosha are present in embryonic neural stem cell generative zones (102, 268), and deletion of DGCR8 results in DiGeorge syndrome, a multisystem disorder associated with significant learning disabilities. During progressive stem cell lineage restriction, dynamic switches in expression profiles of miRNA families occur (141, 164, 167, 275).

The Drosophila ortholog of miR-124 is also entirely restricted to the developing central nervous system, suggesting that this miRNA and others that exhibit expression patterns analogous to those of their vertebrate counterparts have ancient roles in developmental patterning (2). Many other miRNAs are specifically transcribed in subsets of cells in the embryonic brain and the ventral nerve cord in Drosophila, including miR-315, miR-92a, and miR-7 (2), whose fish and mammalian orthologs are also upregulated in brain (213, 302). Other miRNAs display expression in the embryonic peripheral nervous system in Drosophila (2).

A wide variety of miRNAs are localized to mammalian neuronal subtypes, with the highest concentration observed in the cerebral cortex and the cerebellum (164, 167). Additional miRNAs are present predominantly within glial cell subtypes, with other miRNAs exhibiting more global or neural stem/progenitor cell-specific patterns of expression (154, 166, 275). These observations generally suggest that there is extensive cross-talk between transcriptional and posttranscriptional regulatory mechanisms and distinct miRNAs that are active within particular neural cell lineages. During progressive stages of cortical development, a distinct subset of miRNAs displays increased levels of expression while others do not (166).

Neural transcription factors also appear to be miRNA targets (114, 154). Conversely, specific proneural basic helix-loop-helix transcription factors upregulate miRNA expression, including expression of miR-124 which has
the ability to bind to the most abundant miRNA response element in the 3′-UTR and to downregulate the expression of at least 100 mRNAs (154, 180, 297).

In a mouse knockout (KO) model of presenilin 1, the gene mutated in a subset of early familial forms of Alzheimer’s disease (AD), there are profound alterations in embryonic cortical development and precocious neurogenesis (166). Microarray analysis in this presenilin 1 KO model has revealed selective dysregulation of two miRNAs, miR-9 and miR-131. These brain-enriched miRNAs are derived from the arms of the stem of a common miRNA precursor and show independent developmental regulation but coordinate depletion during a late-stage embryonic critical period. miR-9 also regulates the expression of ID2, a negative regulator of neural stem cell lineage maturation, and miR-131 modulates the expression of calcineurin Aβ, a cofactor for calcium-mediated neuronal Sp1 transcription activation (166). Interestingly, Sp1 and other C2H2-type zinc-finger transcription factors have been reported to have a high affinity for its binding motif in RNA:DNA forms (267), suggesting that RNA signaling may control transcriptional activity as well, a possibility supported by a range of other evidence (57, 82, 178, 203).

Lateralization of the chemosensory organs of C. elegans is mediated by the asymmetric expression of the miRNAs miR-273 and lsy-6 with differential translational block of die-1 and default (miR-273) or induced (lsy-6) elaboration of the right- or the left-sided representative of this essential neural structure, respectively (44). The brainspecific miRNA miR-134 is localized to the synaptodendritic compartment of rat hippocampal neurons and negatively modulates the size of dendritic spines by inhibition of translation of the protein kinase Limk1 mRNA (261). Brain-derived neurotrophic factor (BDNF) is thought to relieve the miR-134-mediated translational inhibition with promotion of synaptic development, maturation, and plasticity through modification of the activities of additional transcriptional regulators within the miR-134 complex rather than by dissociation of miR-134 from Limk1 mRNA (261).

B. MicroRNAs in Adult Brain and Brain Disease

miRNAs are also expressed at high levels within the mature and even the senescent brain and function to orchestrate the maintenance of adult neural cell traits, to promote cellular homeostasis and dampen endogenous and exogenous stress responses, and to modulate multiple parameters associated with synaptic plasticity (47, 54, 127, 261, 265, 275). By repressing the expression of genes involved in maintaining the undifferentiated neural cell state, miRNAs promote the fidelity of specific differentiated neural phenotypes (164, 166). Therefore, non-snc-
tioned alterations in the profiles of expression of miRNAs may induce dedifferentiation and cellular transformation.

In the highly malignant glial cell-associated brain tumor, glioblastoma multiforme, there is dramatic overexpression of miR-21, whereas in other neural tumors, including more benign grades of gliomas, there are significantly lesser degrees of miR-21 overexpression (43). Additional experimental studies indicate that aberrant expression of miR-21 specifically blocks gene products essential for glial cell differentiation or apoptosis, thereby favoring tumorogenesis (43).

The largest subgroup of predicted miRNA targets include transcripts encoding synapse-associated proteins such as synapsin 1, synaptotagmin, and the fragile-X mental retardation protein (FMRP) (129). Numerous studies suggest that miRNAs are intimately involved in synaptic tagging to ensure synaptic input specificity during the critical phases of memory formation (146, 197, 257). miRNAs are present in dendritic spines and reversibly interact with the cellular machinery to produce long-lasting changes in synaptic function (146, 261). In response to synaptic activity, changes in the RNA-induced silencing complex (RISC) or conformational alterations in dendritic miRNAs involving accessibility to their 3′-UTRs promote siRNA- and miRNA-mediated silencing (12, 188). miRNAs can participate exclusively in both processes by the actions of Loquacious, the miRNA equivalent of the executor step of RNA interference (RNAi) (242, 311). Interestingly, Arabidopsis miRNAs 173 and 390 are known to target the primary transcripts encoding trans-acting siRNAs (6). These sites of RNA complementarity set the register for the mature siRNA molecules and promote siRNA phasing, thus illustrating the dual functions for miRNA in RISC-mediated silencing (6).

In addition, certain miRNA precursors undergo RNA editing by ADAR1/2 with suppression of processing by Drosha and degradation by Tudor-SN (187, 310). It has also been reported that RNA editing increases the diversity of miRNAs and their targets, thereby modulating miRNA function (28). Some miRNA genes are also subject to imprinting (262). In addition, polymorphisms in the pre-miRNA hairpin region may alter target selection with significant biological consequences (e.g., C→A in mature miR-30c-2) or may disrupt stem integrity and thereby influence miRNA processing (124). Additional variations in their promoter regions have significant implications for miRNA expression levels. These different types of miRNA-associated polymorphisms affect the expression of seminal neurodevelopmental proteins and may be a mechanism for the advent of neurological diseases.

Putative targets for miRNAs include mRNAs encoding proteins involved in all aspects of neural development, maintenance of neuronal function, and neural network plasticity throughout the neuraxis and concurrently in
specific disorders of the developing, mature, and aging nervous system. Predicted miRNA targets include numerous proteins implicated in neurodevelopmental and neurodegenerative diseases (249). Tourette’s syndrome has been shown to manifest clinically as a result of sequence variations in the docking site for miR-189 in the SLIT and Trk-like family member 1 (SLITR1) mRNA (1). It has previously been shown that SLITR1K is required for progressive stages of neuronal maturation and its expression profile is deregulated in several distinct neural tumor subtypes (10, 11). In a type of early-onset Parkinson’s disease (Waisman syndrome) and in a form of X-linked mental retardation (MRX3), there are alterations associated with the gene locus of miR-175 (77). Additional studies have implicated miRNAs in a spectrum of neuropsychiatric diseases associated with developmental pathogenesis (249), and there is evidence that mutations involved in creating or destroying putative miRNA target sites are abundant in mammalian genes and might be important causes of phenotypic variation (50), including psychological variation.

C. Tip of an Iceberg?

The known miRNAs are generally those that are abundant, are highly conserved, and have multiple targets (204), the latter of which accounts for their high evolutionary conservation (204). This in turn implies that there will be many others, perhaps tens or hundreds of thousands of undiscovered miRNAs that are not so constrained and that can evolve quickly or be easily born to explore new connections in regulatory networks, and that act in particular cell types and even individual neurons. The latter is exemplified by the example of lsy-6 in C. elegans, which was only discovered by sensitive genetic screens, and which required extraordinary biochemical analyses to confirm its existence (131). Recent studies have identified many new human miRNAs, a significant number of which appear to be primate specific (21–23). It is also clear that miRNA expression profiles are progressively elaborated as the developmental program unfolds and cellular differentiation proceeds, again suggesting that many if not most may be cell specific (277, 302). Deep sequencing of ~274,000 small RNAs in human colorectal cancers and normal colonic mucosa showed that the levels of different miRNAs can vary by up to four orders of magnitude and that the discovery rate of novel miRNAs was linear between 50,000 and 274,000 tags; these and other similar studies suggest that there are many more low-abundance miRNAs yet to be identified (22, 23, 61). Finally, these conclusions are supported by whole chromosome tiling array transcriptome analyses which indicate that there is on average 1 short (<200 nt) RNA expressed approximately every 3 kb (T. Gingeras, personal communication), as well as the presence of large numbers of plausible miRNA precursor structures in the human genome, many of which appear to be expressed in the brain (L. J. Croft and J. S. Mattick, unpublished observations) and may have specific roles in determining function and cell identity in the nervous system.

It is also likely that there are many hundreds if not thousands of as-yet-undiscovered cell- and tissue-specific snoRNAs and sno-like RNAs, as well as new classes of regulatory RNAs, such as the recently described piRNAs that are specifically expressed in the testis (8, 99), the other tissue apart from brain that shows an extraordinarily rich expression of noncoding RNAs (245). It is also worth noting that many snoRNA and miRNA genes are clustered and that most snoRNAs and many miRNAs are encoded within introns of both protein-coding and noncoding genes (including imprinted genes) (204, 263), testimony to the complexity of the genetic output and the parallel functional and regulatory networks in which these gene products participate (199). The presence of bifunctional or polycistronic RNAs may be much more widespread than previously believed and not restricted to transcripts capable of being processed to miRNAs or snoRNAs. The specificity and complexity of small ncRNA expression and putative mechanisms of action almost certainly play a central role in brain development, mature nervous system functioning, disease states, and neural regenerative responses.

V. OTHER SHORT REGULATORY RNA

An important variation on the theme of short trans-acting RNAs is a double-stranded neuron-restrictive silencing element (dsNRSE) RNA that exhibits a striking structural resemblance to a miRNA (168). However, dsNRSE RNA does not act as a negative regulator of translation but rather as a transcriptional activator of neuronal differentiation genes by converting the neuronal silencer factor (REST/NRSF) and its extensive and ever-changing epigenetic regulatory complex components from a transcriptional repressor in undifferentiated, nonneuronal, and nonneuronal cells to a transcriptional activator during early neuronal lineage elaboration (168). REST has recently been shown to modulate the expression of a subset of miRNAs including the brain-specific miR-124a (54). In undifferentiated and nonneuronal cells, REST inhibits miR-124a expression through the RE-1 promoter gene silencer element, whereas during neuronal maturation alleviation of REST repression allows miR-124a to selectively degrade nonneuronal transcripts, thus providing a regulatory link for REST-independent miRNAs acting in concert with the actions of dsNRSE RNA in complementary transcriptional and posttranscriptional neuronal contexts.
Other small brain-specific trans-acting RNAs are the dendritic BC200 RNA which arose from a monomeric Alu element and is limited to the primate order, and the analogous rodent dendritic BC1 RNA which was also generated by retrotransposition (196, 227). Recent data have suggested that BC1/BC200 may interact with FMRP (130, 314, 315) and may also play a role in translational inhibition (158, 294, 295). As suggested by Brosius (31), we suspect that many so-called repetitive sequences derived from transposons may have in fact acquired functions. We further suggest that many of these functions are elaborated via RNA, and may be intimately involved in mammalian ontogeny, including brain development and function.

VI. LONGER NONCODING RNA

A. Antisense RNAs

Natural antisense RNAs (i.e., RNAs that are expressed from and may potentially interact directly with, and/or regulate the expression of, protein-coding transcripts from the other strand of the same locus) are greatly enriched in the nervous system (161). These antisense RNAs exhibit dynamic developmentally regulated and spatially discrete expression profiles and are observed in over 70% of all protein-coding loci in mouse (137) including those involved in three-dimensional patterning of the neural tube, regional brain morphogenesis, stem cell expansion, proliferation and neurogenic and gliogenic cell divisions, homeostatic and associated stress responses, cell shape, motility and migration, brain feeding, metabolic and reward circuits, and progressive neuronal viability and synaptic maintenance as well as plasticity (161).

Recently, it has been shown that siRNA-mediated knockdown of antisense transcripts can alter the expression of the corresponding sense strand-derived mRNA, although the effects are not predictable, with some sense-antisense pairs showing concordant and others discordant regulation (137, 292). In addition, it appears that the latter may not involve sense-antisense duplex formation, nor the activation of the RNA interference pathway (80). Indeed, these observations indicate that antisense RNAs may regulate the expression of other transcripts in the same locality, including those from the other strand, in complex ways that are yet to be understood, but which may share mechanistic features, such as effects on chromatin structure, in common with ncRNAs involved in regulation of allelic expression at imprinted loci (see below).

There are as yet very few well-described examples of antisense RNAs affecting expression of genes in the nervous system, but this situation is likely to change radically in the coming years. However, some instances are known, although mechanisms remain obscure. In myelin-deficient mice, there is tandem duplication of the myelin basic protein (MBP) gene. The mutated upstream copy is transcribed within the brain as an antisense RNA that is localized in the nucleus but is not polyadenylated (228). In mutant mice, there is normal overall transcriptional activity of the MBP gene but a significant reduction in MBP mRNA concentration in the cytoplasm. These observations indicate that either the processing or transport of MBP mRNA to the cytoplasm is altered by the presence of the antisense RNA.

In the nervous system of the snail Lymnaea stagnalis, a nitric oxide synthase (NOS) pseudogene is transcribed as an antisense RNA complementary to the NOS-encoding mRNA (162). Suppression of NOS enzyme activity in cerebral giant cells is postulated to be caused by hybrid arrest of translation mediated by the trans-acting antisense transcript transcribed by the NOS pseudogene. With the use of a model of long-term memory formation, it has been observed that posttraining expression of the NOS pseudogene is transiently downregulated just before transient upregulation of NOS mRNA and coincident with the critical window for memory formation (145, 163).

These intriguing findings provide evidence that expression of a pseudogene can be regulated by a significant behavioral stimulus. There is accumulating evidence that gene duplication coupled to DNA inversion can produce long sequence trans-acting antisense transcripts (160). There is additional evidence that in both silkworm and Neurospora, core “circadian rhythm” clock genes are regulated by endogenous antisense transcripts (59, 101, 165). Furthermore, susceptibility loci are present within the disabled in schizophrenia 1 (DISC1) gene and in the large antisense DISC2 RNA that is thought to modulate its expression in both schizophrenia and in bipolar illness (211, 212). DISC1 has been implicated in a complex spectrum of nervous system functions including cellular morphogenesis, neuroblast migration and axonal path finding, and vesicular trafficking, and alterations of DISC1 functions during progressive stages of forebrain maturation may underlie the developmental pathogenesis of these selective neuropsychiatric syndromes (134).

B. Other Large Noncoding RNAs

There are also tens of thousands of mRNA-like ncRNAs that are not antisense to known protein-coding genes, although many of these loci also show transcription from both strands, similar to sense-antisense pairs at protein-coding loci. These ncRNAs are transcribed by RNA polymerase II, are polyadenylated, and often exhibit alternate splicing (36, 46, 136). Many of these ncRNAs are developmentally regulated and physiologically respon-
sive, show particular abundance in the brain (123, 245), and appear to be evolving rapidly (230). Unexpectedly, there also appear to be large numbers of nonpolyadenylated large RNAs (46, 152), which have remained hidden from view because of the use of oligo-dT for mRNA purification and to prime reverse transcription in cDNA cloning protocols. It is also possible, if not likely, that many of these ncRNAs, as well as those derived from introns (of both coding and noncoding transcripts), may be subsequently processed to smaller RNAs that have various functional properties and regulatory roles.

In *Drosophila*, large ncRNAs have been implicated in a broad spectrum of developmental processes. These ncRNAs display preferential expression during embryonic development in general and within the nervous system in particular (123). This unusual class of ncRNAs also functions prominently in dosage compensation and chromosome inactivation, forms of genomic imprinting that are particularly important in neural development, adult nervous system function, and the etiology of neurodevelopmental and neuropsychiatric diseases (209, 239, 276). In the *Drosophila* peripheral nervous system, the *bereft* RNA plays important roles in extrasensory organ development and in the adult maintenance of interommatidial bristles of the eye (109).

A number of very long ncRNA species, termed “macronRNAs” or long expressed noncoding regions (ENORs), have recently been identified in mice (88). ENOR loci, including ENOR 28 and ENOR 31, have the capacity to give rise to multiple macronRNAs, all exhibiting preferential enrichment within the nervous system. Many ENOR loci contain host genes for miRNAs and snoRNAs, show evidence of antisense transcription and genomic imprinting, exhibit a predilection for brain-selective expression, and display predominant nuclear localization (88).

Recently, it has been reported that all but 2 of 49 regions of the human genome that are highly conserved among mammals but show accelerated evolution since our divergence from our common ancestor with the chimpanzee (“human accelerated regions” or HARs) are located outside of protein coding sequences and 24% are adjacent to genes involved in neural development (240). Moreover, the most rapidly evolving of these regions HAR1 is part of the first exon of a 2.8 kb spliced ncRNA termed HARF1 that is specifically expressed in Cajal-Retzius neurons in the developing human neocortex (Fig. 1), as well as in ovary and testis. HARF1 is coexpressed with reelin, a protein that is critical for neuroblast migration from paramedian stem cell generative zones and for the specification of the layered structure of the human cortex. In addition, there are two alternatively spliced antisense transcripts HAR1Ra and HAR1Rb, which also contain the HAR1 region in their first exon and which are expressed in the brain and testis, respectively (240). The temporal and spatial pattern of expression of HAR1R suggests that it may have later developmental effects to downregulate HAR1F by antisense-mediated inhibition. Interestingly, reelin exhibits enhanced expres-

![FIG. 1. Expression of the rapidly evolving noncoding RNA HAR1F in the developing brain. A: in situ hybridization on 19 gestational week (GW) coronal brain sections, illustrating expression of HAR1F in the hippocampal primordium (arrows) and dentate gyrus (arrowheads). B: in situ hybridization on 24 GW coronal brain sections, illustrating expression of HAR1F in the cerebellar cortex (arrows in left panel) and olivary complex (arrows in right panel). HAR1F sense probe detects no obvious signal. [From Pollard et al. (240), with permission from Nature Publishing Group.]]
The Allen Brain Atlas (ABA) is a large-scale gene expression study of the adult mouse brain using high-throughput RNA in situ hybridization to visualize the expression of over 20,000 transcripts at cellular resolution (173). While this project was focused primarily on protein-coding genes, it also includes well over 1,000 probes targeted against noncoding transcripts originating from intergenic, intronic, and antisense regions, as well as imprinted loci. Many of these transcripts are localized in specific neuroanatomical regions, including several neocortical layers, as well as in the hippocampus, rostral migratory stream, and olfactory bulb, which are primary sites of neurogenesis, neuronal differentiation, and maturation in the mouse brain (T. Mercer, M. E. Dinger, S. Sunkin, M. F. Mehler, and J. S. Mattick, unpublished data).

C. Noncoding RNAs in Genomic Imprinting

Imprinted genes play seminal roles in brain development and adult functional activities. Moreover, misregulation of their patterns of gene expression can predispose to a complex array of poorly understood neurodevelopmental and neuropsychiatric diseases (66, 67). Allele-specific genes display preferential expression within the nervous system and are often transcribed from larger genomic regions encompassing multiple tandemly repeated miRNAs and C/D snoRNAs (67, 177, 262, 274). Imprinted loci are capable of giving rise to a complex amalgam of both unspliced as well as spliced longer ncRNAs (58, 67, 88, 226, 274). Antisense RNAs to reciprocally imprinted neighboring protein-coding transcripts are also found within or adjacent to imprinted loci (67, 274). Imprinted genes appear to modulate a broad spectrum of cellular processes within the nervous system including intracellular signaling, RNA processing, growth and cell cycle regulation, genome modifications, transcription factor actions, protein trafficking and processing, membrane-associated receptors, transporters and structural proteins, and ncRNAs (67). The essential functions of imprinted genes throughout nervous system development and mature steady-state functioning are best revealed by analysis of the breadth and diversity of neurological diseases associated with parent of origin effects and disruptions in imprinted loci including schizophrenia, attention-deficit hyperactivity disorder, autism, Tourette’s syndrome, and bipolar disorder (reviewed in Refs. 66, 67).

Imprinted genes exhibit exquisite cell-specific patterns of expression within the brain, suggesting an ongoing role in modulating neural connectivity and activity-dependent synaptic plasticity (reviewed in Ref. 67). The Ube3a gene displays imprinted expression in specific brain regions and cell types including mitral cells of the olfactory bulb, Purkinje cells of the cerebellum, and the hippocampus but displays biallelic expression in other tissues and brain regions (5). Conversely, Igf2, Grb10, and Zim1a are imprinted in many tissue types but exhibit biallelic expression in the CNS (9, 69, 147). The Nnat gene is imprinted predominantly in brain regions alone, whereas Murr1 exhibits temporally restricted brain imprinting, and these expression profiles suggest the dynamic and complex regulation of genomic imprinting during brain development and mature functioning (133, 298).

Some imprinted genes occur in the introns of host genes. For three of these microimprinted domains, the imprinted and host genes are highly expressed in brain particularly within the hippocampus (68, 298). These structure-function interrelationships allow for potentially complex interactions between genes and protein products. Several inserted imprinted genes (Nnat, Nap1l5, Peg13, U2af-rs1) are paternally expressed (293). Other imprinted genes in the CNS display reduced expression from the silent allele, suggesting that brain-specific imprinting may be cell-selective or localized, and seemingly minimal gene expression from the silent allele may represent the combined effects of regions of monoallelic and biallelic expression (237, 238). There are also cell-specific profiles of imprinting in the CNS with Ras-Grf1 and monoallelic expression of Ube3a and the oppositely imprinted antisense Ube3a restricted to neurons, whereas Ube3a and antisense Ube3a expression is biallelic in neural progenitor species and glial cells but monoallelic in neurons (308). The maternally expressed Nesp55 gene displays neuronal specificity (20); the paternally expressed Ndu, Nap1l5, and Peg13 exhibit preferential neuronal specificity (68, 283); and the imprinted 5HT2a receptor gene is localized to both neurons and glia (138).

GT2/MEG3 and RIAN represent two large imprinted ncRNAs preferentially expressed in the brains of mice and humans (110, 305). Interestingly, a miRNA gene cluster is present at the same locus as the mouse GT2, a spliced and maternally expressed ncRNA (262). In addition, a large number of tandemly repeated box C/D snoRNAs are located downstream from GLT2 (67). Moreover, the box C/D snoRNAs M/HBII-13, M/HIII-52, and M/HBII-85, which are preferentially expressed in the nervous system, are clustered at a locus between the SNURF-SNRPN and UBE3A genes that harbors several imprinted genes implicated in the pathogenesis of Prader-Willi and Angelman syndromes (67). H/HBII-52 is known to precisely modulate the A1 RNA editing and alternate splicing of the 5HT (2C) mRNA and may thus represent a novel subfamily of posttranscriptional regulators for protein-coding genes (67). The HBII-85 locus contains additional box C/D snoRNA genes (HBII-436, HBII-437, HBII-438A/B) within the same genomic interval, and this
snoRNA cluster is not expressed in the Prader-Willi syndrome that is associated with a large paternal deletion of the entire imprinted domain (39, 72, 254).

Within the imprinted Dlk1-Gtl2 domain, many potential miRNAs are expressed downstream from a cluster of snoRNAs (67, 262). These ncRNAs are only expressed from the maternally inherited chromosome and are preferentially expressed in the nervous system. In this case, a proximal intergenic differentially methylated region between the Dlk1 and the Gtl2 genes regulates imprinted expression (281). Interestingly, targeted deletion of unmethylated sequences within this region results in the repression of all maternally expressed ncRNAs and biallelic expression of all paternally expressed protein-coding genes; the same deletion transmitted paternally has no obvious effects (67, 183). Moreover, miR-127 and miR-136 are processed from a large maternally expressed Rtl1 antisense ncRNA (264). The Rtl1 gene is paternally expressed and encodes a large open reading frame with strong homology to the Gal and Pol retrotransposon domains (264, 313). Experimental observations strongly suggest that these two miRNAs negatively regulate Rtl1 expression through the mechanism of RNA interference due to their perfect sequence complementarity (183). The profiles of sense/antisense gene organization coupled with reciprocal imprinting of the miRNAs relative to their RNA target gene suggest that small RNAs are involved in Rtl1 genomic imprinting. These overall findings suggest a link between RNA interference, genomic imprinting, and retrotransposon silencing (67, 103).

Other imprinted miRNAs can inhibit RNA translation. The Prader-Willi syndrome is a complex neurodevelopmental disorder presenting with mental retardation, hypothalamic insufficiency, and failure to thrive as a result of three different mutations, deletion of paternally inherited 15q11–13, maternal uniparental disomy, and mutation of the imprinting center (38). IPW is a neural imprinting gene within the Prader-Willi locus that represents a spliced and polyadenylated ncRNA (301). ZNF127 and ZNF127 antisense transcripts are encoded at the IPW locus and are expressed predominantly in brain from the antisense strand (132). Angelman syndrome is caused by three reciprocal mutations to Prader-Willi syndrome and is represented pathologically by cortical atrophy, cerebellar dysmyelination, and Purkinje cell loss (38). Patients with Williams syndrome exhibit profound mental retardation and microcephaly caused by maternal deletion of a region of chromosome 7q11.23, although it is not yet certain whether the syndrome is caused by disruption of imprinted genes (7, 235). These cumulative observations demonstrate that ncRNAs are prominently represented within imprinted loci and clearly have a spectrum of essential functions in the process of genomic imprinting and the selectivity of allelic expression within developing and mature brain regions and neural cell subtypes (reviewed in Refs. 70, 150, 177, 253, 263).

VII. TRANSFER RNA, RIBOSOMAL RNA AND DISEASES OF THE NERVOUS SYSTEM

In addition to their known roles in housekeeping functions such as mRNA translation, transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs) have recently been implicated in numerous distinct and specialized brain functions during development as well as adult life as assessed by the effects of mutations in these two classes of ncRNAs in predisposing to a range of neurodevelopmental, neurodegenerative, and neuropsychiatric diseases (reviewed in Refs. 74, 75, 81). These mutations predominantly affect the mitochondrial genome where two-thirds of pathogenic mutations affect tRNAs that themselves comprise only one-tenth of the entire mitochondrial genome.

Mitochondrial diseases exhibit several unique clinopathological features including heteroplasmy, threshold effects, and mitotic and replicative segregation that may define broader phenotypic links with these classes of ncRNA alterations in disease pathogenesis and clinical progression (74, 75). In general, mutations in both tRNAs and rRNAs can cause a spectrum of neurological diseases including chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS; CPEO with retinal degeneration), and MELAS (mitochondrial encephalopathy with stroke-like syndromes and migraine headaches) and MERRF (myoclonus epilepsy, mitochondrial myopathy, cerebellar ataxia and far less frequently dementia, peripheral neuropathy and hearing loss) syndromes (74, 75). MELAS syndrome and other tRNA-mediated diseases are inextricably linked to a broad series of neuropsychiatric diseases including schizophrenia, personality disorders, major depressive disorders, anxiety disorders, psychosis, and delirium, and these clinical features may be among the presenting symptom complexes that predate by years the onset of classical neurological disorders (81).

tRNAs can more infrequently give rise to other neurological disorders including multisystem atrophy, Leigh’s hereditary optic neuropathy (bilateral optic atrophy), and neurosensory deafness (74, 75). MELAS and MERRF syndromes are usually caused by mutations in specific tRNAs such as tRNA<sup>Lys</sup> and tRNA<sup>Leu</sup>, respectively (74, 75). MERRF has also been associated with mutations in tRNA<sup>Phe</sup> (194), and mutations in tRNA<sup>His</sup> can result in an unusual syndrome that includes encephalitis, arterial strokes, venous infarctions, and rhabdomyolysis (75). A unique form of homoplasmic syndromic deafness can result from mutations in the 12S rRNA or in tRNA<sup>Ser (UCN)</sup> (179, 241, 278). In addition, the penetrance of the disease may be enhanced by polymorphisms of the mitochon-
drial ND protein-coding gene in association with the tRNA\textsubscript{Ser}(UCN) mutation (84, 85). Moreover, pseudouridylation of tRNAs by a homozygous missense mutation of the gene encoding pseudouridine synthase I can give rise to an autosomal recessive mitochondrial myopathy (33). Interestingly, specific alterations in the levels of expression of specific tRNAs (tRNA\textsubscript{Val}, tRNA\textsubscript{Asn}, tRNA\textsubscript{Lys}) and rRNAs (5.8S and 5S rRNAs) have been observed in selective regions of the aging human brain predisposed to undergoing degenerative changes in association with Alzheimer’s disease and its antecedent, minimal cognitive impairment, with the profiles of these ncRNA changes predictive of the two related syndromes (76). Furthermore, motor neuron disease has recently been linked to mutations in tRNA\textsubscript{A} (29).

VIII. CYTOPLASMIC NONCODING RNA IN SYNAPTIC SPECIALIZATION AND FUNCTION

Several distinct classes of ncRNAs are required for axodendritic process outgrowth and maturation and synaptogenesis. These same ncRNA species promote mRNA axonal transport, synaptic targeting, and local protein synthesis within “activated synapses” and thus function as essential molecular constituents mediating synaptic plasticity (reviewed in Ref. 127). The FMRP is an RNA binding protein that may play a seminal role in synaptic plasticity and synapse-selective protein translation. In the fragile-X syndrome, genetic alterations of FMRP cause a characteristic neurodevelopmental disorder consisting of mental retardation, autism, anxiety disorders, and epilepsy or a neurodegenerative syndrome during the “premutation” phase (see below). The FMRP ribonucleoprotein complex(es) may include numerous other molecular species such as FXR1P/2P, nucleolin, YB1/p50, Purα, staufen, IMP1 (RNA transport factor), and kinesin 5 (41, 42, 135, 243). In addition, Purα links the cytoplasmic levels and human ncRNAs BC1/200 to microtubules, and this dynamic ribonucleoprotein subcomplex is actively transported to dendrites to participate in the process of synaptic tagging (227), defined as specific information hubs of coincident electrical and molecular activities essential to encode synapse-specific information for precise signal propagation within activated neural networks (130, 307). Purα further interacts with two additional ncRNAs, TAR RNA element of HIV1 and signal recognition particle (SRP) RNA, and this association enhances DNA replication and transcription and membrane translocation (48, 89, 130, 287). YB1 regulates alternate splicing, transcription and translation, DNA repair, and stress responses (42, 156). Moreover, YB1 interacts with MECP2, the gene mutated in the neurodevelopmental disorder Rett’s syndrome (X-linked mental retardation, psychosis, autism, Angelman’s syndrome, and neonatal encephalopathy) (312). MECP2 is known to orchestrate methylation-dependent transcriptional repression, which is probably RNA directed (25), and more recently has been implicated in alternate RNA splicing (312).

Neuronal stimulation increases intracellular calcium levels and activates calpain, which, in turn, liberates dicer and eIF2c at postsynaptic densities (188). These processes act on miRNA precursors and sense/antisense RNA pairs to modulate stabilization and translational of local mRNAs (188, 229, 260). Synaptic protein synthesis is regulated by activity-dependent downregulation of the RISC pathway and proteasomal-mediated degradation (12). miRNA target sequences are present in the 3’-UTRs of the kinesin and the calcium-calmodulin protein kinase II (CaMKII) genes and miR-280 and miR-289 can interact with the 3’-UTRs of the CaMKII and other genes that are targets of RISC-mediated translational silencing (12).

Specific forms of synaptic plasticity also act in concert with FMRP-containing ribonucleoprotein complexes to fine-tune the kinetics of local protein synthesis (120, 127). Additional classes of ncRNAs including rRNAs and tRNAs are also present within discrete dendritic domains and contribute to the modulation of RNA translation and signal integration from multiple synaptic inputs (148, 153, 285). Local protein synthesis promotes retrograde messenger trafficking from the synapse to the cell body to activate mRNA synthesis and to concurrently “mark” activated synapses (19, 125). Thus distinct profiles of synaptic activation give rise to unique molecular signatures and contribute to the speed and efficacy of intracellular transport and utilization processes embedded within a multi-tiered rapid and reversible regulatory cascade including differential utilization of multiple ncRNA subclasses. BC1 is also intimately involved in neural development, somadendritic levels of BC1 are reversibly modulated by neuronal activity, and BC1 may cooperate with FMRP to regulate local dendritic translation initiation (32, 219, 314, 315).

RNA-mediated mechanisms may also be involved in both normal and pathological features of prion proteins. It is now well established that certain spongiform encephalopathies are orchestrated by transmissible proteinaceous agents termed prions (PrP\textsuperscript{Sc}) that undergo self-propagation by promoting conformational changes in the normal cellular (PrP) species (51). Recent studies indicate that only single-stranded RNA, but not RNA/DNA homo- or heterodimers, has the potential to stimulate PrP\textsuperscript{Sc} elaboration and propagation in isolated brain preparations (71). These observations suggest that host-encoded RNA species may be involved in the etiology of a spectrum of diverse prion diseases as well as novel types of normal neurological functions. Interestingly, a neuronal member of the cytoplasmic polyadenylation element binding protein (CPEB) family that regulates mRNA translation also...
exhibits prionlike properties (270). In contrast to other CPEB family members, *Aplysia* neuronal CPEB possesses an NH$_2$-terminal extension that shares molecular and biophysical properties with prion species (65). Moreover, full-length CPEB undergoes epigenetically driven changes in conformational properties during conversion to the active state that are self-perpetuating and display the greatest capacity to promote CPEB-mediated local mRNA translation. A brain-specific form of CREB in mice (mCREB-3) differs from other mammalian CREB isoforms in that it contains an additional 2,500 bp of 3'-UTR and a smaller NH$_2$ terminus with a Q-rich domain postulated to be capable of forming prionlike switches (65).

In *Aplysia*, neurotransmitter-mediated enhancement of CPEB levels promotes long-term synapse-selective modifications through the independent properties of spatial restriction (synapse specificity) as well as persistence (duration) (269, 270). These observations suggest that selective RNA-associated prionlike properties of CPEB may facilitate the activation of dormant mRNA initially elaborated within the cell soma and distributed globally but selectively translated locally within activated synapses (16). These epigenetically mediated plasticity mechanisms may underlie the long-term changes in synaptic strength associated with memory storage as well as other dynamic neural state transitions such as cellular differentiation and even transcription.

**IX. RNA-MEDIATED MECHANISMS UNDERLYING SPECIFIC NEUROLOGICAL DISEASES**

Neurodevelopmental and neurodegenerative diseases associated with trinucleotide repeat expansion may be caused by complex combinations of abnormal RNA-mediated transcriptional silencing, trans-dominant RNA-mediated pathogenic mechanisms, and ncRNA-mediated RNA interference (reviewed in Refs. 90, 92). Fragile-X syndrome results from dramatically expanded (>200) CCG repeats in the 5'-UTR of the *Fmr1* gene and a loss of function mechanism with complete transcriptional silencing (108). In contrast, smaller (60–200) trinucleotide repeat expansions at the *FMRP* gene locus are called “premutations” but have recently been linked to a complex neurodegenerative disease called fragile-X tremor/ataxia syndrome (FXTAS) associated with tremor, cerebellar ataxia, Parkinson’s disease, dementia, cognitive deterioration, peripheral neuropathy, proximal muscle weakness, autonomic system dysfunction, and multisystem atrophy (289). In FXTAS there is a pathological increase in *Fmr1* transcripts and reduced translational efficiency with sequestration and misfolding of cellular proteins, suggesting an RNA-mediated toxic gain-of-function mechanism (284, 303).

Interestingly, the *FMRP* gene premutation is also associated with a discrete developmental disorder characterized by mental retardation, autism, and learning disorders, further expanding our view of the complexity of RNA-mediated nervous system dysfunction and the role of critical developmental periods in mediating pathogenesis (289). The premutation neurodegenerative disease variant exhibits striking gene dosage- and repeat length-dependent clinicopathological effects (128). Furthermore, an rCGG-repeat binding protein (rCGGBP) has recently been identified, and sequestration of specific rCGGBPs has been linked to pathological neuronal death (90). A related syndrome, fragile-X syndrome E, is caused by CCG repeats in the 5'-UTR of the *FMRP2* gene with associated hypermethylation of CpG islands and transcriptional silencing (107, 155, 217). *FMRP2* plays important roles in nervous system development, neural cell differentiation, sensory perception, synaptic plasticity, and memory formation (106).

Myotonic dystrophy (MD) is predominantly a muscle and multisystem disorder but exists in two forms with different profiles of nervous system dysfunction, including DM1 with mental retardation, executive dysfunction, and visuospatial and memory deficits and DM2 with predominant frontal lobe working memory and executive dysfunction (62). DM1 exhibits CTG expansion within the 3'-UTR of the dystrophia myotonica protein kinase (DMPK), whereas DM2 is coupled to CCTG expansion in intron 1 of the zinc finger protein ZNF9 (30, 87, 184, 193, 244). Mutant RNAs in DM1 and DM2 contribute to disease pathogenesis through different degrees and types of repeat length expansion and by differential interactions with several RNA-binding proteins of the muscleblind-like (MBNL) family (126, 231). These abnormal interactions result in selective nuclear sequestration of MBNL proteins and interference with their roles as modulators of alternate splicing. Several deregulated neuronal transcripts with splicing defects have been identified in DM1, including tau, amyloid precursor protein, and the N-methyl-D-aspartate (NMDA) NR1 receptor, and these abnormal molecular profiles may contribute to disease manifestations and to the complex phenotypes seen in MD (126). These overall findings suggest the effects of a trans-dominant RNA-mediated pathogenic mechanism.

RNA-mediated motor neuron degeneration in low-molecular-weight neurofilament (NF-L) mutant mice is mediated by a major RNA instability determinant in NF-L (34, 225). p190 RhoGEF is a neuron-enriched guanine exchange factor that binds to the NF-L destabilizing element, the 3'-UTR of NF-L mRNA, and may thus link neurofilament expression to pathways modulating neuronal homeostasis (34). *BC1* and the low-molecular-weight form of NF-L mRNA exhibit mutually exclusive binding to p190 RhoGEF, suggesting a potential role for *BC1* in the etiology of motor neuron disease (93). Interestingly, neu-
ropathological examination in the setting of superoxide dismutase 1 (SOD1) mutations has revealed abnormal protein aggregates containing mutant SOD1, NF-L, and p190RhoGEF (181, 182). Motor neuron disease-associated mutations in SOD1 result in differential splicing of peripherin pre-mRNA and the generation of neurotoxic forms of peripherin (248). Frontotemporal dementia with parkinsonism associated with chromosome 17 (FTDP-17) is also characterized by alternate splicing of the microtubule-associated protein tau (MAPT) gene and altered spatiotemporal expression of specific differentially spliced MAPT exon 10-associated isoforms, suggesting a potential role in pathogenesis of abnormally activated RNA binding proteins perhaps in the context of alterations of specific ncRNAs (14, 63, 90). A different form of FTD-17 has recently been linked to a series of diverse mutations in the progranulin gene including alternate splicing, intron retention, and production of mutant mRNA transcripts that predispose to nuclear retention and RNA degradation or nonsense mediated RNA decay (17, 60). Progranulin is involved in gender-specific hypothalamic development and in numerous other nervous system developmental events, and overexpression is associated with various types of high-grade and invasive malignancies including glioblastoma multiforme (60, 64, 111). Interestingly, BC200 RNA is dramatically downregulated in post mortem brains of Alzheimer’s disease patients (189). These latter findings implicate diverse RNA-associated mechanisms in the pathogenesis of neurodegenerative diseases.

Spinocerebellar ataxias (SCAs) may also result from distinct RNA-mediated pathological processes. SCA8 is caused by CTG expansion of the 3’-UTR of an untranslated antisense RNA with significant overlap with the Kelch-like 1 (KLHL1) gene (159, 224). The gene mutation may cause the clinicopathological manifestations of this neurodegenerative disease by misregulation of the KLHL1 actin-binding protein or by the toxic consequences of the expanded RNA in ways similar to DM (92, 221). Interest-

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**Fig. 2.** The complexity of RNA transactions in the nervous system. White and pale blue boxes indicate constitutively spliced exonic non-protein-coding and protein-coding sequences, respectively. Pink and dark blue boxes indicate alternatively spliced exonic non-protein-coding and protein-coding sequences, respectively. Green octagons indicate snoRNAs, and purple diamonds indicate miRNAs. Solid lines indicate known interactions and pathways. Dotted lines indicate potential interactions and pathways.
ingly, using SCA8 as a sensitized background in a modifier screen assay revealed the identity of four novel ncRNAs with predominant neuronal expression (221). SCA10 results from an unstable ATTCT repeat expansion within the 3'-end of a large intron of a gene of presently undefined function that may promote transcriptional silencing or a different RNA-associated toxic process (198). SCA12 follows from CAG expansion in the noncoding 5'-promoter and/or 5'-UTR of the PPP2R2B gene, encoding a brain-selective regulatory subunit of protein phosphatase 2A (116). Therefore, disease pathogenesis may be mediated by distinct trans-dominant RNA or alternate toxic gain-of-function mechanisms determined, in large measure, depending on the precise location of the expanded trinucleotide repeat (90, 92, 115).

These observations suggest that different classes of neurological diseases may be caused by distinct RNA-mediated mechanisms including those that encompass alternate splicing, RNA interference, transcriptional silencing, and translational suppression and trans-dominant abnormal RNA interactions with RNA binding proteins that promote aberrant conformational changes and protein sequestration, altered molecular functioning, impaired species interactions, and deregulated clearance.

X. CONCLUSION

The list of verified targets of RNA editing and of the number and functional subclasses of noncoding RNAs involved in the nervous system has steadily increased and likely still represents only a small fraction of the total transcriptome devoted to RNA-mediated mechanisms underlying the ontogeny and functional complexity of mammalian brain functions in health and disease. We hope that this review raises awareness of the central roles that RNA editing, RNA modification, and the enormous numbers of both small and large noncoding RNAs, and the complex regulatory networks in which they participate, play in neural network development, steady-state function, plasticity and metaplasticity, dysfunction, degeneration, and regeneration, as well as stimulate the design of new experiments and the search for ncRNA candidates in genetic studies. It has become increasingly apparent that the vast majority of the human genome is devoted to RNA-mediated regulatory circuitry (199, 203) and that the traditional view that most genes and genetic information are expressed as proteins, which is largely true in the prokaryotes but not in the complex eukaryotes, has led to a fundamental misunderstanding of the nature of the genetic programming of human differentiation and development. We suggest that this is especially pertinent in the human nervous system, where the evolutionary requirements of brain form and function, neural network integrity, and bioenergetic conservation mandate that RNA transactions be most versatile, multilayered, and nuanced (Fig. 2). We also suggest that brain development, brain plasticity, and the formation of the information networks underlying learning, memory, and complex cognitive functions are intimately connected to environmentally driven recoding of the transcriptome and possibly even the genome itself in response to experience.

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