

Emerging roles of non-coding RNAs in brain evolution, development, plasticity and disease

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Abstract | Novel classes of small and long non-coding RNAs (ncRNAs) are being characterized at a rapid pace, driven by recent paradigm shifts in our understanding of genomic architecture, regulation and transcriptional output, as well as by innovations in sequencing technologies and computational and systems biology. These ncRNAs can interact with DNA, RNA and protein molecules; engage in diverse structural, functional and regulatory activities; and have roles in nuclear organization and transcriptional, post-transcriptional and epigenetic processes. This expanding inventory of ncRNAs is implicated in mediating a broad spectrum of processes including brain evolution, development, synaptic plasticity and disease pathogenesis.

Non-coding RNAs

(ncRNAs). RNA molecules belonging to an increasing number of different classes that function explicitly as RNAs, rather than as proteins, in a wide variety of regulatory, structural and functional processes.

Our understanding of the architecture, activity and regulation of the eukaryotic genome has been revolutionized by the recent advent of advanced sequencing technologies and findings from large-scale consortia focused on characterizing functional genomic elements, such as ENCODE¹ and FANTOM^{2,3}. We now recognize the extraordinary complexity and flexibility of the genome. It encodes not only protein-coding genes with multiple transcription start sites, alternative promoter and enhancer elements, splicing initiation and donor sites, as well as variable 3'-untranslated regions (UTRs), but also unexpectedly large numbers of non-coding RNAs (ncRNAs), which have myriad regulatory and other functions and similarly serve as substrates for transcriptional and post-transcriptional diversification (BOX 1). Moreover, high-resolution transcriptomic studies have revealed, surprisingly, that the vast majority of the genome is transcribed in both sense and antisense orientations and expressed in a highly cell type-, subcellular compartment-, developmental stage- and environmental stimulus-specific manner. Each nucleotide can participate in context-dependent transcription that is mediated by specific RNA polymerases that are responsible for giving rise to multiple interlaced and overlapping transcripts⁴. These distinct RNAs can be coordinately or independently regulated, and they can act autonomously or be functionally interrelated, with one RNA modulating the expression and activity of other transcripts derived from the same

genomic locus. It is also clear that the strict dichotomy between protein-coding and non-coding transcripts is false. Some ncRNAs contain open reading frames and can be translated and, in addition to encoding proteins, bifunctional RNA transcripts (such as the steroid receptor RNA activator and tumour protein 53 (TP53) mRNA) participate in meaningful cellular regulatory and functional processes, rather than serving simply as *passé* intermediates for translation^{5–7}.

This renaissance in RNA biology is of prime importance for the CNS because neural cells are highly transcriptionally active, exhibiting robust expression of ncRNAs^{8,9}, and also because ncRNAs have played a part in the evolution of human brain form and function. Indeed, the fastest evolving regions of the primate genome are non-coding sequences that can give rise to ncRNAs that are primarily implicated in modulating neural development genes¹⁰. In addition, because of their potential roles in regulating individual genes, as well as large gene networks (see below), ncRNAs confer neural cells with the capacity to exert very precise control over the spatiotemporal deployment of genes, which is crucial for executing complex neurobiological processes. For example, microRNAs (miRNAs), such as miR-124 and miR-9/9*, are highly integrated into the cAMP responsive element-binding protein (CREB), repressor element 1 (RE1)-silencing transcription factor (REST) and REST corepressor 1 (CoREST) transcriptional networks that mediate neural cell fate decisions^{11,12}.

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Box 1 | Life cycle of non-coding RNAs

Non-coding RNAs (ncRNAs) are subject to diverse transcriptional and epigenetic regulatory mechanisms, biogenesis and maturational pathways, post-transcriptional processing, conformational changes and intra- and inter-cellular trafficking, imbuing the non-coding transcriptome with extraordinary functional diversity and environmental responsiveness. ncRNA expression is controlled by transcriptional and epigenetic factors, including those that coordinately regulate protein-coding genes (repressor element 1-silencing transcription factor (*REST*), cAMP response element-binding (*CREB*), tumour protein 53 (*TP53*) and zinc finger protein 143 (*ZNF143*))^{11,20,138,139}. Specific RNA polymerase enzymes transcribe ncRNAs⁴. Various classes of ncRNAs can also target each other for post-transcriptional regulation^{31,140}. Furthermore, a range of post-transcriptional processes (such as alternative splicing, polyadenylation, 5' capping, non-templated modifications and RNA editing) can modify ncRNAs^{2,24,141}. The roles of these modifications are mostly uncharacterized. Nevertheless, they are likely to have important functional consequences, particularly in the nervous system. For example, adenosine-to-inosine RNA editing targets ncRNA transcripts, including microRNAs (miRNAs), leading to alterations in miRNA–target mRNA interactions, and targets ncRNAs derived from retrotransposons (*Alu* and LINE-1 (L1) sequences), which are the principal substrates for primate RNA editing¹⁴¹. Intriguingly, these sequences have undergone substantial evolutionary expansion in primates; the highest levels of RNA editing are present in human brain¹⁴², suggesting that RNA editing of retrotransposons mediated human brain evolution, has seminal roles in brain functioning and might even promote encoding of salient environmental information back into the neuronal genome. Moreover, ncRNAs can undergo nuclear–cytoplasmic, nuclear–mitochondrial and axodendritic trafficking via ribonucleoprotein complexes, which promote the coordinated spatial and temporal distribution and functioning of particular combinations of ncRNAs, mRNAs and RNA-binding proteins¹⁴³. Additionally, ncRNAs can be involved in intercellular communication through transport to adjacent nerve cells, more distant somatic sites and the germline via exosomes released by multiple cell types and via other transport processes^{133,134}. These observations imply that understanding ncRNAs and their roles in the nervous system will require interrogating, in greater detail, the life cycles of these molecules and the mechanisms responsible for coordinating these dynamic processes.

The evolving non-coding RNA landscape

tRNAs and ribosomal RNAs (rRNAs) are examples of well-known classes of ncRNAs. However, modern studies have demonstrated that these represent only the 'tip of the iceberg'. The existence of many additional classes of ncRNAs has more recently been recognized (FIGS 1, 2; TABLE 1). Most laboratory techniques for isolating RNA are based on size fractionation, which has led to the designation of recently identified classes of ncRNAs as long or small. There are also alternative approaches that can distinguish biologically relevant ncRNAs, such as capturing molecules associated with RNA-binding proteins (RBPs)¹³. Algorithms using sequence conservation, epigenetic marks, structural and other features can also predict ncRNAs^{14,15}. Thus, it is likely that many novel classes remain to be discovered, including those expressed at low levels and in a highly context-specific manner¹⁶. The following sections discuss the major classes of ncRNAs, including the most recently identified, abundant and versatile class, long ncRNAs (lncRNAs).

Long non-coding RNAs. lncRNAs are transcripts of at least 200 nucleotides in length, although they can be orders of magnitude longer. lncRNAs are transcribed from intergenic regions (large intergenic ncRNAs (lin-cRNAs))^{17,18}; in antisense, overlapping, intronic and bidirectional orientations relative to protein-coding genes; from gene regulatory regions (UTRs¹⁹, promoters²⁰ and

enhancers²¹); and from specific chromosomal regions (telomeres²²) (FIG. 1a). Intriguingly, lncRNAs are also derived from the mitochondrial genome²³ (FIG. 1b). lncRNAs are subject to post-transcriptional processing, potentially undergoing 5' capping, polyadenylation, alternative splicing, RNA editing and trafficking^{2,24}. Many lncRNAs exert regulatory effects on the genomic loci they are derived from or on neighbouring loci. For example, lincRNAs can have enhancer-like activity²¹. By contrast, other lncRNAs, such as HOX transcript antisense RNA (*HOTAIR*)²⁵ and X (inactive)-specific transcript (*XIST*)²⁶, promote the establishment of repressive chromatin environments across large genomic regions and even entire chromosomes, respectively. Indeed, lncRNAs have a broad range of functions, including roles in transcriptional and epigenetic mechanisms via the recruitment of transcription factors and chromatin-modifying complexes to specific nuclear and genomic sites; alternative splicing and other post-transcriptional RNA modifications through the assembly of nuclear domains containing RNA-processing factors; nuclear–cytoplasmic shuttling; and translational control²⁷. lncRNAs can also act as precursors for small ncRNAs, such as small nucleolar RNAs (snoRNAs) and miRNAs.

An elegant framework for categorizing the emerging functions of lncRNAs was recently proposed. It describes lncRNAs as: signals for integrating temporal, spatial, developmental and stimulus-specific cellular information; decoys with the ability to sequester a range of RNA and protein molecules, thereby inhibiting their functions; guides for genomic site-specific and more widespread recruitment of transcriptional and epigenetic regulatory factors; and scaffolds for macromolecular assemblies with varied functions²⁷.

Small non-coding RNAs with roles in RNAi pathways.

The most important small ncRNAs are those involved in post-transcriptional regulation of target RNAs via RNAi. These include miRNAs, endogenous small interfering RNAs (endo-siRNAs) and PIWI-interacting RNAs (piRNAs) (FIG. 2a,b). Mature miRNAs are 20–23 nucleotide single-stranded RNAs (ssRNAs), canonically derived from longer primary transcripts (pri-miRNA)^{28,29}. pri-miRNAs are processed by the DROSHA (also known as ribonuclease 3)-containing microprocessor complex, exported into the cytoplasm by exportin 5, and cleaved by the DICER1 ribonuclease. Mature miRNAs are loaded onto Argonaute proteins and integrated into diverse multi-component RNA-induced silencing complexes (RISCs). miRNAs bind to imperfectly complementary sequences, predominantly in 3'-UTRs of target mRNAs, leading to repression or degradation of these transcripts. Not only can an individual miRNA regulate hundreds of different mRNAs but multiple miRNAs can also target a single mRNA.

This model for understanding miRNA processing and mechanism of action is still evolving. In fact, DROSHA- and DICER1-independent biogenesis pathways have been described. miRNA precursors are subject to post-transcriptional modification via RNA editing, which can affect their maturation and target gene

Open reading frames

DNA sequences that are present between start, or initiation, codons and stop, or termination, codons. It is implied that these sequences are translated into proteins.

Chromatin

The structure of the genome in the nucleus formed by DNA, histones, non-histone proteins and associated factors that can undergo dynamic changes locally and more globally into open and closed conformations, which promote the execution of specific cellular programmes, such as transcription and DNA replication and repair.

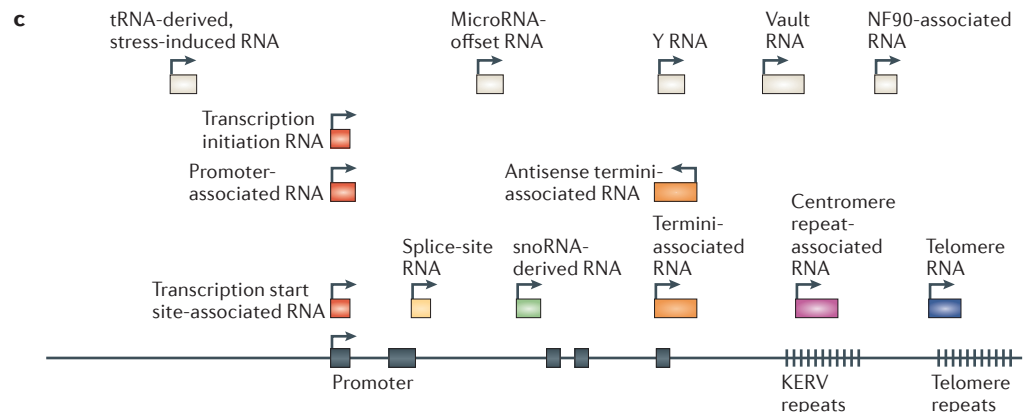
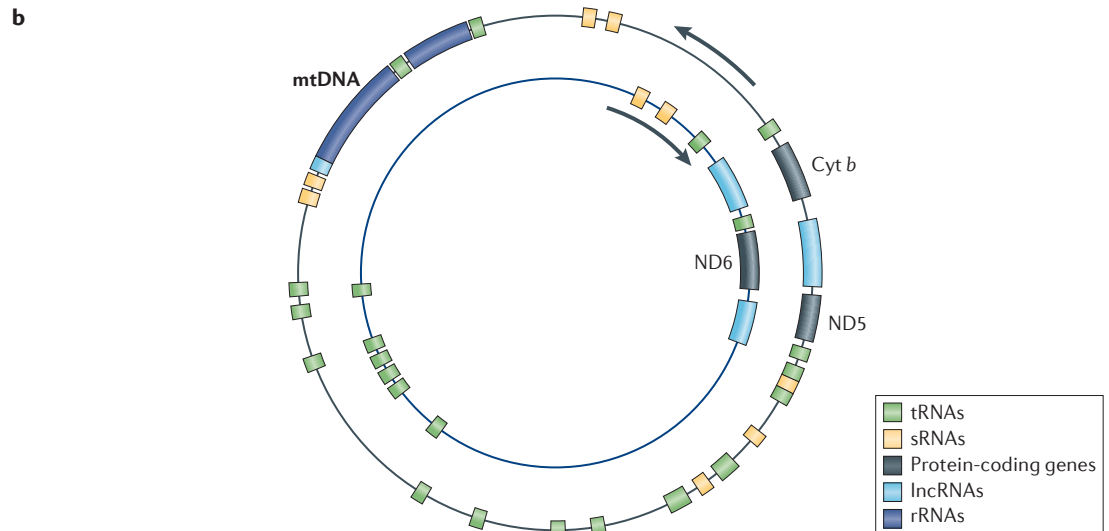
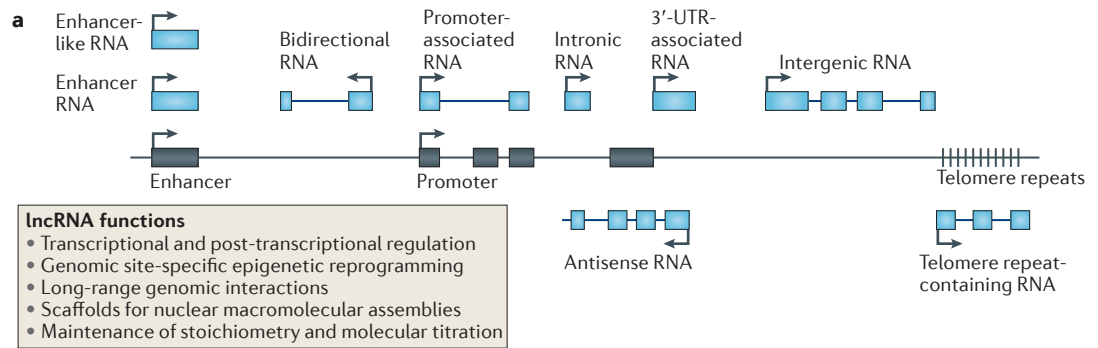


Figure 1 | Emerging classes of non-coding RNAs. **a** | Long non-coding RNAs (lncRNAs; shown in blue) mediate a broad array of genomic and cellular functions and are independently transcribed from: intergenic regions; in antisense, overlapping, intronic and bidirectional orientations to protein-coding genes (black); from gene-regulatory regions, including gene promoters, enhancers and untranslated regions (UTRs); and from specific chromosomal regions, including telomeres (arrows indicate direction of transcription). **b** | The mitochondrial genome contains small ncRNAs (such as ribosomal RNA (rRNA), tRNA and others) and lncRNAs transcribed from both heavy (outer) and light (inner) strands. Cytochrome b (Cyt b) and NADH dehydrogenase 5 (ND5) and ND6 are mitochondrial protein-coding genes that are associated with mitochondrial lncRNAs transcribed from the complementary mitochondrial DNA (mtDNA) strand. **c** | Small ncRNAs mediate RNAi and act as guides for RNA modifications (see FIG. 2), and additional small ncRNAs are derived from protein-coding gene (black) regulatory regions and gene boundaries, including 5' regulatory regions (red), gene termini (orange), intron–exon junctions (yellow) and introns (green). They are also derived from structural components of chromosomes, including centromeres (purple) and telomeres (blue), and can be the cleavage products of other ncRNAs or originate from other sources (white). KERV, kangaroo endogenous retrovirus; NF90, nuclear factor 90; snoRNA, small nucleolar RNA; sRNAs, small RNAs.

Epigenetic mechanisms

Multilayered cellular processes that modulate gene expression and function in response to interoceptive and environmental stimuli during development, adult life and ageing, including DNA methylation, post-translational histone modifications, ATP-dependent nucleosome and higher-order chromatin remodelling, non-coding RNA deployment and nuclear reorganization.

Nuclear domains

A range of dynamically forming macromolecular nuclear assemblies found prominently in neural cells and consisting of chromatin and diverse factors involved in transcriptional and epigenetic regulation and post-transcriptional RNA processing, such as transcription factories, Cajal bodies, promyelocytic leukaemia nuclear bodies, nuclear stress bodies, nuclear speckles and paraspeckles.

Argonaute proteins

Proteins of the AGO and PIWI subfamilies that interact with microRNAs and endogenous small interfering RNAs and with PIWI-interacting RNAs, respectively, and have key roles in mediating post-transcriptional RNA silencing.

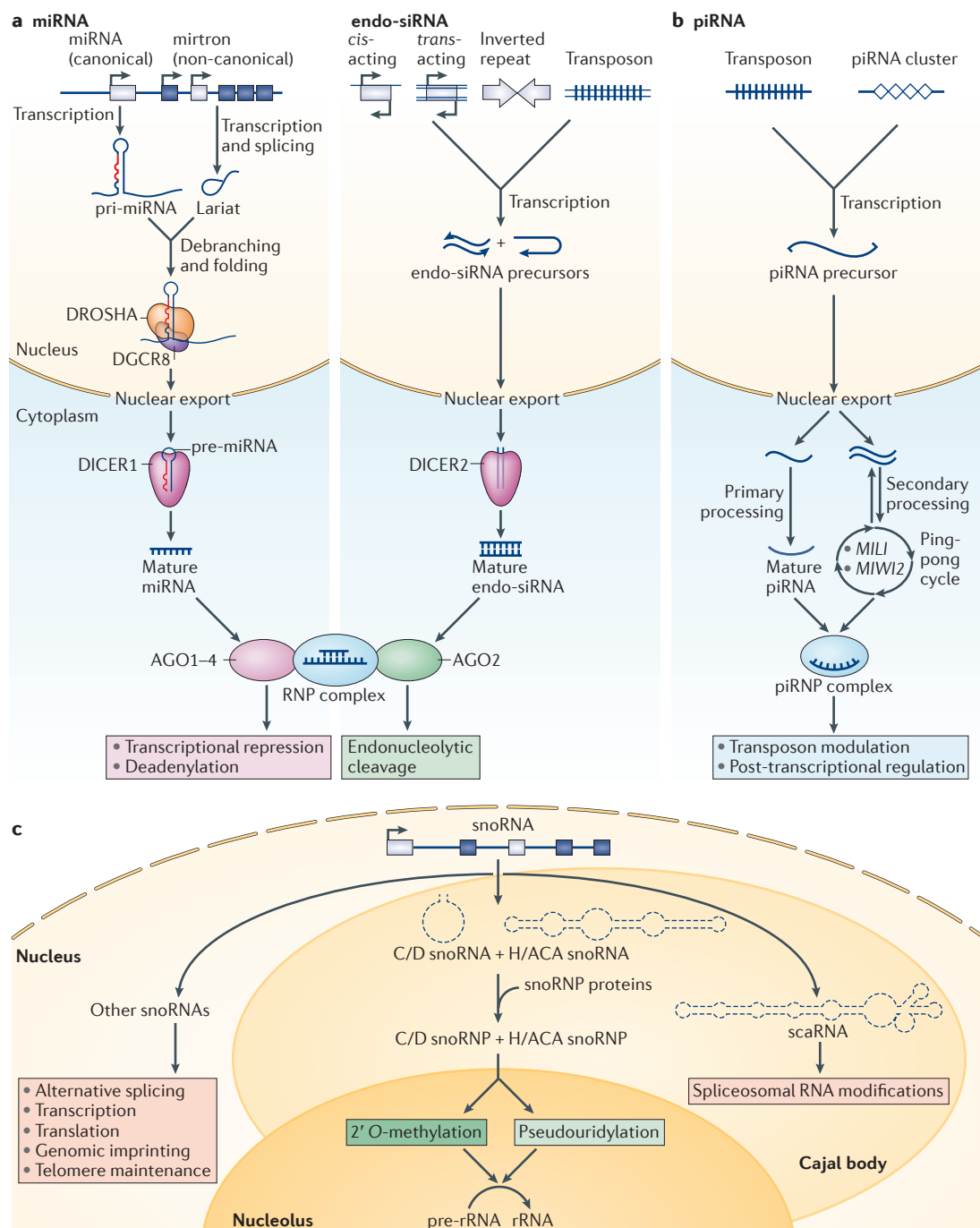


Figure 2 | Non-coding RNAs mediating RNAi and RNA modifications. Biogenesis and functions of non-coding RNAs mediating RNAi are shown. **a** | Left panel: microRNAs (miRNAs) are transcribed as primary miRNA (pri-miRNA; canonical pathway) or from introns (mirtrons; non-canonical pathway). After pri-miRNA and mirtron processing by the DROSHA–DiGeorge syndrome critical region 8 (DGCR8) complex or lariat debranching or folding enzymes, respectively, and nuclear export, precursor miRNAs (pre-miRNAs) are processed by DICER1 and undergo ribonucleoprotein (RNP) assembly with Argonaute proteins 1–4 (AGO1–4). Right panel: endogenous small interfering RNAs (endo-siRNAs) are generated from cis- or trans-acting sense–antisense pairs, inverted repeats and transposons. endo-siRNAs undergo nuclear processing and export, cytoplasmic processing by DICER2, and RNP assembly with AGO2. miRNAs promote deadenylation and translational repression and endo-siRNAs promote endonucleolytic cleavage, respectively. **b** | PIWI-interacting RNAs (piRNAs) are generated from transposons and piRNA clusters. piRNA precursors undergo nuclear processing and export, primary or cyclic secondary processing (by the PIWI proteins MILI and MIWI2) and piRNP complex assembly. piRNAs mediate transposon modulation and post-transcriptional regulation. **c** | Roles of small nucleolar RNAs (snoRNAs) are shown. snoRNAs are transcribed from introns, processed into C/D and H/ACA snoRNAs, undergo small nucleolar ribonucleoprotein (snoRNP) assembly in Cajal bodies and promote methylation and pseudouridylation of pre-ribosomal RNA (pre-rRNA) in the nucleolus. Small Cajal body-specific RNAs (scaRNAs) promote similar spliceosomal RNA modifications and other snoRNAs display additional functions.

Table 1 | Examples of the diversity and functionality of emerging classes of non-coding RNAs

Class	Description	Refs
Antisense lncRNAs	lncRNAs derived from antisense transcription, present at ~70% of mammalian genomic loci and implicated in the regulation of sense protein-coding genes	3
Antisense termini-associated short RNAs	Small ncRNAs with 5' poly-U tails originating from 3' termini of genes in antisense orientation, suggesting transcription by RNA-dependent RNA polymerase	45
Centromere repeat-associated small interacting RNAs	Small ncRNAs (34–42 nucleotides) derived from centromeric repeats with putative roles in local epigenetic modifications and heterochromatin formation	47
Endogenous small interfering RNAs	Small DICER-dependent ncRNAs (21–26 nucleotides) associated with Argonaute proteins (AGO2) and involved in post-transcriptional and epigenetic silencing of protein-coding genes and transposons	33
Enhancer RNAs	lncRNAs transcribed from enhancer domains of, and expressed coordinately with, activity-dependent neuronal genes	109
Enhancer-like long ncRNAs	lncRNAs exhibiting enhancer activity, particularly for genes regulating development and differentiation	21
Large intergenic RNAs	lncRNAs derived from intergenic regions that act as guides for recruiting PRC2, CoREST and other chromatin-modifying complexes	17,18
lncRNAs	Large family of ncRNAs (>200 nucleotides) with diverse functional roles	24
MicroRNA-offset RNAs	Small ncRNAs (~20 nucleotides) produced from microRNA precursors and exhibiting independent expression relative to associated microRNAs, implying distinct functional roles	46
MicroRNAs	Small DICER-dependent ncRNAs (20–23 nucleotides) associated with Argonaute proteins (AGO1–4) and involved in post-transcriptional silencing of protein-coding genes and ncRNAs	28,29
Mitochondrial ncRNAs	Small ncRNAs and lncRNAs generated from both strands of the mitochondrial genome, regulated by nuclear-encoded mitochondrial proteins and expressed in cell type- and tissue-specific patterns	23,51
PIWI-interacting RNAs	Small ncRNAs (26–30 nucleotides) associated with the PIWI subclass of Argonaute proteins and involved in silencing of mRNAs and transposons	34
Promoter-associated long RNAs	lncRNAs transcribed from promoter domains of protein-coding genes, particularly cell cycle modulators, and capable of recruiting regulatory factors	20,150
Promoter-associated small RNAs	Small ncRNAs (20–200 nucleotides) possessing 5' ends that coincide with the transcription start sites of protein-coding genes and ncRNAs	43
Small nucleolar RNAs	Small ncRNAs derived from intronic regions with roles in promoting RNA modifications, including pseudouridylation (H/ACA snoRNAs) and methylation (C/D snoRNAs), as well as pre-mRNA processing	36
Small RNAs derived from small nucleolar RNAs	Small ncRNAs derived from 3' ends of H/ACA snoRNAs (20–24 nucleotides) and 5' ends of C/D snoRNAs (17–19 nucleotides and >27 nucleotides) with microRNA-like functions	41
Small nuclear factor 90-associated RNAs	Small ncRNAs (117 nucleotides) associated with the nuclear factor 90 RBP and exhibiting accelerated evolution and expansion in hominids and region-specific expression in human brain	151
Splice-site RNAs	Small ncRNAs (17–18 nucleotides) derived from splice sites of highly transcribed genes, and expressed in developmental stage- and region-specific patterns	46
Telomere small RNAs	Small DICER-independent telomere-specific ncRNAs (~24 nucleotides)	48
Telomere repeat-containing RNAs	lncRNAs transcribed from telomeric repeats and dynamically regulated during the cell cycle with roles in heterochromatin formation and telomere functioning	22
Termini-associated short RNAs	Small ncRNAs originating from 3' termini of protein-coding genes and ncRNAs	24
Transcription initiation RNAs	Small ncRNAs (18 nucleotides) originating downstream of transcription start sites of protein-coding genes, implicated in modulating CTCF localization and nucleosome density	44,52
Transcription start site associated RNAs	Small ncRNAs (20–90 nucleotides) originating from the -250 to +50 position relative to the transcription start site of protein-coding genes	152
tRNA-derived RNA fragments	Small ncRNAs (19–40 nucleotides) derived from cleavage of mature tRNAs constitutively by DICER and in response to stress by angiogenin (in humans), promoting stress granule assembly and inhibiting mRNA translation	49
3'-untranslated region-associated ncRNAs	lncRNAs derived from 3'-untranslated regions of protein-coding transcripts and exhibiting independent developmental stage- and tissue-specific expression profiles	19
Vault RNAs	Small ncRNAs (88–100 nucleotides) integral to the vault RNP complex with putative roles in multidrug resistance, apoptosis resistance and innate immunity	153,154
Y RNAs	Small ncRNAs (~100 nucleotides) required for DNA replication, cleaved into microRNAs and implicated in Ro RBP localization and function	155,156

CoREST, repressor element 1-silencing transcription factor corepressor 1; CTCF, CCCTC-binding factor; lncRNA, long non-coding RNA; ncRNA, non-coding RNA; PRC2, Polycomb repressive complex 2; RBP, RNA-binding protein.

interactions³⁰. miRNAs can also target and repress other ncRNAs. For example, miR-671 silences the expression of an antisense lncRNA transcribed from the cerebellar degeneration-related protein 1 locus³¹. Moreover, high-resolution RNA-sequencing analyses, including those carried out using human brain tissues, have revealed most miRNAs have length and sequence heterogeneity³². These miRNA variants (isomiRs) are present in developmental stage- and tissue-specific profiles and selectively associate with particular Argonaute RBPs, implying nuanced and context-dependent functional roles.

endo-siRNAs are 21–26 nucleotide double-stranded RNAs (dsRNAs) that are cleaved from longer dsRNA intermediates in a DICER2-dependent manner and incorporated into RISC, in which they function as single-stranded entities³³. endo-siRNAs are derived from various genomic locations and are implicated in gene regulation and genome defence via silencing of mRNAs and transposon-derived ncRNAs, respectively, as well as other emerging functions. In contrast to miRNAs, endo-siRNAs act on RNA molecules containing perfectly complementary sequences. piRNAs are 26–30 nucleotide ssRNAs that are DICER-independent and generated via biogenesis pathways, including a series of amplification steps, termed the ‘ping-pong’ cycle³⁴. piRNAs derived from transposon elements are implicated in regulating transposon activity, whereas piRNAs derived from piRNA clusters are implicated in modulating gene expression. Although piRNAs were initially found in germ cells, recent studies have established that piRNAs are expressed in somatic cells, including neurons³⁵.

Small non-coding RNAs as guides for modifying other RNAs. snoRNAs guide RNA-modifying enzyme complexes to other RNA molecules (such as rRNAs) in the nucleolus^{36,37} (FIG. 2c). These functions are mediated by the formation of duplexes between snoRNAs and RNAs with complementary sequences. The H/ACA and C/D box snoRNAs promote pseudouridylation and methylation, respectively. Additionally, snoRNAs have other functions. For example, snoRNAs from the brain-specific snoRNA C/D box 115 cluster promote alternative splicing through interactions with non-canonical protein partners^{38,39}. Small Cajal body RNAs are variants of snoRNAs that mediate alternative splicing in nuclear Cajal bodies⁴⁰. Interestingly, the majority of snoRNA loci can also give rise to miRNA-like small RNAs, suggesting the existence of complex crosstalk between snoRNA-mediated RNA processing and RNAi pathways^{41,42}.

Additional classes of small non-coding RNAs. Additional classes of poorly characterized small ncRNAs (FIG. 1b,c) include those derived from gene-regulatory regions and gene boundaries (subclasses of promoter-associated small RNAs⁴³, such as transcription initiation RNAs (tiRNAs)⁴⁴, termini-associated short RNAs, antisense termini-associated short RNAs⁴⁵ and splice-site RNA (spliRNA)⁴⁶); structural components of chromosomes (centromere-associated RNAs and telomere small RNAs^{47,48}); cleavage of other ncRNAs (tRNA fragments⁴⁹); and other sources (mitochondrial ncRNAs^{50,51} and miRNA-offset RNAs⁴⁶).

Functional studies have begun to interrogate these factors. For example, deleting tiRNAs that are associated with binding sites for RNA polymerase II and CCCTC-binding factor (CTCF; also known as transcriptional repressor CTCF) alters CTCF binding and nucleosome density at genomic loci proximal to sites of tiRNA biogenesis⁵². In addition, the biological processes associated with ncRNAs may be inferred by studying their genomic contexts. For example, spliRNAs, which are associated with splice sites of alternatively spliced genes, may have a role in modulating developmental stage- and tissue-specific alternative splicing. Ongoing studies are focused on understanding the evolution, biogenesis and functions of these diverse classes of ncRNAs and their roles in the CNS and other organ systems.

Evolutionary aspects of brain organization

Increasingly large proportions of non-coding sequences constitute the genomes of organisms with correspondingly greater degrees of developmental complexity⁵³. It has further been suggested that an expansion in the inventory of ncRNAs — particularly miRNAs, which have been continuously acquired throughout vertebrate evolution and remain fixed within most lineages — was responsible for the emergence of vertebrate complexity⁵⁴.

Comparative genomic and transcriptomic analyses suggest that ncRNAs are partly responsible for evolutionary innovations in brain and the cognitive and behavioural repertoires of higher organisms. For example, non-coding sequences associated with human neural genes exhibit prominent signatures of positive selection and accelerated evolution¹⁰. The highly accelerated region 1A (*HAR1A*) lncRNA is derived from one of 563 genomic regions designated human accelerated regions, because they evolved rapidly since divergence from the great apes. *HAR1A* expression correlates with that of *reelin*, suggesting that it similarly coordinates the establishment of regional forebrain organization. By contrast, evolutionarily constrained genomic elements also give rise to ncRNAs. For example, *Evf2* (also known as DLX6 antisense RNA 1) is an lncRNA transcribed from an ultra-conserved enhancer that affects forebrain development by modulating expression of the distal-less homeobox 5/6 transcription factors⁵⁵. Loss of function of *Evf2* in mice leads to a transient decrease in the population of GABAergic interneurons within the early postnatal hippocampus and dentate gyrus. Although the numbers of these interneurons later normalize, defects in synaptic inhibition are present throughout life, highlighting the role of *Evf2* in neuronal functioning. Analysis of other highly conserved lncRNA transcripts in birds, marsupials and eutherian mammals reveals remarkable similarities in the spatiotemporal expression profiles of orthologous lncRNAs and indicates ancient roles during brain development⁵⁶.

Recent studies also implicate miRNAs in mediating neural gene expression changes during evolution. Indeed, a range of miRNAs are conserved only in primates, exhibit lineage-specific expansion or are human-specific and are preferentially expressed in brain^{57–60}. One interesting study revealed that a substantial portion of intergenic

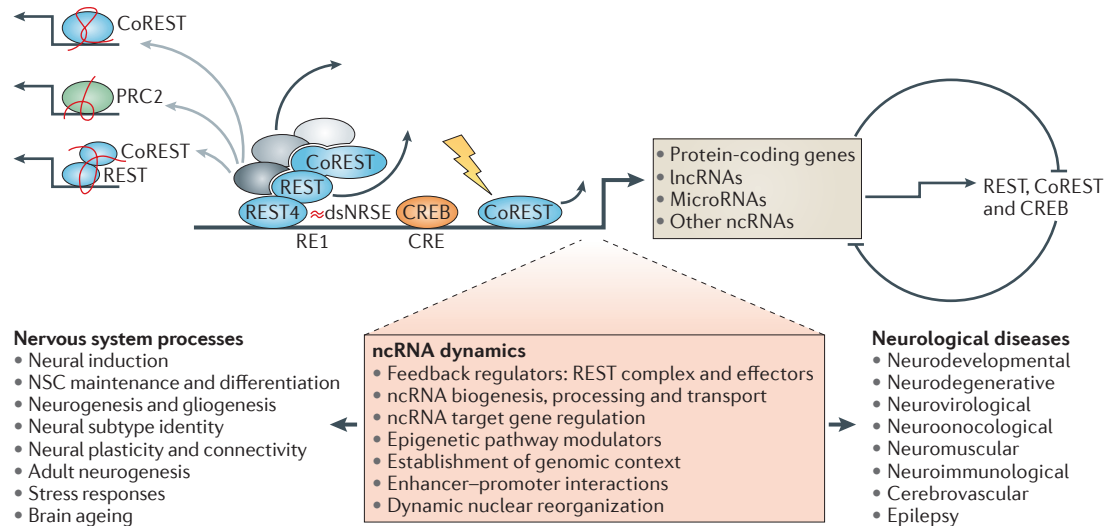


Figure 3 | Non-coding RNA dynamics mediate diverse nervous system processes and neurological disease states. Non-coding RNAs (ncRNAs) engage in a complex range of molecular and cellular functions (shown in the pink shaded box). They are also highly integrated, at multiple levels, into the mechanisms and circuitry that underlie neurobiological processes in health and disease. The expression of microRNAs, long ncRNAs (lncRNAs) and possibly other classes of ncRNAs, is similar to the expression of protein-coding genes: it is subject to developmental, constitutive and activity-dependent regulation (yellow lightning bolt) by key modulators of neural gene transcription (including repressor element 1 (RE1)-silencing transcription factor (REST), REST co-repressor 1 (CoREST)) and cAMP response element-binding (CREB)). In turn, ncRNAs influence the expression of these and other seminal neural factors via gene silencing, forming bidirectional regulatory relationships (curved lines, figure right), and by affecting their post-transcriptional RNA processing (alternative splicing (REST4)). ncRNAs can also influence the activity and deployment of neural factors. For example, the small modulatory ncRNA double-stranded neuron-restrictive silencer element (dsNRSE) (red waves) associates with and regulates the REST complex and its modular cofactors (grey ovals), and lncRNAs (red strings) promote the genome-wide deployment of chromatin-modifying complexes (including REST, PRC2 (Polycomb repressor complex 2) and CoREST–REST) (grey arrows). CRE, cAMP response element; NSC, neural stem cell.

transcripts conserved between human, chimpanzee and rhesus macaque brains are alternative or extended 3'-UTRs of known genes and represent putative sites for differential miRNA regulation⁵⁹. A corresponding analysis of the prefrontal cortex and cerebellum of adult humans, chimpanzees and rhesus macaques identified substantial and parallel degrees of divergence in the expression of miRNAs⁵⁷. This divergence in miRNA expression seems, in part, to explain differences in brain mRNA and protein expression levels between species. Indeed, a recent comparative genomic analysis that reported the existence of 220 candidate RNA structural families, 280,000 non-coding regions from transposable elements and >1,000 primate- and human-accelerated regions⁶¹ suggests that ncRNAs served as central effectors of brain evolution. Moreover, miRNAs that exhibit human-specific expression profiles preferentially target genes involved in neural functions. These range from neural stem cell (NSC) proliferation and differentiation to axon guidance and long-term potentiation (LTP) (discussed below).

Neural development, maintenance and plasticity

ncRNAs are expressed in precise regional, cellular, sub-cellular and temporal patterns in developing and adult brains, reflecting their widespread and diverse influence. Whereas most studies have focused on defining neurobiological roles for miRNA⁶², recent studies have

also begun to characterize the expression and function of lncRNAs^{63–65} and other classes of ncRNAs⁶⁶. lncRNAs that are found in developing and adult brains, including those found in specific cortical laminae, have expression profiles that correlate with factors involved in promoting brain development and organization and in transcriptional regulation of neural genes. For example, *SOX2OT* (sex-determining region Y-box 2 overlapping transcript) and *SOX2DOT* (sex-determining region Y-box 2 distal overlapping transcript) are lncRNAs transcribed from the same genomic locus as *SOX2* and are implicated in its regulation⁶⁷.

Other classes of ncRNAs are also likely to have important roles in shaping brain development. For example, one remarkable study recently identified thousands of retrotransposon reintegration sites for LINE-1 (L1), *Alu* and composite SVA (SINE, VNTR and *Alu*) elements in the human caudate and hippocampus⁶⁸. These findings are consistent with previous reports suggesting that retrotransposon activity is important for neural development and plasticity^{69–71}. In addition, they also indicate that retrotransposon-derived ncRNAs and ncRNAs that are responsible for regulating retrotransposition events (via endo-siRNAs and piRNAs) mediate somatic mosaicism in neurons and act as regulatory control systems for the establishment, maintenance and plasticity of the transcriptional architecture of the brain.

Retrotransposon

Mobile genetic element comprising a substantial proportion of the human genome transcribed into RNA intermediates and subsequently reintegrated into the genome, including LINE and SINE subclasses, such as *Alu* elements.

Somatic mosaicism

The presence of distinct genotypes in different somatic cells and tissues of an individual organism. Mosaicism arises from somatic mutations that can be generated by endogenous factors, such as mobile genetic elements and exogenous factors.

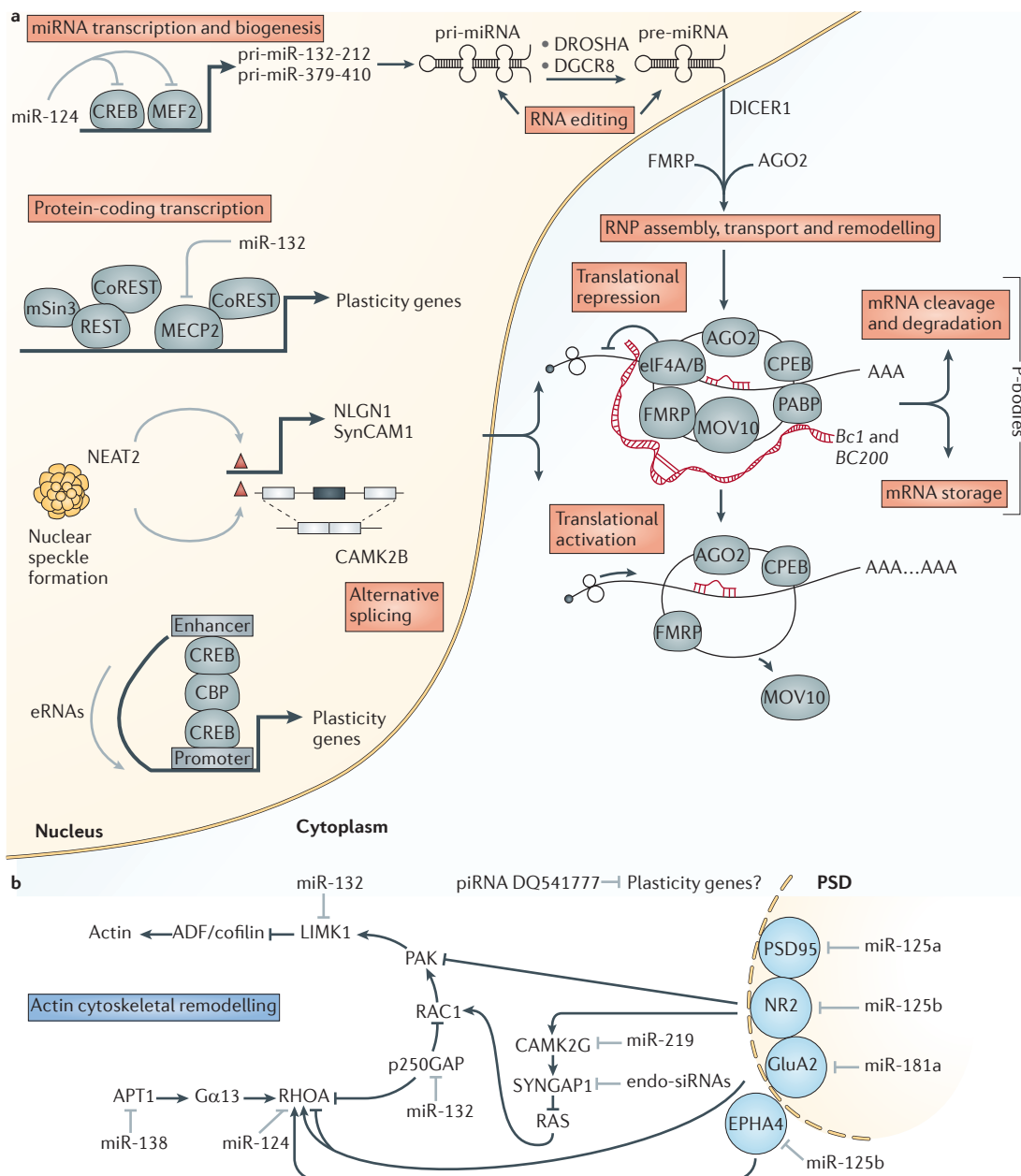


Figure 4 | Role of non-coding RNAs in synaptic plasticity. **a** | Diverse non-coding RNA (ncRNA) mechanisms are involved in activity-dependent processes underlying synaptic plasticity (shown in pink shaded boxes). MicroRNAs (miRNAs) contribute to synaptic plasticity through transcription of primary miRNA (pri-miRNA) clusters; processing of pri-miRNAs (by DROSHA and DiGeorge syndrome critical region 8 (DGCR8)) and pre-miRNAs (by DICER1); RNA editing; ribonucleoprotein (RNP) assembly, transport and remodelling; translational repression and activation; and associated mRNA storage, cleavage and degradation in P-bodies. miRNAs also modulate transcription of pri-miRNA clusters and protein-coding plasticity genes through regulation of activity-dependent transcription factors (including cAMP response element-binding (CREB) and myocyte enhancer factor 2 (MEF2)) and methyl-CpG binding proteins (including methyl-CpG-binding protein 2 (MECP2)). The long ncRNAs, brain cytoplasmic RNA 1 (*Bc1*) and its primate analogue *BC200* (red), promote translational repression by binding to RNP components (including eukaryotic translation initiation factor 4A/B (eIF4A/B), fragile X mental retardation 1 protein (FMRP) and polyadenylate binding protein (PABP)). Nuclear-enriched abundant transcript 2 (NEAT2) forms nuclear speckles, which are nuclear domains that regulate transcription of plasticity genes (such as, neuroligin 1 (*NLGN1*) and synaptic cell adhesion molecule 1 (*SynCAM1*)) and alternative splicing of plasticity genes (such as calcium/calmodulin-dependent protein kinase type II- β (*CAMK2B*)). Activity-dependent enhancer RNAs (eRNAs) promote transcription of plasticity genes through enhancer–promoter interactions. **b** | Within the spine head and shaft, modulation of local protein synthesis of neurotransmitter receptors, scaffolding proteins, calcium-dependent signalling molecules and actin cytoskeletal remodelling factors by miRNAs, endogenous small interfering RNAs (endo-siRNAs) and PIWI-interacting RNAs (piRNAs) contributes to synaptic plasticity by regulating spine morphology and synaptic transmission. ADF, actin-depolymerizing factor (also known as gelsolin); AGO2, Argonaute 2; APT1, acyl-protein thioesterase 1; CBP, CREB-binding protein; CoREST, REST co-repressor 1; CPEB, cytoplasmic polyadenylation element-binding protein; EPHA4, Eph receptor A4; GluA2, AMPA-type glutamate receptor subunit 2 (also known as GluR2); Gα13, guanine nucleotide-binding protein- α 13; LIMK1, LIM domain kinase 1; MOV10, Moloney leukaemia virus 10 (also known as putative helicase MOV-10); mSin3, paired amphipathic helix family proteins; NR2, glutamate receptor, ionotropic, *N*-methyl d-aspartate 2; PAK, p21 protein (CDC42/RAC)-activated kinase; PSD, postsynaptic density; RAC1, Ras-related C3 botulinum toxin substrate 1; REST, repressor element 1-silencing transcription factor; RHOA, RAS homologue family member A; RISC, RNA-induced silencing complex; SYNGAP1, synaptic RAS GTPase-activating protein 1.

Neural stem cell maintenance and differentiation. ncRNAs have important roles in regulating stem cell maintenance and differentiation programmes, including neural lineage restriction, cell fate specification and terminal differentiation. Numerous studies have focused on defining how miRNA factors are incorporated into the complex transcriptional and epigenetic circuitry regulating neural cell fate decisions⁶². A key experimental strategy involves targeting the miRNA biogenesis factor DICER1. *Dicer1*-knockout animals are associated with a range of neural developmental defects, including abnormalities in brain size and cytoarchitecture and extensive apoptosis^{72–76}. Complementary approaches have elucidated the seminal neurobiological roles of individual miRNAs (let-7, miR-9, miR-10, miR-17, miR-34, miR-124, miR-125, miR-132, miR-324-5p and miR-326)⁶². For example, in zebrafish, miR-9 modulates midbrain–hindbrain boundary (MHB) patterning during development, the balance between maintenance and differentiation of neural stem and progenitor cells, and their migration⁷⁷. miR-9 promotes the progression of neurogenesis in the MHB progenitor pool by targeting the antineurogenic genes (*her5* and *her9*)⁷⁸. In the mouse, miR-9 has a bidirectional negative feedback relationship with the orphan nuclear receptor TLX, which mediates NSC self-renewal and proliferation, thereby promoting neural differentiation⁷⁹. Moreover, introducing miR-9 into the embryonic brain leads NSCs to differentiate prematurely and migrate into the cortical plate. In addition to TLX, miR-9 targets forkhead box protein G1 (FOXG1) and promotes differentiation of Cajal–Retzius cells in the medial pallium⁸⁰. Mice with ablation of miR-9 exhibit impairments in the production of Cajal–Retzius cells and early born cortical neurons, suppression of progenitor proliferation in ventricular and subventricular zones, defects in neocortical lamination, enhancement of neural progenitor cell (NPC) proliferation in the subpallium, shifting of the pallial–subpallial boundary and misrouting of thalamocortical and corticofugal axons⁸¹.

The extent to which miRNAs serve as key nodes in networks responsible for establishing cell identity is highlighted by reports showing that the expression of a small number of miRNAs can promote reprogramming of somatic cells into induced pluripotent stem cells (iPSCs)^{82,83} and, conversely, how other miRNAs can act as barriers for somatic cell reprogramming⁸⁴. Two seminal studies demonstrated that the introduction of miR-124 together with either a particular combination of transcription factors or miR-9/9* in human fibroblasts can reprogramme them into neuronal cells^{85,86}. The mechanisms of action for these two miRNAs may involve modulating the composition of ATP-dependent Brg1/Brm-associated factor (BAF) chromatin-remodelling complexes, which are comprised of specific combinations of subunits and are essential for particular stages of neuronal lineage maturation⁸⁷. miR-124 and miR-9/9* are also crucial for modulating neural gene expression because they are integrated with the circuitry of CREB, REST and CoREST, which are master transcriptional and epigenetic regulators of neural genes and neural cell fate decisions^{11,12} (FIG. 3). miRNAs also regulate glial lineage

elaboration. For example, *Dicer1* depletion leads to disruptions in the maturation of oligodendroglial (OL) and astroglial lineage species, which can be rescued by reintroducing particular miRNAs^{73–75}. Given the emerging roles of REST and CoREST in NSC maintenance, lineage restriction, neuronal and glial subtype specification and differentiation⁸⁸, these observations suggest the existence of intricate regulatory relationships between these multifunctional factors and ncRNAs in promoting glial lineage specification and maturation analogous to developmental processes in neuronal cells.

Complementary efforts have been directed towards characterizing the parts played by other classes of ncRNAs in the establishment of neural cell identity. For example, isolating small ncRNAs from hippocampal NSCs identified a small double-stranded ncRNA derived from the *RE1* (also known as *NRSE*) REST-binding sequence, which is implicated in modulating REST activity and promoting hippocampal neurogenesis⁸⁹. By contrast, genomic approaches first identified dynamic expression profiles for substantial numbers of lncRNAs in developing neural cell types⁹⁰. Subsequent studies focused on determining the functions of a few of these lncRNAs in neural cell fate specification and maturation^{91,92}. We used a custom designed microarray platform to identify hundreds of lncRNAs that are dynamically regulated during GABAergic neurogenesis and OL lineage elaboration⁹³. Many of these lncRNAs are transcribed from genomic loci associated with, and exhibit expression patterns that correlate with, key neural developmental genes. Another functional study analysed chromatin-state maps and reported the presence of more than 1,500 lncRNAs in NPC cells (and other cell types), including those associated with hippocampal development, OL maturation and GABAergic neuronal function¹⁷. An RNA-seq analysis carried out using a human iPSC paradigm showed that more than 1,500 lncRNAs are dynamically regulated during differentiation of iPSCs into ‘glutamatergic neurons’ (REF. 94). A large proportion of these lncRNAs are implicated in recruiting chromatin-remodelling complexes (including REST, CoREST and Polycomb repressive complex 2) to their genomic sites of action^{18,95} (FIG. 3). These observations provide insight into the complex interrelationships between ncRNAs and multifunctional epigenetic and transcriptional regulatory factor complexes coordinating neuronal and glial lineage elaboration.

Synaptic development, maintenance and plasticity.

Numerous aspects of synaptic function are influenced by ncRNAs. Indeed, there is a bidirectional relationship between the two: ncRNAs mediate synaptic processes, and synaptic activity influences ncRNA expression, intracellular transport and function^{62,96,100} (FIG. 4). For example, various miRNAs target CREB, which is vital for LTP and memory¹¹. In turn, miRNAs can also be regulated by CREB activity. Specific miRNAs are enriched in distinct subcellular compartments and can participate in the spatiotemporal control of protein synthesis underlying axon guidance, dendritic elaboration and synaptic plasticity⁹⁷. Reciprocally, various miRNAs

are subject to regulation by LTP and long-term depression^{98,99}. Examining individual miRNAs reveals diverse roles in modulating dendritic growth and arborization (miR-124, miR-125b, miR-132, miR-134, miR-137 and miR-485), synapse formation (let-7, miR-125b, miR-132, miR-137, miR-138 and miR-485) and synaptic function and plasticity (miR-1, miR-132, miR-134, miR-181a, miR-219, miR-284 and miR-485)^{62,96,100}. Manipulating miRNA biogenesis and maturation pathways similarly highlights the importance of miRNAs in synaptic structure and physiology. For example, in *Drosophila melanogaster*, hypomorphic mutations of miRNA pathway components *drosha*, *dgcr8* (DiGeorge syndrome critical region 8; also known as *pasha*) and *Dicer1* lead to reduced levels of miRNAs and defects in synaptic transmission in photoreceptor neurons, but no neural developmental effects¹⁰¹. In mice, using conditional and inducible strategies to inactivate *Dicer1* in forebrain neurons yields alterations in dendritic spine morphology^{72,102}. Mice with monoallelic deletions of *Dgcr8* have decreased levels of expression for a subset of miRNAs involved in synaptic development and functioning¹⁰³. Accordingly, pyramidal neurons in the cortex of these mice exhibit impairments in dendritic morphology and excitatory synaptic transmission. These observations suggest that other short ncRNAs, which are dependent on these biogenesis and maturation pathways, also have roles in establishing these abnormal synaptic phenotypes.

miRNAs and associated factors are often packaged into cytoplasmic RNA–protein complexes (neuronal RNA granules) that mediate subcellular targeting, metabolism and effector functions⁹⁶. Studies of RBPs — many of which are linked to neurological diseases — have therefore been used to elucidate how miRNAs promote synaptic development and plasticity. The fragile X mental retardation 1 protein (FMRP) and related family members (FXR1P (also known as FMR1) and FXR2P (also known as FMR2)) provide salient examples. This family of RBPs is integrated with miRNA pathways at multiple levels and promotes the stabilization, dendritic targeting and local synaptic translation of neuronal mRNAs^{104,105}. These RBPs interact with miRNA biogenesis (through DICER1) and effector (RISC) mechanisms, directly associate with particular miRNAs and are even subject to regulation by miRNAs. In fact, FXR1P is involved in the biogenesis of miR-9 and miR-124 (REF. 106) and, in turn, its expression is modulated by various miRNAs¹⁰⁷. Ataxin 2 (ATX2) is another example of a protein involved in neurological disease that links RNA granules and miRNA pathways with synaptic functions. ATX2 and its homologues are RBPs that interact with a range of RNA processing factors (including polyadenylate binding protein (PABP) and maternal expression at 31B (ME31B)) and have established roles in RNA granule assembly and translational regulation. A recent study further demonstrated that *D. melanogaster* ATX2 is required for synaptic plasticity in a long-term olfactory habituation paradigm¹⁰⁸. It suggested that ATX2 specifically regulates the Argonaute 1 (AGO1)–RISC pathway of miRNA-mediated translational repression through effects mediated by ME31B and PABP, which interact

with the core RISC proteins, GW182 (also known as TNRC6A) and AGO1.

Other ncRNAs are also implicated in modulating synaptic development, maintenance and plasticity. One intriguing report identified thousands of ncRNAs in neuronal cells derived from enhancer elements, called enhancer RNAs (eRNAs)¹⁰⁹. The transcription of eRNAs is activity-dependent and correlates with the expression of proximally located protein-coding genes, suggesting that the process of eRNA transcription and/or the eRNA transcripts are responsible for promoting activity-dependent gene transcription. Additionally, specific lncRNAs located in the nucleus or those that are trafficked to dendritic compartments can mediate essential synaptic processes. For example, nuclear-enriched abundant transcript 2 (*NEAT2*; also known as *MALAT1*) is a nuclear lncRNA that modulates synaptogenesis by regulating synaptic gene expression (of neuroligin 1 and synaptic cell adhesion molecule 1 (*SynCAM1*); also known as *CADMI*) and alternative splicing (of calcium/calmodulin-dependent protein kinase II- β) via sarcoplasmic reticulum-splicing factors in nuclear speckle domains¹¹⁰. By contrast, rodent brain cytoplasmic RNA 1 (*Bc1*) and its primate analogue *BC200* (also known as *BCYRN1*) are actively transported to the somatodendritic domains of neurons, where they repress local protein synthesis. They disrupt translational initiation complex assembly by inhibiting the catalytic activity of eukaryotic initiation factor 4A and binding to eukaryotic initiation factor 4B, thus preventing its interaction with the 18S rRNA of the ribosome¹¹¹. Moreover, mice with deletion of *Tsx*, a lncRNA transcribed from the X-inactivation locus, exhibit changes in associative learning and memory for conditioned fear, suggesting that *Tsx* modulates short-term hippocampal memory¹¹². A number of atypical piRNAs¹¹³ are expressed in mouse brain in specific regional, cellular and subcellular distributions, and suppression of the most highly expressed hippocampal dendritic piRNA, *DQ541777*, reduced dendritic spine size, suggesting a role for this factor in activity-dependent translational regulation⁶⁶. Moreover, high-throughput sequencing of short ncRNAs from hippocampi of mice undergoing olfactory discrimination training, a hippocampal-dependent task, found that a number of short ncRNAs increase more than 100-fold with training, including various endo-siRNAs, snoRNA-derived ncRNAs and other small RNAs¹¹⁴. Conspicuously, a considerable number of these endo-siRNAs are derived from genomic loci encompassing genes that regulate synaptic plasticity, and the most abundant siRNAs identified in this study are associated with a gene involved in synapse development and plasticity, synaptic RAS GTPase-activating protein 1 (*SYNGAP1*), which encodes a RAS GTPase-activating protein that is highly expressed at the postsynaptic density and implicated in modulating α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor trafficking and excitatory synaptic transmission. The *SYNGAP1* gene locus is also linked to mental retardation and autism, highlighting the potential for ncRNAs to be derived from disease-associated loci and to affect disease pathways.

Table 2 | Roles of non-coding RNAs in nervous system disorders

Paradigms for linking non-coding RNAs to disease	Refs
Genetic variation in non-coding RNA genes	116–121
Genetic variation in disease-associated protein-coding genes affects interactions with non-coding RNAs	122–124
Epigenetic deregulation of non-coding RNA genes	125
Perturbations in factors that modulate non-coding RNA biogenesis, maturation and function	126
Genomic context links non-coding RNA genes to disease-causing genes and susceptibility loci	127
Differential expression of non-coding RNAs in disease-related central and peripheral tissues	130–134

Neurological disease pathogenesis

It is well known that defects in ncRNAs can lead to disease¹¹⁵. For example, mitochondrial tRNA mutations are the primary causes of mitochondrial encephalomyopathies¹¹⁶. However, our appreciation for the evolving ncRNA landscape and the central roles ncRNAs have in neurobiological processes has had a major impact on the study of CNS disease mechanisms and the development of novel clinical applications. Indeed, a rapidly expanding body of evidence suggests that miRNAs and other classes of ncRNAs, along with associated factors, are involved in the pathophysiology of every major class of neurological and psychiatric disorder (TABLE 2; see [Supplementary information S1](#) (table)).

Genetic variation in ncRNA genes can promote CNS disease onset. For example, eliminating the *Bcl* gene in mice leads to neuronal hyperexcitability, increased cortical gamma frequency oscillations and susceptibility to epileptogenesis¹¹⁷. Expansion repeats in the *ATXN8OS* lncRNA gene are partly responsible for the pathology of spinocerebellar ataxia type 8 through a toxic RNA gain-of-function mechanism that includes formation of ribonuclear inclusions in the cerebellum and deregulation of muscleblind-like splicing regulator 1-mediated alternative splicing¹¹⁸. Moreover, genome-wide association studies have demonstrated that the 9p21 genomic locus is a hotspot for the risk of stroke, glioma, plexiform neurofibroma and other disorders^{119–121}. This locus encodes the cyclin-dependent kinase inhibitors *CDKN2A* and *CDKN2B* and the lncRNA *ANRIL* (also known as *CDKN2B-AS1*), but analysing single-nucleotide polymorphisms (SNPs) and transcript levels suggests that, whereas multiple sites within the 9p21 locus may influence disease vulnerability, variations in *ANRIL* and levels of *ANRIL* expression correlate with disease most significantly. These observations underscore the concept that genetic variation at a single locus can affect the expression and function of many distinct protein-coding and non-coding transcripts, and studies of genetic epidemiology must be interpreted in this context.

Correspondingly, genetic variation present in either miRNAs or their targets can change regulatory interactions between these molecules and, thereby, affect disease processes^{122–124}. For example, a risk of Parkinson's

disease conferring an SNP in the fibroblast growth factor 20 (*FGF20*) gene disrupts the binding of miR-433 to the *FGF20* mRNA 3'-UTR. Deregulated expression of *FGF20* is correlated with increased levels of α -synuclein, which can lead to Parkinson's disease. Similarly, a Tourette's syndrome-linked variant within the miR-189 binding site of the 3'-UTR of *SLIT* and *NTRK*-like (*SLITRK1*) leads to increased repression of *SLITRK1* (REF. 124). These findings have important implications for characterizing disease-linked non-coding DNA regions because an individual miRNA can target multiple protein-coding transcripts and because a single protein-coding transcript can be modulated by multiple miRNAs. Furthermore, these observations support the more general hypothesis that genetic variation can alter biophysical relationships between ncRNA transcripts and their DNA, RNA and protein partners, considerably or more subtly influencing regulatory and functional interactions and modifying disease susceptibility.

Additionally, epigenetic deregulation of ncRNA genes can be associated with CNS diseases¹¹⁵. For example, it has been suggested that DNA methylation modulates the expression of the tumour suppressor miRNAs miR-124 and miR-137 in anaplastic astrocytoma and glioblastoma¹²⁵. ncRNAs are also subject to epigenetic regulation via mechanisms other than DNA methylation, such as histone and chromatin modification, and it is likely that future studies will reveal that disease-linked abnormalities in ncRNA activity are associated with deregulation of these processes. Perturbations in factors responsible for modulating ncRNA metabolism and function can also lead to pathology. For example, age-related macular degeneration is characterized by decreased levels of *DICER1* in the eye that result in impaired degradation of *Alu* retrotransposon-derived ncRNAs, which are directly toxic to the retinal pigmented epithelium¹²⁶.

Genomic context can also link ncRNA genes to CNS disease-causing genes and susceptibility loci. In some cases, mechanistic relationships between overlapping or proximally located ncRNA and protein-coding genes have been identified. For example, *SCA7/ATXN7* antisense RNA 1 (*SCAANT1*) is an lncRNA transcribed antisense to the gene mutated in spinocerebellar ataxia type 7 (*ATXN7*), and it functions as a repressor of *ATXN7* transcription¹²⁷. Convergent bidirectional transcription has similarly been observed at a number of loci associated with neurological diseases and is, notably, implicated in promoting instability of expansion repeats¹²⁸.

Last, ncRNAs can be differentially expressed in disease-related central and peripheral tissues associated with a range of CNS disorders, including neurovascular, neurodevelopmental, neurodegenerative, neuropsychiatric and neuroimmunological disorders and brain tumours^{62,129}. It is debatable to what extent these correlations represent causal relationships. Nevertheless, target genes of deregulated ncRNAs are often involved in cellular pathways linked to the pathophysiology of corresponding disease states. For example, a recent study demonstrated that in peripheral blood mononuclear cells from patients with Parkinson's disease, miRNAs were differentially expressed, with higher

Bidirectional transcription
Transcription that occurs on both the positive and negative strands of DNA simultaneously, where the direction of RNA polymerase progression along each strand is either is convergent or divergent.

Box 2 | Live cellular imaging of non-coding RNAs

The development of techniques for live cellular imaging and high-resolution analysis of single RNA molecules is crucial for better understanding the *in vivo* spatiotemporal dynamics of non-coding RNAs (ncRNAs). Strategies for RNA imaging in live cells include using light microscopy (wide-field, confocal or total internal reflection microscopy) to detect fluorescence of reporter probes (molecular beacons or MS2 labels). Oligonucleotide-based molecular beacons fluoresce upon hybridization with target RNAs. MS2 labelling exploits the high-affinity interaction between the MS2 bacteriophage coat protein and its cognate MS2 RNA-binding sequence. The MS2 protein can be fused with a fluorescent protein and used to label RNAs engineered with MS2-binding sequences. One interesting study investigated the behaviour of the human telomere repeat-containing RNA (*TERRA*) ncRNA, using a molecular beacon-based approach¹⁴⁴. It was observed that *TERRA* localizes to telomeric DNA and forms a parallel G-quadruplex structure, which has roles in modulating telomerase activity and gene expression. Other studies have uncovered how the X (inactive)-specific transcript (*XIST*) long ncRNA (lncRNA), mediates X chromosome inactivation with MS2 labelling¹⁴⁵ and how the nuclear paraspeckle assembly transcript 1 (*NEAT1*) lncRNA establishes paraspeckle nuclear domains using direct visualization of paraspeckle proteins and fluorescence recovery after photobleaching¹⁴⁶. Various studies have also reported imaging microRNAs (miRNAs) with fluorescent proteins, luciferase reporters and molecular beacons¹⁴⁷. One described imaging miR-124 during neuronal differentiation using a molecular beacon with an miR-124-binding sequence¹⁴⁸. Intriguingly, this molecular beacon was linked to a magnetic nanoparticle, enabling the imaging of miRNAs in live animals by magnetic resonance imaging. The same authors also reported how a molecular beacon targeting the glioma-promoting miR-221 can be conjugated to a magnetic nanoparticle and to an aptamer that specifically binds to nucleolin, which is expressed on glioma cells¹⁴⁹. This 'theragnostic' device can potentially be used to target glioma cells selectively and to image and even inhibit miR-221 activity. The widespread adoption of these and other innovative quantitative real-time *in vitro* and *in vivo* imaging technologies — those exploiting different probe designs (nanoparticles, quantum dots, organic dyes and chemical tags) and advanced optical methods — will help to characterize specific patterns and kinetics for dynamic ncRNA transcription, processing, transport and molecular interactions in health and disease and to develop novel diagnostics and therapeutics.

levels of miRNAs for predicted target genes that are involved in pathways associated with the disease, including those in the protein ubiquitylation and glycosphingolipid biosynthesis pathways¹³⁰. This example suggests that ncRNA expression profiles in peripheral tissues might reflect CNS disease states. Evidence from other disorders, such as stroke, has in fact demonstrated that miRNA expression profiles present in the brain after cerebral ischaemia correspond with those in the blood (in animal models¹³¹ and in stroke patients¹³²). Moreover, ncRNAs derived from neural cells can circulate in the blood and cerebrospinal fluid in membrane-bound exosomes^{133,134}. These overall observations suggest that ncRNAs might represent peripheral biomarkers for CNS diseases.

However, whether safe and effective diagnostic and treatment strategies for neurological and psychiatric diseases can be developed by targeting ncRNAs (and their

biogenesis and effector pathways) is yet to be determined. miRNA-profiling assays are already commercially available for cancer diagnosis and prognostication. They are also being developed for other ncRNAs and other systemic disorders. Therapeutic approaches targeting ncRNAs are also being explored, including those focused on designing and delivering oligonucleotide molecules with the ability to repress or replace ncRNAs¹¹⁵ and on identifying small molecules that can influence ncRNA biogenesis and effector pathways¹³⁵. Although these strategies are promising, selective delivery into the CNS remains the most considerable challenge, and it has not been addressed effectively although methods using viral delivery systems, chemical modification and conjugation strategies, aptamers, nanotechnologies and barrier modulation are currently being investigated.

Perspectives

The CNS is defined by extraordinary degrees of cellular diversification, synaptic and neural network connectivity and plasticity, and responsiveness to interoceptive and environmental signals. It is now clear that these features are mediated not only by cell type-, developmental stage- and stimulus-specific profiles of protein-coding mRNA expression, post-transcriptional modifications, localization and translation but also by ncRNAs that have similarly complex life cycles and a diverse and interrelated range of regulatory and functional roles in transcriptional, post-transcriptional, epigenetic and nuclear processes^{136,137} (FIG. 3). In fact, because the proportion of non-coding DNA sequence present in the genome correlates with organismal complexity⁵³ (in contrast to protein-coding genes), it is intriguing to speculate that ncRNAs preferentially mediated the accelerated and asymmetric evolution of the human CNS and underlie the unique functional repertoire of the brain. Further elucidating how ncRNAs operate at molecular, cellular and more hierarchical (neural network activity and topology) levels may therefore provide novel insights for answering seminal neurobiological questions (BOX 2). Chief among these may be uncovering the genetic and evolutionary bases of complex CNS diseases, which have remained elusive. Indeed, the concept that each nucleotide — in addition to being involved in diverse DNA-protein, DNA-RNA and DNA-DNA interactions in local and more global chromatin environments within the nucleus — can give rise to multiple ncRNAs with divergent functions suggests a paradigm in which genetic variation in even a single base pair can both subtly and more dramatically affect the execution of myriad cellular programmes, potentially accounting for how complex CNS disease states unfold and co-morbidities arise.

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Competing interests statement

The authors declare no competing financial interests.

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