Non-coding RNAs with essential roles in neurodegenerative disorders

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The importance of various classes of regulatory non-protein-coding RNA molecules (ncRNAs) in the normal functioning of the CNS is becoming increasingly evident. ncRNAs are involved in neuronal cell specification and patterning during development, but also in higher cognitive processes, such as structural plasticity and memory formation in the adult brain. We discuss advances in understanding of the function of ncRNAs in the CNS, with a focus on the potential involvement of specific species, such as microRNAs, endogenous small interfering RNAs, long intergenic non-coding RNAs, and natural antisense transcripts, in various neurodegenerative disorders. This emerging field is anticipated to profoundly affect clinical research, diagnosis, and therapy in neurology.

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Introduction

The idea that the genome exerts its functions only via classical genes and proteins seems more and more a naive oversimplification of a fascinating system of feed-forward and feedback loops that involves various RNA molecules. Indeed, a large part of the genomic code is only transcribed as RNA and is never translated into protein. Only in the past decade has non-protein-coding RNA (ncRNA) been recognised to have important functions. The phenomenon of so-called pervasive transcription[1–3] provides the transcriptome and the proteome with an incredibly complex layer of regulation and fine-tuning.

ncRNAs are abundantly expressed in mammalian CNS in specific spatiotemporal patterns.[4–7] MicroRNAs (miRNAs) and other ncRNAs are features of virtually all neuronal activity, including neurogenesis, neuronal patterning, neurotransmission, and synaptic plasticity (figure 1).[8–14] They maintain cellular homoeostasis with time and energy efficiency, which is particularly relevant in the nervous system, where rigorous requirements for continuous signalling and plasticity necessitate the rapid and accurate buffering of gene products along long processes and to distant synapses (figure 1).[8,10,15] The implications of this knowledge for the understanding of neurological and psychiatric diseases have only begun to be clarified compared with those for other diseases, and possible medical applications in diagnosis and therapy are not yet being sufficiently explored. Notably, the number of scientific publications on the roles of ncRNAs in cancer pathology is around ten times higher than that on their involvement in physiological and pathological processes in the CNS. In cancer, miRNAs, long intergenic non-coding RNAs (lincRNAs), and other long non-coding RNAs (lncRNAs) exert oncogenic or tumour-suppressing effects on tumorigenesis, invasion, angiogenesis, and metastasis.[16–19]

In this Review we provide an overview of ncRNA biology in the CNS and discuss possible roles in neurological disorders, with a focus on neurodegeneration (webappendix). We draw lessons from pioneering translational studies in cancer and cardiovascular disease and note where more thorough and systematic work is needed in human tissues and in animal models before findings can be translated into daily clinical practice for neurological disorders.

Biology of ncRNAs

Molecules of ncRNA are roughly classified into two size groups: small (<400 nucleotides) and long (>400 nucleotides). Small ncRNAs comprise well characterised infrastructural RNA, such as ribosomal RNAs, transfer RNAs, and small nuclear/spliceosomal RNAs. Other types of small ncRNAs are regulatory RNAs, such as miRNAs, small interfering RNAs (siRNAs), piwi-interacting RNAs, splice junction-associated RNAs, and small nucleolar RNAs.[20] Long ncRNAs can exceed 100 000 nucleotides and cover a wide range of heterogeneous regulatory molecules, such as lincRNAs,[21] natural antisense transcripts (NATs),[22] and non-coding RNA repeats (table, figure 2).[33] In many cases, however, ncRNAs have mixed characteristics and do not clearly fall into any of these categories.

Why 98·8% of the human genome consists of non-coding DNA (ncDNA) has puzzled researchers for many years.[39] High-resolution and high-throughput screening technologies, such as microarrays and next-generation sequencing (deep sequencing), have shown that in fact most of this ncDNA encodes ncRNA.[2,39] Strong evidence, including conservation of promoter sequences, dynamic expression during development and disease, and expression patterns and responsiveness to stimuli that are specific to tissue, cell, and subcellular structures, argues for the functionality of these ncRNAs.[2,20] Nevertheless, further studies are needed to address the extent to which each of the transcripts is operational and to identify the transcripts that affect specific molecular pathways in a physiological context.

The ncRNA machinery involves RNA–RNA, RNA–DNA, and RNA–protein interactions, and affects transcription, stability, processing, and translation of messenger RNA (mRNA), alternative splicing, and epigenetic events, such as RNA editing and chromatin modification.[9,10] These regulatory phenomena are effected on neighbouring transcripts (in cis) or at loci distant from their own transcription (in trans) and depend mainly on

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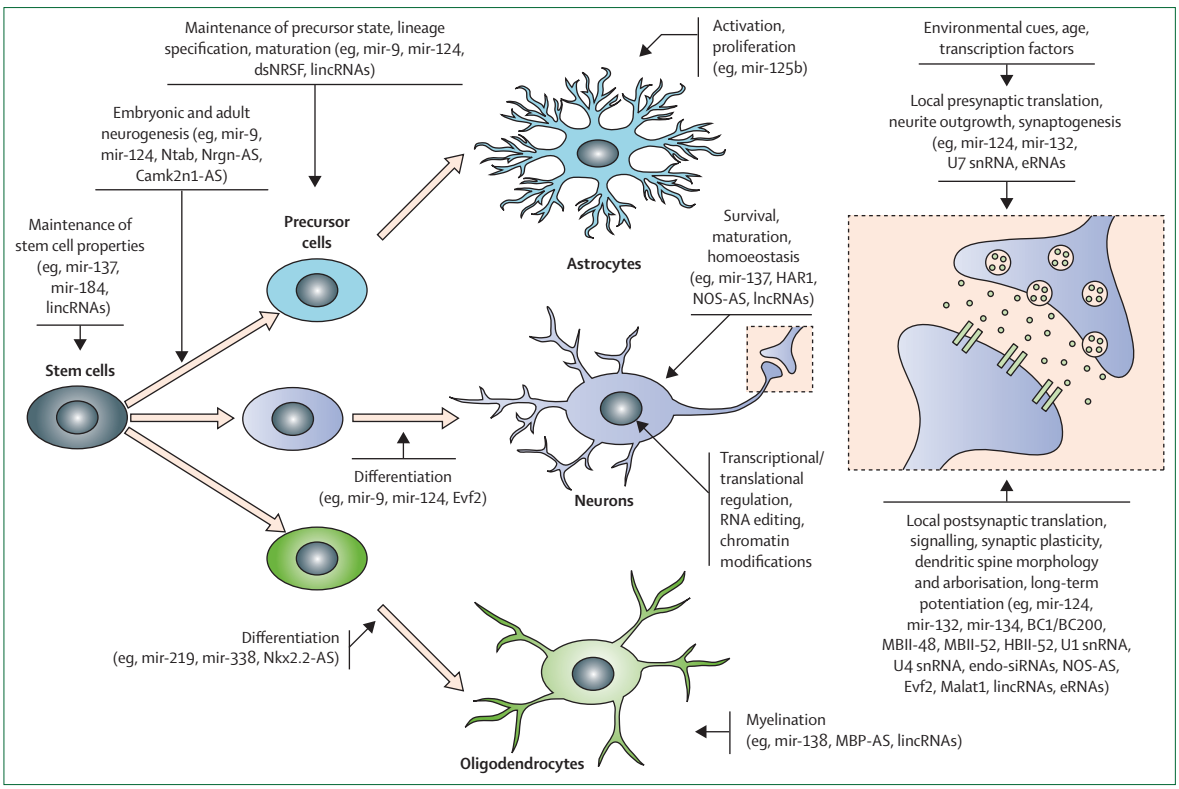

***Figure 1*: Physiological roles of regulatory ncRNAs in the CNS**
Examples of ncRNAs involved in the physiology of the CNS and affecting processes ranging from neuronal development to maturation. ncRNAs can be involved in the maintenance of the stem cell status of neuronal stem cells and their differentiation into neuronal, astrocytic, or oligodendroglial precursors, and also in the maintenance, lineage specification, maturation, and differentiation of precursor cells into their respective cell types. Additionally, ncRNAs affect processes such as activation and proliferation of astrocytes, survival, maturation, and homoeostasis of neurons, and myelination of mature oligodendrocytes. At the level of the synapse, ncRNAs might regulate local translation, neurite outgrowth, synaptogenesis, neurotransmitter signalling, synaptic plasticity, and long-term potentiation. ncRNA=non-protein-coding RNA. lincRNA=long intergenic non-coding RNA. lncRNA=long non-coding RNA. snRNA=small nuclear RNA. eRNA=enhancer RNA. endo-siRNA=endogenous small interfering RNA. BC1=brain cytoplasmic RNA of 150 nucleotides. BC200=brain cytoplasmic RNA of 200 nucleotides.

the complementarity between the ncRNA sequence and DNA or RNA molecules. In many cases, structural changes in regulatory or structural proteins can also be induced by the secondary structure of particular ncRNAs;[2] this phenomenon is similar to the mechanism of action of regulatory proteins. Notably, ncRNAs are regulated by the CNS in an activity-dependent manner, which results in rapid changes in their expression.[10,15]

In view of their broad biology and function, it is unsurprising that the numbers of ncRNAs recognised as contributing to key pathogenic mechanisms are increasing. Below we look at the characteristics and roles of specific ncRNA species in the context of neurological diseases.

miRNAs and neurodegenerative disorders

miRNAs were discovered in *Caenorhabditis elegans* almost 20 years ago,[40] and they now represent the most extensively studied class of ncRNAs. This species of RNA is particularly important for the fine-tuning of neuronal networks.[41] miRNA genes are transcribed mainly by RNA polymerase II. The resulting long primary transcript can contain several miRNA stretches, but subsequent processing by a series of enzymes, including nuclear Drosha and cytoplasmic Dicer, results in one or more double-stranded, mature miRNA molecules of around 21 nucleotides in length. One of the strands becomes incorporated into the RNA-induced ribonucleoprotein silencing complex and, after base pairing with the 3′-UTR of the target mRNA, induces most of the time mRNA degradation or repression.[23,24,42] Of note, translation activation has also been reported.[43] Each of the roughly 700 miRNAs identified so far in the human genome can theoretically bind to a few hundred mRNAs.[44] This feature raises the question of whether all the interactions are equally important; in many reported examples, one particular miRNA has affected one specific mRNA that is responsible for most of the biological effects exerted. For instance, in phenotype rescue experiments the mir-310–313 cluster regulated synaptic strength at the neuromuscular junction mainly via one target, Khc-73,[11] whereas activity-dependent dendritic growth, which was mediated by mir-132, was attribtued to the translational regulation of the Rho family GTPase-activating protein p250GAP.[45]

A rather crude and general way to investigate the biological relevance of the miRNA machinery in the CNS is to genetically delete the miRNA-processing enzyme Dicer. This enzyme (a classical protein) controls final maturation of precursor miRNA into the tiny operational miRNAs. The full deletion of Dicer in mice is embryonic lethal,[46] whereas conditional inactivation of Dicer selectively in Purkinje cells or in midbrain dopaminergic, cortical, or hippocampal neurons leads to degeneration of the cells.[47] Follow-up studies have investigated which miRNAs are specifically involved in these degenerative phenotypes. Changes in miRNAs have been detected in various neurodegenerative disorders (eg, Alzheimer's disease,[48–54] Parkinson's disease,[55,56] Huntington's disease,[57–60] and prion disease[61,62]), and proteins involved in these diseases can be regulated by miRNAs. Thus, the hypothesis that changes in miRNAs are involved in the neurodegenerative disease process, either causally or as part of positive feedback loops, is gaining ground. Available studies in patients, however, have only provided snapshots of the disease process and have not shown how miRNAs change with disease progression. The observed alterations might also be only epiphenomena of the underlying disease process. In view of the important regulatory roles of miRNAs in brain physiology, the reality is probably that some miRNA changes are part of the disease process and others are due to the loss of cells or other secondary alterations. To gain understanding of both possibilities is crucial and might yield novel diagnostic markers and deeper insight into the neurodegenerative disease process itself.

Alzheimer's disease

The list of protein targets relevant to neurodegenerative diseases is rapidly growing. In Alzheimer's disease, two of the most important proteins are extensively controlled (at least in vitro) by miRNAs: amyloid precursor protein (APP), which carries the amyloid-β peptide that precipitates in the amyloid plaques, and BACE1, which cleaves APP in the generation of amyloid-β peptide (figure 3 and webappendix). Of note, post-mortem analysis of brains from patients with Alzheimer's disease has revealed increased levels of BACE1 but not of its mRNA,[50,67,68] which indicates post-transcriptional regulation of the protein under pathological conditions. The marked decrease in the expression of miR-29a/b-1 and miR-107 and increased concentrations of BACE1 as Alzheimer's disease progresses suggest a direct functional link between these miRNAs and the protease in the disease process.[49,50] Several neuronal miRNAs are differentially regulated after exposure to amyloid-β peptide, which suggests that aberrant miRNA function contributes to the elusive toxic effects of amlyoid-β peptide in patients with Alzheimer's disease.[69] The link to tau pathology in this disease remains less clear, but deletion of Dicer in adult mouse brain led to changes in tau phosphorylation and neurodegeneration, which supports a potential link between dysfunctional miRNA pathways and tangle pathology during the disease course.[70] In one report, mir-132 was noted to be involved in the abnormal tau splicing pattern in sporadic progressive supranuclear palsy, which is a neurodegenerative tauopathy.[71]

Parkinson's disease

Evidence for a direct or indirect role of miRNAs in the pathogenesis of Parkinson's disease is accumulating (figure 4 and webappendix). One of the pathological hallmarks of this disease is fibrillar aggregates (Lewy bodies) in the dopaminergic neurons of the substantia nigra that contain α-synuclein. The expression of α-synuclein is fine-tuned by specific miRNAs (figure 4 and webappendix). Furthermore, a report has implicated the most important gene in Parkinson's disease, *LRRK2*, in the negative regulation of let-7 and mir-184* functions in dopaminergic neurons. The respective targets of these

	Origin	Mechanisms and functions
Short		
miRNAs[23,24]	Sense, intergenic or intronic	Incorporate into RISC (miRISC), base pair to 3′-UTR of mRNA targets, and mainly induce translational repression or deadenylation and degradation
endo-siRNAs[24,25]	Sense or antisense, intergenic or exonic	Incorporate into RISC (siRISC), base pair to mRNA target, and induce degradation or heterochromatin formation
piRNAs[26]	Sense or antisense, intergenic	Epigenetic and possible translational control via complementarity with DNA or RNA sequences
PASRs[1]	Sense, intergenic (promoter region)	Unknown
TASRs[1]	Antisense, intergenic (3′-UTR end of genes)	Unknown
snoRNAs[27]	Sense, intergenic or intronic	Pre-RNA processing or nucleoside modification (2′-O-ribose methylation and pseudouridylation) of other RNA molecules
snRNAs[28]	Sense, intergenic or intronic	pre-mRNA splicing
tiRNAs[29,30]	Sense or antisense, intergenic (5′-UTR transcription initiation sites)	Possibly promotes transcription via epigenetic regulation
spliRNAs[30]	Sense, exonic (splice donor site)	Possible epigenetic regulation
Long		
lincRNAs[31]	Sense or antisense, intergenic	Epigenetic regulation
NATs[32]	Antisense, exonic or intronic	mRNA transcription, splicing, stability, and translation, epigenetic modifications, and precursors of endo-siRNAs
RNA expansion repeats[33]	Sense or antisense, exonic or intronic	Epigenetic regulation and RNA toxic effects
ENORs[34]	Sense or antisense, intergenic	Transcriptional regulation, genomic imprinting, precursors of other short and long ncRNAs
eRNAs[35]	Sense, intergenic (enhancer region)	Transcriptional regulation
PALRs[1]	Sense, overlap with promoter and first exon of genes	Unknown

miRNAs=micro RNAs. RISC=RNA-induced silencing complex. mRNA=messenger RNA. endo-si RNAs=endogenous small interfering RNAs. piRNAs=piwi-interacting RNAs. PASRs=promoter-associated short RNAs. TASRs=termini-associated short RNAs. snoRNAs=small nucleolar RNAs. snRNAs=small nuclear RNAs. tiRNAs=transcription initiation RNAs. spliRNAs=splice junction-associated RNAs. lincRNAs=long intergenic non-coding RNAs. NATs=natural antisense transcripts. ENORs=expressed non-coding regions. eRNAs=enhancer RNAs. PALRs=promoter-associated long RNAs.

***Table*: Non-protein-coding RNA species**

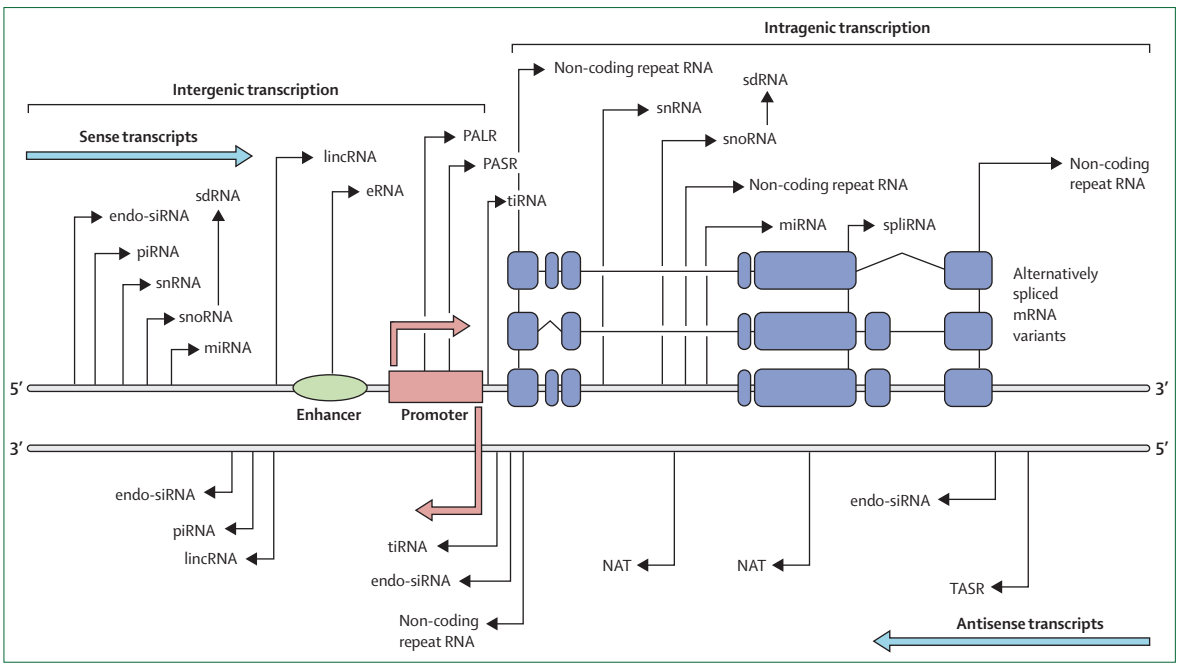

***Figure 2*: Complexity of transcription patterns of ncRNAs**
The two strands of genomic DNA are displayed together with the basic building blocks of a gene. ncRNAs can be transcribed by one of three different RNA polymerases (RNA polymerase I, II, or III) from intergenic (DNA sequences between two genes) and intragenic (intronic or mostly unstable exonic transcripts) genomic regions. Transcription occurs in patterns nested in and overlapping with protein-coding transcripts but also in regions where no classical genes are found (even heterochromatin sequences[2]). Both sense and antisense orientations of the genome are used.[2,8,9,36,37] Some ncRNAs undergo further modifications similar to authentic mRNAs, including polyadenylation (conferring stability)[38] or 5′ end nucleotide capping (which provides stability but is also important for cellular transport and localisation of the transcripts).[36] Notably, sometimes ncRNAs undergo alternative splicing.[9] Clearly, these RNA molecules are processed and regulated in ways as complex as those that affect proteins. ncRNA=non-protein-coding RNA. endo-siRNA=endogenous small interfering RNA. piRNA=piwi-interacting RNA. snRNA=small nuclear RNA. snoRNA=small nucleolar RNA. miRNA=microRNA. sdRNA=sno-derived RNA. lincRNA=long intergenic non-coding RNA. eRNA=enhancer RNA. PALR=promoter-associated long RNA. PASR=promoter-associated short RNA. tiRNA=transcription initiation RNA. spliRNA=splice junction-associated RNA. mRNA=messenger RNA. NAT=natural antisense transcript. TASR= termini-associated short RNA.

miRNAs, E2F1 and DP, are in turn overexpressed, which results in defective cell division and eventually cell death.[75] These observations need confirmation in mammalian brain to definitively establish a role for LRRK2 in this pathway. Finally, mir-133b, a dopaminergic neuron-specific miRNA that is downregulated in Parkinson's disease, forms a negative feedback loop with the transcription factor PITX3, which in the CNS is expressed exclusively in midbrain dopaminergic neurons.[55] Although a direct role of mir-133b in the loss of dopaminergic neurons is not supported by this report, its downregulation might counteract a neuroprotective mechanism.

Other neurodegenerative disorders

The pathogenic mechanism in Huntington's disease is not completely understood. Trinucleotide expansion in the gene that encodes huntingtin is, however, known to impair the cytoplasmic binding of this protein to REST/NRSF, which represses transcription of neuronal genes in non-neuronal cells. This effect could partly explain the increased nuclear translocation of REST/NRSF and the consequent aberrant decrease in neuronal gene expression in the cortex of patients with Huntington's disease.[58,60] Several miRNAs are regulated by REST/CoREST.[60] miR-9 and miR-9* are thought to be part of a double negative feedback loop, as they are regulated by the REST repressor complex but they also target REST and CoREST expression.[57] Multiple studies of miRNAs in mouse and human brain tissue have provided further links to neurodegenerative diseases, including spinocerebellar ataxia type 1,[76,77] amyotrophic lateral sclerosis,[78] frontotemporal dementia,[79] prion diseases,[61,62] spinal muscular atrophy,[80] and myotonic dystrophy type I.[81] Collectively, these data clearly support pivotal functions for miRNAs in neurodegeneration via interaction with and regulation of crucial players in the pertinent pathways or through their cardinal roles in regulating the survival and function of neurons, which affect mitochondrial function,[82,83] apoptosis,[52] inflammation,[84] and synaptic plasticity.[85]

Single-nucleotide polymorphisms

Few studies have investigated the importance of single-nucleotide polymorphisms (SNPs) identified in genome-wide association studies for ncRNA biology. Many SNPs reside in the non-coding parts of the genome. Somewhat incorrectly, the increased disease risk associated with an identified SNP is frequently interpreted to imply roles for protein-encoding genes near the polymorphism.

Of the thousands of SNPs that have been characterised, many occur at experimentally validated target sites of miRNAs, but the variation in actual miRNA sequences is low.[86–89] Most of those SNPs were predicted to create novel miRNA-binding sites. Potentially, such polymorphisms might contribute to the phenotypic differences between individuals.[86,89] Cancer research is at the forefront in the analysis of this phenomenon,[90] but the study of these SNPs in neurodegeneration has only just started. For example, bioinformatic analysis has shown that seven genes associated with the risk of Alzheimer's disease bear SNPs in their 3′-UTRs at miRNA-binding sites, which suggests a possible role for variable miRNA regulation of these genes in the course of the disease.[91] Additionally, aberrant regulation of APP expression by miRNAs owing to the introduction of SNPs in the 3′-UTR has been related to the pathophysiology of Alzheimer's disease.[92] A polymorphism in an miRNA-binding site in *GRN* was proposed to result in decreased progranulin concentrations, which might in turn represent a risk factor in frontotemporal dementia and Alzheimer's disease.[79,93] A 3′-UTR SNP associated with Parkinson's disease and translational control by miR-433, however, did not correlate with a specific population of patients with this disease.[94]

Future challenges

An increase is expected in the number of functional studies in mouse models that directly explore the effects on disease processes of loss of specific miRNAs in neurons or glial cells. The generation of a complete mouse embryonic stem cell library with all miRNA genes conditionally targeted[95] makes this task highly feasible. Major challenges are the expansion of the number of patients assessed and stratification of the data by disease stage, brain area, and quality of material used. Single-cell analysis will also become very important in the identification of cell-specific changes. The ability to detect alterations in miRNAs in the brain by analysis of cerebrospinal fluid, or even better the analysis of plasma,

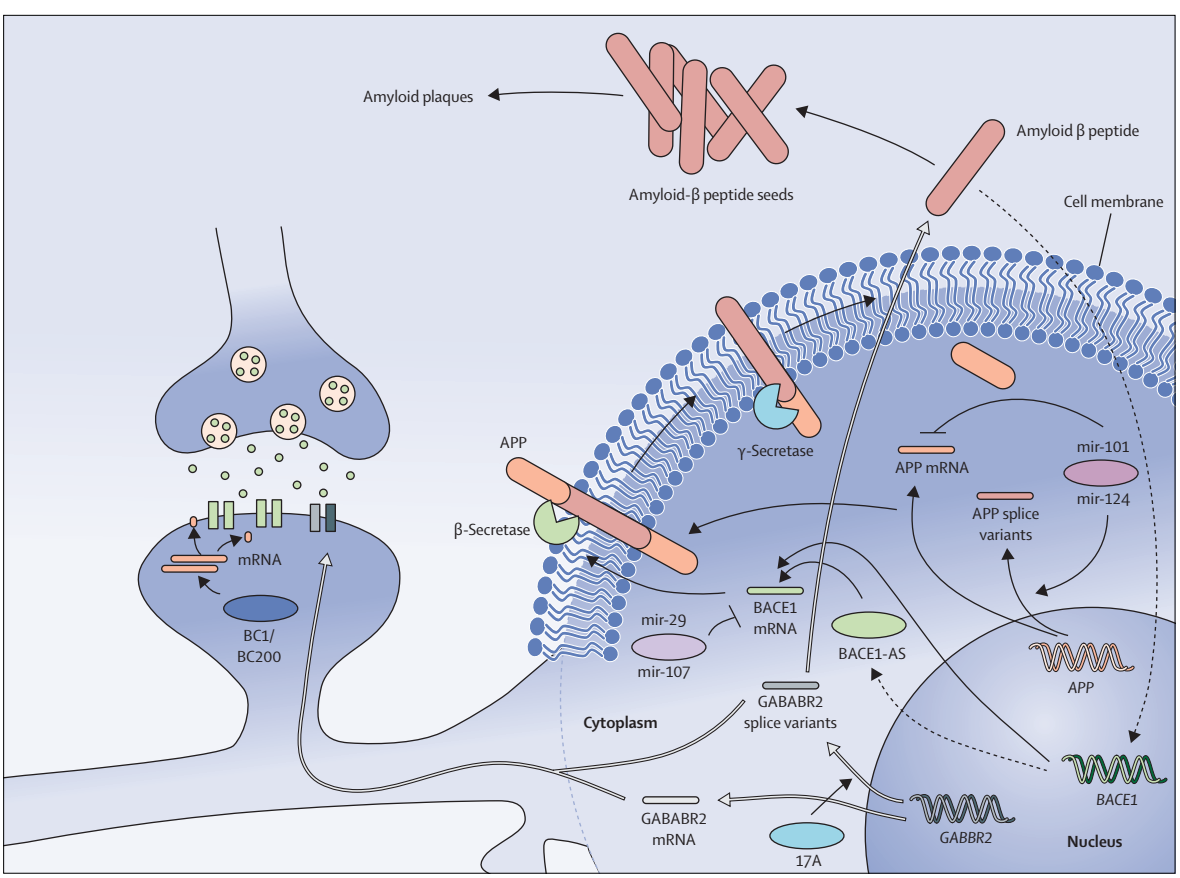

***Figure 3*: Regulatory ncRNAs in Alzheimer's disease**

Some of the paradigms of ncRNA regulation in the pathogenesis of Alzheimer's disease that involve microRNAs, natural antisense transcripts, and other ncRNAs are shown. More specifically, APP mRNA is regulated by miR-101,[63,64] miR-106a, miR-520c,[65] miR-124,[53] and let-7,[66] and BACE1 mRNA is regulated by miR-298, miR-328,[67] miR-107,[49] and miR-29a/b-1.[50] BACE1-AS reacts to deposition of amyloid-β peptide and in turn binds to and stabilises BACE1 mRNA, resulting in increased BACE1 expression and APP processing. BC1 and BC200 reside in synapses, where they are involved in activity-dependent translational regulation. Finally, 17A, an antisense small ncRNA, affects the alternative splicing of the GABABR2 receptor, which leads to aberrant $GABA_B$ signalling and increased production of amyloid-β peptide. APP= amyloid precursor protein. ncRNA=non-protein-coding RNA. BC1=brain cytoplasmic RNA of 150 nucleotides. BC200=brain cytoplasmic RNA of 200 nucleotides. BACE1-AS=BACE1 antisense transcript. mRNA=messenger RNA.

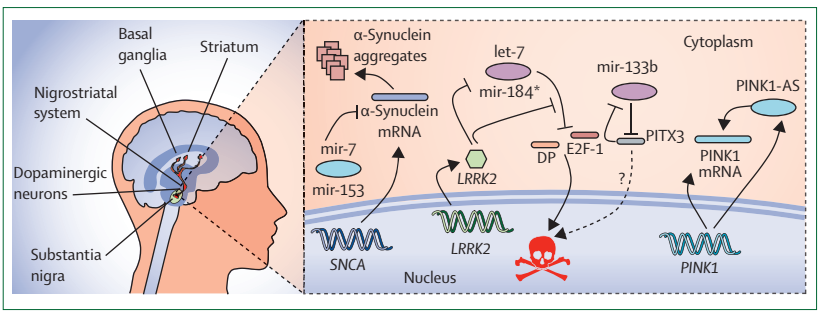

***Figure 4*: Regulatory ncRNAs in Parkinson's disease**
Examples of ncRNAs possibly directly or indirectly involved in the dopaminergic phenotype of Parkinson's disease. The expression of α-synuclein is fine-tuned by specific microRNAs (eg, mir-7,[72,73] mir-433,[74] and mir-153[72]). Under pathogenic conditions *LRRK2* mediates a microRNA-dependent transcriptional regulation mechanism in dopaminergic neurons, whereas mir-133b is involved in a negative feedback loop that results in perturbed transcriptional control. Finally, PINK1-AS and PINK1 mRNA levels are co-regulated during mitochondrial biogenesis. ncRNAs=non-protein-coding RNAs. mRNA=messenger RNA.

would remarkably improve the diagnostic toolbox for Alzheimer's disease, Parkinson's disease, Huntington's disease, and prion and other neurological diseases.

Other small regulatory ncRNAs

The number of small regulatory ncRNAs identified in the CNS is rapidly growing. siRNAs that occur naturally in plants have been used in neurology research to manipulate expression of genes, for instance, in the context of antisense therapies. Endogenous siRNAs have also been noted in mammalian cells. They apparently originate from transposable elements, cis-natural antisense transcripts, mammalian pseudogenes, and long foldback transcripts called hairpin RNAs.[25] In contrast to miRNAs, siRNAs require perfect sequence complementarity with their targets. Furthermore, they are derived from long, double-stranded RNAs and their maturation process is independent of Drosha. One study revealed with deep sequencing that expression of many endogenous siRNA-like RNAs strikingly changes after hippocampus-dependent learning.[96] Some of these short RNAs align to important neuronal proteins, such as BACE1 and hSIRT3 and, therefore, they are proposed to be functionally involved in learning-associated neuronal processes. Further experimental data are needed, however, to confirm the role of endogenous siRNAs in cognition and memory.

Certain small nucleolar RNAs identified in mouse brain have been implicated in learning and memory,[27,97] and small nuclear RNAs in the spliceosome, which is responsible for the splicing of precursor mRNA in eukaryotes, seem to have additional functions.[28] The assembly of small nuclear RNA–ribonucleoprotein complexes is directed by the survival of motor neuron protein (SMN), whereas deficiency of SMN leads to spinal muscular atrophy with characteristic motor neuron degradation.[98] Regulation of alternative splicing in the brain by small ncRNAs has been described,[99] as has altered expression of piwi-interacting RNAs in response to induced ischaemia in rat brains.[100]

Some newly identified small RNA species include promoter-associated short RNAs and termini-associated short RNAs, which originate, respectively, from regions at the 5′ or 3′ end of genes,[1] tiny RNAs associated with transcription initiation sites,[29] and RNAs associated with splice junctions.[30] Many of these small ncRNAs seem to originate from longer sense or antisense transcripts, which indicates a complex system of cis-regulatory and trans-regulatory interplay.[101] Tiling arrays (high-density microarrays consisting of oligonucleotide probes that cover the whole genome of a given species) have revealed that almost half of the protein-coding genes in the human genome are flanked by promoter-associated short RNAs and termini-associated short RNAs (22–200 nucleotides long), some of which are expressed at the same level as their overlapping genes. This finding supports claims of biological relevance,[1] but their functions in the CNS have not yet been investigated.

Two small ncRNAs of particular note are a brain cytoplasmic RNA of 200 nucleotides (BC200) and one of 150 nucleotides (BC1; figure 3). They are transcribed by RNA polymerase III and are specifically expressed in primate and rodent brains, respectively.[102,103] These two ncRNAs are actively transported to dendrites, where they participate in activity-dependent translational regulation during synaptogenesis, and in control of the excitation–repression equilibrium in the synaptodendritic compartment, which promotes neuronal plasticity.[103,104] Upregulation and cellular mislocalisation of BC200 have been seen in regions of disease-affected brain in patients with Alzheimer's disease. Thus, BC200 seems to have a reactive or even causative role in neurodegeneration related to Alzheimer's disease, possibly by perturbed translational control at the synapse.[105]

Finally, a unique, small, double-stranded, non-coding RNA has been identified that interacts with and converts the REST/NRSF complex from repressor to activator. This action triggers the expression of neuron-specific genes in adult hippocampal stem cells and directs them towards a neuronal identity.[106]

lincRNAs

Despite their abundance in the genome, only a handful of lincRNAs have so far been functionally characterised. This species of ncRNA is transcribed by RNA polymerase II from genomic loci located between two genes, and varies in length from 2000 to 20 000 nucleotides.[31] lincRNAs display limited sequence conservation across species, which raises the question of whether they represent merely transcriptional noise.[107,108] However, in a study that used a chromatin-immunoprecipitation sequencing approach (ultra-high-throughput sequencing used initially to detect transcription factors associated with transcriptionally active DNA regions), more than 1000 evolutionarily conserved lincRNAs were predicted that might counteract the function of transcription factors and, therefore, repress transcription.[31]

Another chromatin-immunoprecipitation sequencing study identified a brain-specific cluster of lincRNAs that could be implicated in brain ageing, hippocampal development, oligodendrocyte myelination, synaptic transmission, and signalling pathways involving the transcription factor CREB, GABA, and G-protein-coupled receptors.[31] Experimental evidence from a transcriptomics analysis in mice reveals a role for AK044422, a lincRNA abundantly expressed in brain tissue, in lineage specification of GABAergic neurons.[109] Of note is the fact that AK044422 RNA additionally serves as an miRNA precursor. A follow-up study identified almost 3300 lincRNAs and suggested that a common function is binding to and guidance of chromatin remodelling complexes (enzymatic complexes that catalyse chromatin modifications and lead to transcriptional silencing or activation), such as the polycomb repressive complex 2 and CoREST (a primary cofactor of REST), which has been implicated in Huntington's disease.[110–112]

NATs

NATs are RNA polymerase II transcripts that originate from the opposite strand of annotated, protein-coding sense mRNAs. Many of them have mRNA-like properties, such as 5′ capping, 3′ polyadenylation, and alternative splicing. Various studies have suggested that more than 70% of human and mouse genes undergo such antisense transcription.[32] NATs might also encode proteins, although most represent non-coding transcripts. The mechanisms of action of NATs involve both cis and trans interactions with complementary sense transcripts,[113,114] and affect a wide variety of cellular processes, including transcription, splicing, stability, and translation of mRNA, epigenetic modifications (leading to an increase or decrease in the levels of the sense mRNA transcript), and generation of endogenous siRNAs.[32,115]

Many NATs have been specifically detected in the CNS and have been postulated to regulate important neuronal processes, such as stem cell renewal and proliferation, stress responses,[116] cortical neuron specification and migration,[117] oligodendrocyte differentiation,[118] myelination,[119] synaptic transmission, plasticity,[120,121] and long-term memory formation.[122] BACE1 antisense transcript, a conserved non-coding transcript, becomes upregulated when cells are exposed to the amyloid-β peptide (figure 3).[123] It interacts with BACE1 transcript and seems to stabilise this mRNA, which leads to increased BACE1 expression and a further increase in amyloid-β production through a positive feed-forward loop.[123] BACE1 and BACE1 antisense transcripts show similar expression patterns and their levels are raised in brain samples from patients with Alzheimer's disease and in *APP* transgenic mice.[123] BACE1 antisense transcript is thought to act by masking the binding site of mir-485-5p on BACE1 mRNA; miRNAs repress translation of their target mRNA.[124]

Another amyloid-responsive NAT is the neuronal NAT-Rad18, which is upregulated after exposure of rat cortical neurons to amyloid-β peptide and leads to post-transcriptional downregulation of the DNA repair protein Rad18.[125] This finding indicates that RNA control machinery is involved in the DNA damage repair system in Alzheimer's disease. Additionally, a NAT against apolipoprotein E might be particularly relevant to this disease: levels of both apolipoprotein E and apolipoprotein E-AS1 increase in response to CNS injury, and the latter might regulate expression of the former.[126]

One of the genetic forms of Parkinson's disease is caused by mutations in the mitochondrial gene *PINK1*.[127] Antisense transcripts of the PINK1 locus have been identified in human beings and mice and are concordantly regulated with PINK1 mRNA during mitochondrial biogenesis in vivo.[128,129] In Huntington's disease, a NAT called HAR1 targets REST/NRSF and has been implicated in the pathogenesis of the disease, as greatly decreased levels have been seen in the striatum of patients.[130]

Collectively, these data suggest that NATs are important for the fine-tuning of gene expression. In view of their abundance in the CNS, these transcripts are expected to influence almost all important signalling pathways in neurobiology, and identification of many more naturally occurring antisense transcripts with regulatory roles in a variety of neurodegenerative disorders is anticipated.

Other long regulatory ncRNAs

New classes and subclasses of lncRNAs with distinct and overlapping genomic and functional features are frequently being reported. For instance, long expressed non-coding regions (also known as macroRNAs or ENORs) constitute a class of very long sense or antisense, mainly nuclear, ncRNAs that are specifically enriched in the CNS and function through antisense transcriptional regulation and genomic imprinting. They serve as precursors of other lncRNAs, miRNAs, and small nucleolar RNAs.[34]

Enhancer RNAs represent a separate class of lincRNAs and are transcribed by RNA polymerase II from activity-regulated enhancers (DNA sequences that can activate gene transcription from remote positions) that are frequently associated with the transcription of neuronal genes.[13,35,131] A brain-specific enhancer RNA, termed Evf2, is transcribed from an intergenic enhancer locus and acts through recruitment of positive (DLX family) and negative (MECP-2) transcription factors to the enhancer region, thereby increasing the transcription of the downstream gene. Of note, this first lncRNA knockout mouse model showed that Evf2 might act both in cis and in trans to control the transcription of the homoeodomain transcription factors Dlx-5 and Dlx-6 and also of the enzyme Gad1 (necessary for the conversion of glutamate to GABA) in the developing mouse brain. This effect ensures normal development of GABAergic inhibitory interneurons in the hippocampus, and proper synaptic activity in the embryonic and adult brain.[132]

Promoter-associated long RNAs are non-coding RNA transcripts that originate from the same genomic loci as

Search strategy and selection criteria

We searched PubMed with the search terms "non coding RNA", "non coding RNA and neurodegeneration", "non coding RNA and disease", and "non coding RNA and central nervous system" for papers published from 1987 to November, 2011. We also manually searched the reference lists of published work to identify further relevant references. Only papers published in English were reviewed. The final reference list was generated on the basis of originality and relevance to the broad scope of this Review.

promoter-associated short RNAs and overlap with the 5′ end and the first exons and introns of certain genes.[1]

An RNA toxic gain-of-function mechanism involving a variety of non-coding sense, antisense, intronic, or exonic RNA repeats has been implicated in several nucleotide repeat expansion disorders. These include different spinocerebellar ataxias,[133–136] myotonic dystrophies,[137,138] Huntington's disease-like 2,[139] and fragile X tremor ataxia.[140]

Many more lncRNA species have been detected in specific regions of the CNS. These include Gomafu[141] (an mRNA-like, brain-specific repetitive ncRNA that is preferentially expressed in rat brain and further gives rise to a small nucleolar RNA),[142,143] Ntab,[144] Sox2dot, an isoform of a non-coding Sox2-overlapping transcript that is enriched in regions of adult neurogenesis,[145] and metastasis-associated lung adenocarcinoma transcript 1 (a highly abundant neuronal ncRNA that has been implicated in synapse formation).[146] The latter lncRNA was identified as a binding target of TAR DNA-binding protein 43, a nuclear protein that forms inclusion bodies in frontotemporal lobar degeneration and amyotrophic lateral sclerosis.[147,148]

Conclusions and future directions

Although still in its early days, the study of ncRNAs in neurological disease, and in neurodegeneration in particular, is clearly a research area of great potential. We expect that further in-depth study of the specific patterns and expression profiles of these ncRNAs in different diseases will ultimately result in the identification of new diagnostic markers. The initial expression-profiling studies of miRNAs have shown the potential of this approach. The available information, however, should be interpreted with caution. Differences in brain sampling area, variability between individuals, insufficient clinical stratification,[47] and, in many cases, inherent bias related to the platforms used to detect the RNA molecules, could introduce obscure errors that make comparisons difficult. Moreover, to what extent the altered expression of RNA in the brain will be reflected in alterations that can be measured in biological fluids, such as CSF or plasma, remains unclear. miRNAs, for instance, have been detected in serum and plasma,[149] but their origins remain controversial. One possibility is secretion from cells via microvesicles and exosomes (membranous vesicles released from the cell surface that contribute to cell-to-cell communication) in peripheral blood or CSF.[150–154] Some studies have validated changes in miRNA concentrations in body fluids in the context of cancer and cardiovascular disorders,[155,156] which suggests that further work in neurological diseases might be fruitful.

Further study of ncRNA biology might eventually result in novel therapeutic applications. So-called RNA-based drugs, which are synthetic RNA molecules generated to mimic the mechanisms of action of endogenous ncRNAs—typically siRNAs or miRNAs—have been developed for many diseases.[157] The best developed and most studied approach is RNA interference, which involves synthetic double-stranded siRNAs, short hairpin RNAs, and artificial miRNA precursors that are mainly expressed by viral vectors and intended to suppress the translation of specific disease-related molecules.[157] Antisense molecules that antagonise miRNAs, such as antagomirs, locked nucleic acids, and morpholinos, have generally been chemically modified to improve specificity and plasma half-life in vivo.[158] Of note, the first miRNA-based therapeutic agent, a mir-122 locked nucleic acid antisense drug, is currently being tested in a phase 2 clinical trial in chronic hepatitis C patients.[159] While RNA-interference technology might become useful for the treatment of malignant and cardiovascular diseases,[160,161] neurobiology has its own particular issues, the most important being the blood–brain barrier.[162] Advances in siRNA delivery into the brain have, however, been reported.[163] Finally, issues concerning toxic effects in the CNS and off-target effects have to be seriously considered.[164–166]

An exponential increase in knowledge with regard to the role of ncRNAs in the CNS seems likely. We predict that this research will rapidly translate into increasingly precise diagnostic applications for neurology clinics. The prospects for novel treatments are realistic, but new and more efficient brain-targeting strategies are required. The versatility and straightforward nature of RNA chemistry, however, makes rapid translation into therapeutic strategies a reasonable hope.

Contributors

ES and BDS jointly did literature searches, prepared the figures and tables, and wrote and revised the paper.

Conflicts of interest

We declare that we have no conflicts of interest.

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