Long Noncoding RNAs in Development and Regeneration of the Neural Lineage

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Long noncoding RNAs (lncRNAs) are gathering increasing attention toward their roles in different biological systems. In mammals, the richest repertoires of lncRNAs are expressed in the brain and in the testis, and the diversity of lncRNAs in the nervous system is thought to be related to the diversity and the complexity of its cell types. Supporting this notion, many lncRNAs are differentially expressed between different regions of the brain or in particular cell types, and many lncRNAs are dynamically expressed during embryonic or postnatal neurogenesis. Less is known about the functions of these genes, if any, but they are increasingly implicated in diverse processes in health and disease. Here, we review the current knowledge about the roles and importance of lncRNAs in the central and peripheral nervous systems and discuss the specific niches within gene regulatory networks that might be preferentially occupied by lncRNAs.

Tens of thousands of loci in mammalian genomes produce long noncoding RNAs (lncRNAs), which are generally less abundant (by roughly an order of magnitude) and much more tissue-specific than protein-coding genes. A typical mammalian cell type expresses (at one transcript per cell, on average) at least ∼50% of all protein-coding genes, but only approximately 1000 distinct lncRNAs (<5% of the currently annotated lncRNAs). Most lncRNAs are produced from regions distal to other genes, but some, enriched with the more highly and broadly expressed ones, are produced in close proximity to promoters of other genes (most commonly in a divergent orientation) or from other “genic” regions. Another subset of lncRNAs, which is also rather abundant, serves as precursors for small RNAs, such as microRNAs and small nucleolar RNAs (snoRNAs), and the interplay between the two classes has been reviewed elsewhere (Ulitsky 2018).

Most lncRNAs are spliced, and produce “linear” isoforms that start with a cap and end with a poly(A) tail, which help stabilize the RNA, but there is an increasing appreciation of the diversity of circular RNAs (circRNAs), which are lncRNAs produced by backsplicing (Ebbesen et al. 2016). We will focus here only on linear RNA forms, as functions of the circular forms in the neural system were recently reviewed (Sekar and Liang 2019).

Studies describing where lncRNAs are expressed, utilizing techniques used to measure mRNA expression, have overall progressed rapidly. There is also an increasing number of reports on what lncRNAs do in the nervous system, described below. It is overall much less clear how lncRNAs function, but various mechanisms have been proposed for both cis and trans actions. Overall, there are no reasons to think that the mechanisms utilized by lncRNAs in the nervous system are different than those used in other tissues, but there are so far few similarities between mechanisms proposed for different lncRNAs (for a recent review, see Kopp and Mendell 2018), and so transfer of mechanistic understanding from one gene to others remains difficult.

There are several reasons to be interested in what lncRNAs do specifically in the nervous system. One is that the brain expresses a particularly rich repertoire of lncRNAs, rivaled only by that expressed in germ cells. Together with the rich cell type diversity of the nervous system, it is possible that lncRNAs often regulate cell fate decisions in the brain, and so neuronal systems can be particularly appealing for dissecting such functions. Another reason is the common diseases that affect the brain, and in which lncRNAs are commonly dysregulated (see below). From this perspective, lncRNAs can act as interesting biomarkers, potential therapeutic targets, or even potential therapeutic agents (although delivery of large RNAs remains a formidable challenge). As biomarkers, lncRNAs can be particularly appealing because of their tissue specificity, although the low abundance of the vast majority of lncRNAs will likely make it difficult to robustly detect them in distal tissues (e.g., detect in the blood lncRNAs arising from neural cells in the brain).

DIFFERENTIAL EXPRESSION OF lncRNAs IN THE BRAIN AND DURING NEURAL DIFFERENTIATION

Several large-scale resources of gene expression are now available and describe where lncRNAs are expressed at various resolutions (Table 1). Both human and mouse brains express a relatively rich repertoire of lncRNAs (Ravasi et al. 2006; Cabili et al. 2011; Derrien et al. 2012), many of which display unique temporal and spatial expression patterns within the central nervous system.
SUBCELLULAR EXPRESSION OF lncRNAs

The complex morphology of cells in the nervous system opens the question of where within the neuronal cells lncRNAs preferentially reside and act. For individual lncRNAs this is typically addressed using fluorescence in situ hybridization (FISH), recently with single-molecule approaches, which found highly variable localization patterns for lncRNAs, ranging from a single focal region in the nucleus to mRNA-like distribution in the cytoplasm (Cabili et al. 2015). More systematically, physical fractionation of cells followed by high-throughput sequencing can be used to quantify subcellular localization of all transcripts (Sterne-Weiler et al. 2013). In neurons, it is of particular interest to ask whether some lncRNAs transit to the neurites, axons, or dendrites, as there they can have unique and specific functions in neuronal pathways. Using mouse ESC (mESC)-derived neurons and RNA-seq on neurite and soma fractions, the Chekulaeva laboratory recently found that 12 of the 550 tested lncRNAs were at least twofold enriched in neurites (Zappulo et al. 2017) (∼2% compared to ∼7% of mRNAs using similar criteria), suggesting that at least in this cell type, a modest fraction of lncRNAs travels to neurites. Additional studies identified specific lncRNAs in axonal fractions (Briese et al. 2016) or sequenced RNA from axons or dendrites but did not specifically describe lncRNAs (Minis et al. 2014; Nijssen et al. 2018; Farris et al. 2019). lncRNA-centric analysis of these and new data will likely shed light on how prevalent are lncRNAs in neuronal processes.

METHODS FOR STUDYING lncRNA FUNCTIONS IN THE NERVOUS SYSTEM

Whereas the approaches described above are useful for describing where lncRNAs may act, understanding the functions of lncRNAs requires perturbations, which are generally difficult in the nervous system. Challenges include cell accessibility, the postmitotic state of most relevant cells, and the related transfection difficulties. When considering lncRNAs, these constraints meet additional ones, derived from low and sometimes nuclear-enriched expression of most lncRNAs, and their common overlap with other functional elements, such as other genes or enhancers. These difficulties can be addressed by an increasingly diverse toolbox that now contains many modalities that are used in lncRNA research (Fig. 1). These can be broadly divided into genome-editing approaches, which nowadays rely almost exclusively on CRISPR–Cas9 and which are typically applied in either mouse zygotes (for generating transgenic animals) or mESCs (for either generating transgenic animals or deriving cellular models that can then be differentiated to neuronal cells). In principle, it is also possible to edit the genomes of neuronal cell lines, but the typically large and variable number of chromosomes in these cells make it difficult to obtain homozygously edited cells. Genome editing has obvious advantages in its capability to completely ablate (CNS) and during neuronal and glial cell differentiation (Mercer et al. 2008, 2010; Ramos et al. 2013; Goff et al. 2015). The genome-wide expression patterns of mouse lncRNAs have been studied in cells isolated from different brain tissues, in neural stem cells (NSCs) isolated from the subventricular zone (SVZ) and differentiated in vitro for four days, and in embryonic stem cells (ESCs) differentiated into neural progenitor cells (NPCs) (Ramos et al. 2013). Cells in different regions of the brain, as well as cells at different stages of differentiation, had distinct lncRNA expression patterns. Mean expression specificity score in these data sets was 0.57 for lncRNAs compared to 0.45 for mRNAs, demonstrating that lncRNAs exhibit greater brain region and temporal specificity than mRNAs (Ramos et al. 2013). Another study generated a map of expression dynamics and regulatory effects of 13 lncRNA loci in mouse brain, using mouse models where the lncRNA locus was replaced with a LacZ cassette, and found dynamic and broad expression profiles in the brain, with some lncRNAs presenting highly restricted expression in few brain regions (Goff et al. 2015). These data suggest that some lncRNAs have the potential to carry out specific functions in different types of neural cells and during different stages of acquisition of a neuronal cell identity.

Table 1. Large-scale resources of long noncoding RNA (lncRNA) expression in the nervous system

<table>
<thead>
<tr>
<th>Name</th>
<th>URL</th>
<th>Tissues</th>
<th>Methodology</th>
<th>lncRNA annotations</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTEx</td>
<td><a href="https://gtexportal.org/home/index.html">https://gtexportal.org/home/index.html</a></td>
<td>54 human tissues, including 13 brain regions</td>
<td>RNA-seq</td>
<td>GENCODE/Ensembl</td>
</tr>
<tr>
<td>EBI expression atlas</td>
<td><a href="https://www.ebi.ac.uk/gxa/home">https://www.ebi.ac.uk/gxa/home</a></td>
<td>Various studies, including atlases of tissues and cell lines and differential expression</td>
<td>RNA-seq</td>
<td>GENCODE/Ensembl</td>
</tr>
<tr>
<td>ENCODE</td>
<td><a href="https://www.encodeproject.org/">https://www.encodeproject.org/</a></td>
<td>Mouse tissues (mostly adult) and human and mouse cell lines</td>
<td>RNA-seq</td>
<td>GENCODE</td>
</tr>
<tr>
<td>Eurexpress</td>
<td><a href="http://www.eurexpress.org/ec/">http://www.eurexpress.org/ec/</a></td>
<td>Mouse in situ images</td>
<td>FISH</td>
<td>Various (mapped to FISH probes)</td>
</tr>
<tr>
<td>Kaessmann laboratory</td>
<td><a href="https://kaessmannlab.org/ncrna_app/">https://kaessmannlab.org/ncrna_app/</a></td>
<td>Developmental time course in 7 tissues in 7 species</td>
<td>RNA-seq</td>
<td>De novo, reconstruction based on RNA-seq data</td>
</tr>
</tbody>
</table>
some editing approaches, like introduction of polyadenylation
methods (Bassett et al. 2014; Kopp and Mendell 2018).
Methods for the study of long noncoding RNAs (lncRNAs). (A) Transgenic animals can be generated using introduction of
genome engineering reagents into pluripotent stem cells, mouse zygotes, or early embryos, to induce deletions of lncRNA loci or parts
thereof, and insertion of various “stop” signals or insertion ofloxP sites that can be used for conditional manipulations. The resulting cells
can then be used to derive and breed animal models. The indicated reagents can then be introduced into wild type (WT) or transgenic
animals in the embryonic (e.g., using electroporation) or adult stages. Primary cells can be derived from embryonic or adult neural tissues
and used in culture, when the indicated methods of manipulation are available and can be applied prior to differentiation of the cells into
more mature populations or in vitro injury models. (B) Embryonic stem cells (ESCs) can be manipulated using the engineering methods
described in A or by introducing the indicated reagents, and then differentiated into neural progenitor cells (NPCs) and then glial or
neuronal cells. (C) Neuronal cell lines, such as Neuro2a/N2a, can be transfected with various reagents and then differentiated into
postmitotic neuronal cells. (siRNA) Small interfering RNA, (shRNA) short hairpin RNA, (ASOs) antisense oligonucleotides.
loci and the resulting uniform cellular population. However,
several caveats have to be considered. First, as
lncRNA loci may overlap other elements, pinpointing
perturbations, ideally using the same system and the same
methods (Bassett et al. 2014; Kopp and Mendell 2018).
Some editing approaches, like introduction of polyadenylation
sequences, are considered relatively “safe” as they
do not delete any DNA sequences, but recent discrepancies
between the results obtained after editing the
Hand2us locus hint that even this approach should be
interpreted with caution (Anderson et al. 2016; Han
et al. 2019). Promoter deletions are commonly used for
lncRNA inactivation and provide a useful compromise
between deletion of a relatively short stretch of DNA
(compared to whole locus deletion) while having reasonable
likelihood to eliminate transcription, although activation
of cryptic promoters is often observed (Lavalou et al.
Another caveat is that successful elimination of lncRNA
production still makes it difficult to distinguish between
the function of the RNA product and the act of its tran-
scription (Kopp and Mendell 2018). The recent descrip-
tion of introduction loci of self-cleaving ribozymes into
lncRNA loci is an exciting progress in this direction (Tuck
et al. 2018), but the efficiency of these elements in reduc-
gene expression is variable, and it is not yet clear how
fast the cleavage occurs. Because of that, it is not yet clear
if self-cleaving ribozymes may also affect transcription
elongation.

The last major caveat is mostly relevant to editing done
in cultured cells and, in particular, in pluripotent stem
cells. The act of genome editing and, in particular, the
double-strand breaks that it usually involves can trigger
a stress response that may have lasting effects on the cells
and on their ability to later differentiate into different lin-
edges. The general process of establishing stable clones,
which typically requires disruptive actions such as cell
dissociation and several passages, can also have lasting
effects. As a result of these, any edited clone will differ
from the original cells and from other clones in ways that
may or may not relate to the genetic changes that were
acquired. Analysis of multiple independently established
clones, as well as of control clones that experienced a very
similar procedure (ideally, editing in a bystander locus),
can help alleviate these concerns, but this remains very
laborious and is still not routinely performed.

Transient introduction of perturbation reagents, such as
siRNAs or antisense oligos, typically does not suffer from
these caveats but is associated with many other concerns.
These perturbations may not lower expression levels be-
low those required for function, may lead to off-target
effects (which are well-understood for RNAi, but almost
everly unexplored for antisense oligonucleotides [ASOs]
or GapmeRs), and the introduction of the reagents via
transfection or infection may activate a stress response.
Because of these concerns, it is essential to deploy and
compare different combinations of perturbation tech-
niques, which often include genome editing and transient
perturbations, ideally using the same system and the same
phenotypic assays, although it is not always possible, in
particular given the challenges involved in working with
neuronal cells.

FUNCTIONS OF lncRNAs IN DEVELOPMENT
OF THE NERVOUS SYSTEM

Different in vivo and in vitro models have been used to study the functions of lncRNAs during neuronal differen-

Figure 1. Methods for the study of long noncoding RNAs (lncRNAs). (A) Transgenic animals can be generated using introduction of
genome engineering reagents into pluripotent stem cells, mouse zygotes, or early embryos, to induce deletions of lncRNA loci or parts
thereof, and insertion of various “stop” signals or insertion ofloxP sites that can be used for conditional manipulations. The resulting cells
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tiation and during nervous system development (Table 2). The roles of Cyrano and Megamind, two deeply conserved lncRNAs, were studied during the development of zebrafish embryos by injection of morpholino (MO) antisense oligos, which led to severe defects in brain development (Ulitsky et al. 2011), which were not observed in genetic models later generated for these lncRNAs (Goudarzi et al. 2019; Kok et al. 2015). The function and mode of function of Cyrano was also studied by the generation of knockout (KO) mice (Kleaveland et al. 2018). Loss of Cyrano did not result in any detectable effects on survival, appearance or behavior in mice, but it led to the accumulation of miR-7 and down-regulation of Cdr1as, a circRNA known to regulate neuronal activity (Piwecka et al. 2017; Kleaveland et al. 2018). The function of Megamind (also known as Tunar) was also studied during neuronal differentiation of mESCs. In that system, Megamind knockdown (KD) with shRNAs reduced the differentiation efficiency, demonstrating its potentially conserved role in the acquisition of neuronal cell fate (Lin et al. 2014).

Several studies found roles for lncRNAs during mouse brain development. KO of Evy2, which is transcribed from the Dlx-5/6 ultraconserved region (Feng et al. 2006; Bond et al. 2009), led to reduced numbers of GABAergic interneurons in early postnatal hippocampus and dentate gyrus (Bond et al. 2009). Pantr2 (line-Brn1b) KO mice have defects in the proliferation of cortical progenitors in the subventricular zone (SVZ) during development (Sauvaageau et al. 2013). In addition to the generation of mouse models, NSCs can be isolated from embryonic, postnatal, or adult mice and used for targeting different lncRNAs, optionally followed by in vitro differentiation. Pnky was studied in the mouse cortex using different methods: electroporation of shRNAs to ventricular zone stem cells during development, infection of shRNAs to cultured NSCs from postnatal mouse brain (Ramos et al. 2015), and generation of conditional KO mice (Andersen et al. 2019), which showed that Pnky interacts with PTBP1 splicing factor and acts in trans to regulate proliferation of NSCs and neurogenesis. In vitro differentiation of mouse embryonic NSCs was used to study the function of lnc-OPC. KD of this lncRNA using shRNAs during differentiation of NSCs to oligodendrocyte progenitor cells (OPCs) significantly repressed OPC markers (Dong et al. 2015). Overall these studies have shown that lncRNAs can modulate progenitor proliferation and differentiation and have profound effects on brain development. lncRNAs are also involved in developmental processes in the postnatal nervous system, such as myelination, retinal development, and neuronal outgrowth. The function of four lncRNAs highly expressed in oligodendrocyte lineage cells, lncOLI-1-4, was studied by siRNA transfection during oligodendrocyte differentiation of primary postnatal OPCs. KD of each of these lncRNAs repressed myelin genes (He et al. 2017). lncOLI-1 was further studied in KO mice, in which severe defects in myelination were observed at P9 and postnatal week 3 (He et al. 2017). During retinal development, Six3OS is transcribed from an independent promoter separated by ~4 kb from the promoter of Six3. Six3OS was studied by overexpression or KD with shRNAs in P0.5 mouse retina and was found to be involved in retinal cell specification by binding factors known to co-regulate target genes with SIX3 and potentially recruiting histone-modifying enzymes to SIX3 targets (Rapicavoli et al. 2011). KD of Six3OS in adult SVZ NSCs during differentiation reduced neurogenesis, suggesting that, in addition to its role in retinal development, this lncRNA has roles also in neuronal differentiation (Ramos et al. 2013). Another lncRNA, BDNF-AS, which was studied both by transfection of siRNAs or ASOs to neurospheres and by intracerebroventricular delivery of ASOs to adult mice was found to regulate neuronal outgrowth and differentiation both in vitro and in vivo (Modarresi et al. 2012).

Other studies focused on in vitro neuronal differentiation of ESCs or neuroblastoma cell lines, which allow the interrogation of several lncRNAs in parallel. ESCs can be differentiated into NPCs and further into different types of neurons and glial cells, and in recent years, they have been used to identify and characterize the functions of lncRNAs in this process. RMST and three additional lncRNAs have been identified in a study based on neuronal differentiation of human ESCs (hESCs) (Ng et al. 2012), which also found that their KD with siRNAs blocked neurogenesis. A later study found that RMST interacts with SOX2 and promotes SOX2 binding to its target sites in the genome during neuronal differentiation (Ng et al. 2013). In haploid mESCs, the overexpression and genetic perturbation of lnc-Nr2fl1 led to dysregulation of hundreds of genes as the cells were differentiated into neurons, with down-regulated genes related to neuronal pathfinding and axon guidance (Ang et al. 2019). Conversely, overexpression of lnc-Nr2fl1 during ESC differentiation and during reprogramming of mouse embryonic fibroblasts (MEFs) to induced neurons (iNSs) led to increased neurite length and increased neural conversion (Ang et al. 2019). Another study focused on the differentiation of mESCs to motor neurons (MNs) (Yen et al. 2018). Differentiated MNs were enriched with Dlk-Dio3 locus-derived lncRNAs including Meg3. The depletion of Meg3 resulted in dysregulation of progenitor and caudal Hox genes (Yen et al. 2018). Neuroblastoma cell lines are another in vitro model of neuronal cells that are generally more accessible to perturbations compared to primary cells. These cells, including the commonly used mouse Neuro2a/N2a and human SH-SY5Y cell lines, express many neuronal markers, and when exposed to specific neurotrophic factors, they further differentiate, exit the cell cycle, and extend neurites. Paupar, which is transcribed from a locus upstream of Pax6, was studied in N2a cells by transfection of shRNAs and was found to act both in cis, by regulating Pax6 expression, and in trans, by binding and regulating Pax6 activity, affecting cell cycle progression and neural differentiation (Vance et al. 2014). A later study found that Paupar binds the KAP1 chromatin regulator and promotes KAP1 occupancy to Pax6 targets (Pavlaki et al. 2018). Interestingly, in pancreatic α cells Paupar appears to regulate splicing and not expression of Pax6 (Singer et al. 2019). Dali, also studied with shRNAs in N2a cells, was reported to locally regulate the transcription of the Pou3f3
<table>
<thead>
<tr>
<th>Name</th>
<th>Systems studied</th>
<th>Methods of perturbation</th>
<th>Phenotype</th>
<th>Suggested mode of action</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyrano/OIP5-AS1</td>
<td>Zebrafish embryos, adult mice</td>
<td>MO antisense oligos, deletion of conserved region and whole gene deletion</td>
<td>Zebrafish: defects in neural tube opening, loss of neurons in the retina and tectum, enlarged nasal placodes. These phenotypes were not observed when the whole locus was deleted. Mouse: Cyrano pairing to miR-7 promotes miR-7 degradation, leading to increased expression of Cdr1 as circRNA in neuronal cell bodies and processes.</td>
<td>Ulitsky et al. 2011; Kleaveland et al. 2018; Goudarzi et al. 2019</td>
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<tr>
<td>Megamin/Tunar</td>
<td>Zebrafish embryo, mESCs</td>
<td>MO antisense oligos, shRNAs</td>
<td>Zebrafish: smaller heads and eyes, enlarged brain ventricles, loss of neurons in the retina and tectum. These phenotypes were not observed when the whole locus was deleted. mESCs: reduced differentiation efficiency</td>
<td>Ulitsky et al. 2011; Lin et al. 2014; Kok et al. 2015</td>
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<tr>
<td>Evf2</td>
<td>Mouse embryos and adults, C17 and MN9D neural cell lines</td>
<td>Insertion of a triple polyadenylation signal into exon 1, overexpression</td>
<td>Reduced numbers of GABAergic interneurons, reduced synaptic inhibition</td>
<td>Bond et al. 2009; Feng et al. 2006; Cajigas et al. 2015, 2018</td>
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<tr>
<td>Pantr2 / linc-Brn1b</td>
<td>Mouse embryos</td>
<td>Replacement of entire gene locus with a lacZ reporter cassette</td>
<td>Reduction in the number of intermediate progenitor cells in the cerebral cortex</td>
<td>Sauvageau et al. 2013</td>
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<tr>
<td>Pnky</td>
<td>Mouse embryos, cultured postnatal NSCs</td>
<td>shRNAs, conditional KO by flanking the entire gene with loxP sites</td>
<td>Cultured NSCs: decreased population of proliferating cells in the ventricular zone, increased neurogenesis In vivo cortical development: malformation of the postnatal neocortex, increasing the number of deep-layer neurons while decreasing the population of upper-layer neurons</td>
<td>Ramos et al. 2015; Andersen et al. 2019</td>
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<tr>
<td>lnc-OPC</td>
<td>Cultures of mouse embryonic NSCs</td>
<td>shRNAs</td>
<td>Decreased expression of OPC markers</td>
<td>Dong et al. 2015</td>
<td></td>
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<tr>
<td>BDNF-AS</td>
<td>Mouse neurospheres, adult mice</td>
<td>siRNAs, ASOs</td>
<td>Increased neurite outgrowth and neuronal differentiation Absence of myelinated axons at P9, reduced numbers of myelinated axons at postnatal week 3</td>
<td>Modarresi et al. 2012</td>
<td></td>
</tr>
<tr>
<td>lncOL1</td>
<td>Postnatal mice, cultured OPCs</td>
<td>siRNAs, deletion of exon 1</td>
<td>Absence of myelinated axons at P9, reduced numbers of myelinated axons at postnatal week 3 3</td>
<td>He et al. 2017</td>
<td></td>
</tr>
<tr>
<td>Six3os</td>
<td>Postnatal mice, cultured adult NSCs</td>
<td>Overexpression, shRNAs</td>
<td>Postnatal mice: following KD, decrease in the fraction of bipolar cells, increase in Muller glia; following overexpression, reduction in syntaxin staining Cultured NSCs: reduced numbers of TuJ1- and Olig2-expressing cells, increased numbers of GFAP-expressing cells</td>
<td>Rapicavoli et al. 2011; Ramos et al. 2013</td>
<td></td>
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<td>RMST</td>
<td>hESCs</td>
<td>siRNAs</td>
<td>Arrested neurogenesis</td>
<td>Ng et al. 2013, 2012</td>
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<td>Name</td>
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<tr>
<td>lnc-Nr2f1</td>
<td>mESCs, reprogramming of MEFs to iNs</td>
<td>KO by insertion of a poly (A) transcriptional termination signal, overexpression</td>
<td>Overexpression: increased neurite length, induction of genes with functions in axon guidance, increased conversion of MEFs to iNs KO: down-regulation of neuronal pathfinding and axon guidance genes</td>
<td><em>lnc-Nr2f1</em> binds to distinct genomic loci regulating neuronal genes.</td>
<td>Ang et al. 2019</td>
</tr>
<tr>
<td>Meg3, Rian, Mirc</td>
<td>mESCs, mouse embryos</td>
<td>Deletion of maternal IG-DMR, shRNAs</td>
<td>Dysregulation of progenitor and caudal Hox genes, motor axon innervation defects</td>
<td><em>Meg3</em> facilitates the binding of PRC2 and Jarid2, leading to global down-regulation of H3K27me3 and aberrant expression of progenitor and caudal Hox genes in postmitotic MNs.</td>
<td>Yen et al. 2018</td>
</tr>
<tr>
<td>Paupar</td>
<td>N2a cells, cultured neurospheres, postnatal mice</td>
<td>shRNAs</td>
<td>Reduced proliferation, increased neurite outgrowth, reduced number and altered morphology of olfactory bulb neurons</td>
<td><em>Paupar</em> binds KAP1 and promotes KAP1 occupancy and H3K9me3 deposition at PAX6 bound loci.</td>
<td>Vance et al. 2014; Pavlaki et al. 2018</td>
</tr>
<tr>
<td>Dali</td>
<td>N2a cells</td>
<td>shRNAs</td>
<td>Reduced neurite outgrowth following differentiation</td>
<td><em>Dali</em> interacts with the POU3F TF and the DNA methyltransferase DNMT1 and directly binds genomic loci to regulate gene expression.</td>
<td>Chalei et al. 2014</td>
</tr>
<tr>
<td>lncND</td>
<td>hESCs, SH-SY5Y cells, mouse embryo</td>
<td>siRNAs, in utero electroporation of human transcript to mouse radial glia cells</td>
<td>SH-SY5Y cells: reduced cell proliferation and increased neuronal differentiation Mouse embryo: expansion of radial glia population</td>
<td><em>lncND</em> sequesters miR-143-3p in NPCs, leading to increased production of NOTCH proteins.</td>
<td>Rani et al. 2016</td>
</tr>
</tbody>
</table>

(MO) Morpholino, (circRNA) circular RNAs, (mESCs) mouse embryonic stem cells, (shRNAs) short hairpin RNAs, (TFs) transcription factors, (N/A) not applicable, (NSCs) neural stem cells, (KO) knockout, (siRNAs) small interfering RNAs, (ASO) antisense oligonucleotide, (OPCs) oligodendrocyte progenitor cells, (KD) knockdown, (hESCs) human embryonic stem cells, (MEFs) mouse embryonic fibroblasts, (iNs) induced neurons, (MNs) motor neurons, (NPCs) neural progenitor cells.
transcription factor (TF) and distally bind active promoters and regulate the expression of genes related to neuronal differentiation through physical association with the POU3F3 protein (Chalei et al. 2014).

The combination of different in vitro and in vivo systems can be used to efficiently characterize the functions of lncRNAs in neuronal differentiation. For example, lncND, a primate-specific lncRNA, was found to regulate Notch signaling during neuronal differentiation by sequestering miR-143-3p (Rani et al. 2016). hESCs were used to characterize the expression dynamics of lncND during neuronal differentiation, siRNA transfection in SH-SY5Y cells was then used to study the functions of lncND during proliferation and differentiation, and, finally, injection of the human lncND transcript to the developing mouse cortex, which resulted in expansion of a radial glia population, supported the hypothesis that lncND contributed to the expansion of radial glia in higher primates (Rani et al. 2016).

THE ROLE OF lncRNAs IN NEUROREGENERATION

Neurons within the peripheral nervous system (PNS) can undergo axon outgrowth, which may lead to substantial functional recovery, while this process is limited within the CNS. Following axotomy, PNS neurons activate a unique regenerative transcriptional program and up-regulate numerous regeneration-associated genes (RAGs) (Curcio and Bradke 2018). The differences in the ability of CNS neurons to activate RAG expression, along with extracellular environmental factors, can explain why CNS neurons do not regenerate successfully (Ma and Willis 2015). Modulating critical hubs of RAG transcription can therefore have important therapeutic implications (Gao et al. 2016).

The next section describes the role of lncRNAs in nerve regeneration following different types of injuries, as demonstrated by in vitro and in vivo methods (Fig. 2 and Table 3).

Figure 2. Long noncoding RNAs (lncRNAs) implicated in response to neuronal injury. Names of lncRNAs are indicated next to the cell types and injury types in which they have been studied.
SNI. Therefore, Silc1 regulates neuroregeneration in cultured cells and in vivo, through cis-acting activation of the Sox11 TF, through a currently unknown mechanism (Perry et al. 2018).

IncRNAs are also important for Schwann cells regulation after PNI. IncRNAs in Schwann cells were profiled using microarrays with RNA from the distal segment of the mouse sciatic nerve, where the Wallerian degeneration process occurs (Pan et al. 2017b). One of these, NON-MMUG014387, promoted mouse Schwann cell proliferation by increasing Cthrc1 expression and activating the Wnt/PCP pathway (Pan et al. 2017a). IncRNA BC088327 was implicated in Schwann cell proliferation in a rat model with SNI after treatment with Heregulin-1β. BC088327 may play a synergistic role with heregulin-1β in repairing PNI (Wang et al. 2018).

The myelination process is also crucial for PNS nerve regeneration. Recently it was found that the EGR2 TF is an important modulator in this process in mice. Egr2 is down-regulated during SNI, whereas Egr2-AS IncRNA, transcribed antisense to the proximal promoter of Egr2, is up-regulated. Overexpressing Egr2-AS in mouse DRG explant cultures results in inhibition of Egr2 mRNA expression and induces demyelination, suggesting that this IncRNA acts in trans. Inhibition of Egr2-AS in vivo using GapmeRs at the time of SNI, rescues the inhibition of Egr2 transcript expression and affects EGR2-regulated genes to delay demyelination. Mechanistically, it was proposed that Egr2-AS recruits H3K27me3, AGO1, AGO2, and EZH2 to the Egr2 promoter following SNI. Furthermore, expression of Egr2-AS is regulated through ERK1/2 signaling to YY1 (Martinez-Moreno et al. 2017). More, expression of Egr2-AS is regulated through ERK1/2 signaling to YY1 (Martinez-Moreno et al. 2017).

An additional important process in PNS regeneration is Schwann cell migration, which can precede and enhance axonal repair by guiding axon reinnervation and controlling synaptic formation. Silencing of the TNXA-PS1 IncRNA in vitro and in vivo by siRNAs promoted Schwann cell migration. Additional experiments showed that TNXA-PS1 might exert its regulatory role by sponging miR-24-3p/miR-152-3p and affecting Dusp1 expression (Yao et al. 2018).

### IncRNAs in Spinal Cord Injury

Primary spinal cord injury (SCI) is commonly caused by direct trauma or pathological alterations; it is much more complex than a PNS injury and thus involves a greater role for noncoding RNA, particularly long noncoding RNAs (lncRNAs). The myelination process is also crucial for PNS nerve regeneration. lncRNAs are also important for Schwann cells regulation after PNI. lncRNAs in Schwann cells were profiled using microarrays with RNA from the distal segment of the mouse sciatic nerve, where the Wallerian degeneration process occurs (Pan et al. 2017b). One of these, NON-MMUG014387, promoted mouse Schwann cell proliferation by increasing Cthrc1 expression and activating the Wnt/PCP pathway (Pan et al. 2017a). IncRNA BC088327 was implicated in Schwann cell proliferation in a rat model with SNI after treatment with Heregulin-1β. BC088327 may play a synergistic role with heregulin-1β in repairing PNI (Wang et al. 2018).

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### Table 3. Roles of long noncoding RNAs (lncRNAs) in neuroregeneration

<table>
<thead>
<tr>
<th>Name</th>
<th>Systems studied</th>
<th>Methods of perturbation</th>
<th>Phenotype</th>
<th>Suggested mode of action</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC089918</td>
<td>SNI in rat model and DRG culture cells</td>
<td>siRNAs</td>
<td>Negative effect on neurite outgrowth of DRG culture cells</td>
<td>N/A</td>
<td>Yu et al. 2013</td>
</tr>
<tr>
<td>Uc.217</td>
<td>SNI in rat model and DRG culture cells</td>
<td>siRNAs</td>
<td>Negative effect on neurite outgrowth of DRG culture cells</td>
<td>N/A</td>
<td>Yao et al. 2015</td>
</tr>
<tr>
<td>Silc1</td>
<td>SNI in mouse and DRG culture cells</td>
<td>siRNAs, KO by deletion of promoter and first exon</td>
<td>Reduction in total axonal outgrowth; delayed regeneration following sciatic injury</td>
<td>cis-acting activation of Sox11</td>
<td>Perry et al. 2018</td>
</tr>
<tr>
<td>NONMMUG014387</td>
<td>SNI in mouse and Schwann cells</td>
<td>Overexpression</td>
<td>Increased proliferation of Schwann cells</td>
<td>Activation of Wnt/PCP pathway</td>
<td>Pan et al. 2017a, Wang et al. 2018</td>
</tr>
<tr>
<td>BC088327</td>
<td>SNI in mouse and Schwann cells</td>
<td>Overexpression, GapmeRs</td>
<td>Increased demyelination</td>
<td>Inhibition of Egr2 expression</td>
<td>Martinez-Moreno et al. 2017</td>
</tr>
<tr>
<td>Egr2-AS</td>
<td>SNI in mouse and DRG explant culture</td>
<td>siRNAs</td>
<td>Reduced Schwann cell migration</td>
<td>Sponging miR-24-3p/miR-152-3p and regulation of Dusp1</td>
<td>Zhang et al. 2018a, Wanga et al. 2018</td>
</tr>
<tr>
<td>TNXA-PS1</td>
<td>Rat SNI and Schwann cells culture</td>
<td>siRNAs</td>
<td>Reduced Schwann cell migration</td>
<td>Sponging miR-130b-5p to regulate PRDM5</td>
<td>Zhang et al. 2018b, Zhang et al. 2018b</td>
</tr>
<tr>
<td>BDNF-AS</td>
<td>Rat acute SCI and hypoxia cellular model, neuronal cell lines</td>
<td>siRNAs</td>
<td>Increased neuronal cell apoptosis</td>
<td>Binding and negative regulation of PRDM5</td>
<td>Zhang et al. 2018b, Zhang et al. 2018b</td>
</tr>
<tr>
<td>DGCR5</td>
<td>Rat acute SCI and hypoxia cellular model, neuronal cell lines</td>
<td>siRNAs</td>
<td>Reduced neuronal apoptosis</td>
<td>N/A</td>
<td>Jiang and Zhang 2018</td>
</tr>
<tr>
<td>SNHG5</td>
<td>Rat SCI and primary cultured astrocytes</td>
<td>siRNAs, overexpression</td>
<td>Enhanced viability of astrocytes and microglia</td>
<td>N/A</td>
<td>Wang et al. 2018</td>
</tr>
<tr>
<td>GAS5</td>
<td>Mouse TBI and primary culture neuronal cells</td>
<td>siRNAs</td>
<td>Increased neuronal apoptosis</td>
<td>Sponging miR-335 to activate Rasa1</td>
<td>Wang et al. 2017, Dai et al. 2019</td>
</tr>
<tr>
<td>CRNDE</td>
<td>Rat TBI</td>
<td>siRNAs</td>
<td>Increased nerve repair</td>
<td>N/A</td>
<td>Yi et al. 2019</td>
</tr>
</tbody>
</table>

(SNI) Spinal nerve injury, (DRG) dorsal root ganglion, (siRNAs) small interfering RNAs, (N/A) not applicable, (KO) knockout, (SCI) spinal cord injury, (TBI) traumatic brain injury.
more complicated than PNI because of extensive cell loss, axonal disruption, glial scar, and shortage of growth-permissive factors (Estrada and Müller 2014). This is followed by secondary injury mechanisms, including glutaminergic excitotoxicity, oxidative stress, increased adaptive immune responses, Wallerian degeneration, and scar tissue formation, leading to further structural and functional disturbances (Ahuja et al. 2017). Various types of ncRNAs were implicated in these processes (Ning et al. 2014; Qin et al. 2018; Li et al. 2019b; Pinchi et al. 2019; Yao and Yu 2019; Zhou et al. 2019). The roles of IncRNAs after SCI have been recently thoroughly reviewed (Li et al. 2019b), and so we will present only a few examples here. BDNF-AS IncRNA is up-regulated in a rat acute SCI model. BDNF-AS KD by siRNAs in neuronal cell lines reduced neuronal cell apoptosis in hypoxic conditions. It was suggested that BDNF-AS associates with miR-130b-5p, which is repressed during SNI. In vivo, BDNF-AS KD with siRNAs inhibited the expression of PRDM5, supposedly through competitive binding with miR-130b-5p, resulting in decreased apoptosis (Zhang et al. 2018a). In contrast, DGCR5 IncRNA was down-regulated in this model and in neurons treated with hypoxia (Zhang et al. 2018b). Using in vitro and in vivo methods it was shown that DGCR5 suppresses neuronal apoptosis through directly binding and negatively regulating PRDM5, thereby ameliorating SCI (Zhang et al. 2018b).

IncRNA SNHG5 is an example of an IncRNA that is induced following SCI and enhances the viability of astrocytes and microglia. SNHG5 overexpression promoted cell viability of both astrocytes or microglia, while its down-regulation by siRNAs led to cell death (Jiang and Zhang 2018). SNHG5 overexpression in vivo results in reduced motor recovery post-SCI in rats. Additional experiments demonstrated that SNHG5 enhances the expression of both KLF4 and eNOS (Jiang and Zhang 2018). Additional IncRNAs that have a proposed role in SCI are IncSCRIR1, Casc7, MALAT1, XIST, and Map2K4 (Li et al. 2019b).

IncRNAs IN TRAUMATIC BRAIN INJURY

Traumatic brain injury (TBI) is a significant source of morbidity and mortality in the adult population. TBI causes secondary biochemical changes that contribute to neurological dysfunction, delayed neuroinflammation, and nerve cell death (Kabadi and Faden 2014). IncRNA dysregulation after TBI was observed in the cerebral cortex and hippocampus (Zhong et al. 2016; Wang et al. 2017). The current progress of studies on IncRNAs in TBI was recently reviewed (Zhang and Wang 2019). Therefore, several IncRNAs, including GAS5, MEG3, and CRNDE, were found to be dysregulated in the brains of HD patients (Johnson 2012).

Different IncRNAs, such as naPINK1, NEAT1, PINK1-AS, BC200, and Sox2OT, were found to be dysregulated in Parkinson’s disease (PD) patients’ brains (Wan et al. 2017). Recently it was found that IncRNA Neat1 is
significantly up-regulated in the midbrain of PD mice, and it was suggested that Neat1 promotes MPTP-induced autophagy in PD by stabilizing PINK1 protein (Yan et al. 2018).

An increasing number of studies report on lncRNAs as being implicated in additional neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), multiple system atrophy (MSA), frontotemporal lobar degeneration (FTLD), and glaucoma (Riva et al. 2016; Quan et al. 2017; Wan et al. 2017; Wang et al. 2017).

CONCEPTUAL ROLES OF lncRNAs IN THE NERVOUS SYSTEM

We summarized here a large number of recent reports on various functional outcomes of lncRNA perturbations in the nervous system. What ties together these observations? One trend is that a large number of the observed phenotypes were related to changes in proliferation of progenitors or their ability to give rise to particular populations. Importantly, these are also likely the easiest phenotypes to score in the nervous system, which provides a possible explanation for their prevalence in the literature.

Beyond the need to regulate the balance between proliferation and differentiation and to specify and then “lock” different fates, cells in the nervous system have several features that may be particularly prone to lncRNA regulation. First, the vast majority of the cells in the adult nervous system are postmitotic and so cannot rely on replication-dependent histone exchange for chromatin regulation. Transcription- or lncRNA-mediated effects thus may replace some of the regulatory processes that rely more heavily on replication-associated mechanisms in more proliferative cells (e.g., in colon or blood). Such mechanisms likely play an outsized role in the ability of neurons to regenerate in the absence of cell proliferation. Another prominent feature of the nervous system is the requirement for rapid changes in gene expression upon specific cues, such as neuronal activity, which requires maintenance of chromatin in a particularly poised state, as well as extensive post-transcriptional regulation, that acts to limit the expression window of genes induced in the early stages of the response or allows fast maturation and export of particularly long genes (Mauger et al. 2016).

Last, the size and complex morphology of neural cells dictate the need for complex post-transcriptional regulation, which may benefit from RNA-based control. Future studies will elucidate which of these or other features of the neural environment preferentially rely on lncRNA function and may explain why neural cells express a particularly vibrant complement of lncRNA genes.

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