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Regulatory Long Non-coding RNAs and Neuronal Disorders

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Two major classes of neurons are responsible for maintaining the balance between excitation and inhibition in the brain. While excitatory projection neurons send “go” signals through the neurotransmitter glutamate, inhibitory local circuit interneurons send “stop” signals through the neurotransmitter gamma-aminobutyric acid (GABA). To form GABA, GABAergic interneurons produce glutamate decarboxylase (GAD), the rate-limiting enzyme converting glutamate into GABA. It has recently become clear that glutamatergic and GABAergic synapses utilize fundamentally different mechanisms¹. GABAergic interneuron diversity has recently been classified into groups based on morphology, gene expression and electrophysiology². Given such diversity, it would be expected that GABAergic interneurons have multiple regulatory functions beyond that of a simple on/off switch³. It has recently become clear that GABAergic interneurons have diverse functions such as neuronal proliferation, migration and differentiation during development, and temporal synchrony and refinement of local cortical circuits^{4–8}. Therefore, loss of the “stop” signal from altered GABAergic interneuron transmission would ultimately be expected to result in abnormal brain function.

In support of this idea, altered GABAergic regulated circuits have been implicated in different neurological disorders such as schizophrenia, autism, Tourette's syndrome, Rett syndrome, and epilepsy. In epilepsy, decreased inhibition resulting from mutations in genes encoding ion channels or GABA receptors can cause uncontrolled neuronal firing^{9,10}. In addition, disruption of *Dlx1* and *Arx1* homeodomain transcription factors critical for GABAergic interneuron migration during development causes epilepsy in mice and humans, respectively^{11,12}. A consistent finding among schizophrenic patients is reduction in prefrontal cortex GAD67, the enzyme required for GABA synthesis¹³. Also, single nucleotide polymorphisms¹⁴ in the 5' regulatory region of the *GAD1* gene that codes for GAD67 have been linked to childhood onset schizophrenia. In addition, it has been proposed that increased hypermethylation of the *GAD1* promoter may cause decreased GAD67 expression in schizophrenic patients^{15,16}.

In autism spectrum disorders (ASD)¹⁷, it has been proposed that reduced minicolumn width in specific cortical layers may result from altered GABAergic function^{18–20} ultimately disrupting connectivity. It has also been proposed that Rett syndrome, an ASD caused by mutations in the methyl CpG-binding protein MECP2 in humans, may result from altered imprinting of the *Dlx5* gene, a member of a family of transcription factors critical for GABAergic interneuron differentiation²¹. Importantly, MECP2 mutant mice carrying an exon 3 deletion display decreased inhibitory cortical activity²². Therefore, multiple reports

support the idea that altered GABAergic function can cause a variety of neurological diseases.

Although single genetic loci have been linked to complex mental disorders such as autism and schizophrenia, these diseases are thought to result from the interaction of multiple genes and/or environmental factors. Evidence that a complex mental disorder can result from mutation of a single gene came from studies on Rett syndrome, an ASD that specifically affects neurons and causes Rett specific behavioral phenotypes^{23–25}. Despite the fact that *Mecp2* is a single gene, its ability to bind a common DNA modification still supports the idea that multiple gene targets are involved in the etiology of complex mental disorders. While the identification of *MECP2* established a link between aberrant epigenetic modification and a complex mental disorder, the mechanism by which *MECP2* mutations specifically affect neurons and cause Rett-specific behavioral phenotypes is still not clear.

Increasing evidence in the literature supports a role for non-protein coding RNAs (ncRNAs) in neurological disease. ncRNAs are functional RNA molecules, such as transfer RNA, ribosomal RNA, snoRNA, microRNA, siRNA, piRNA, and long non-coding RNA. While large-scale genomic studies reveal that ncRNAs are abundantly expressed, studies on individual ncRNAs reveal novel roles in transcriptional regulation and DNA methylation control^{26–28}. In addition to multiple recent reviews, a comprehensive review of ncRNAs specifically involved in retinal development has recently been published²⁹. Table 1 describes some of the known non-coding RNAs with possible implications in neurological disorders. Specifically, an anti-sense beta-secretase 1 RNA (*BACE1-AS*) stabilizes *BACE1* RNA, resulting in elevated amyloid-beta protein in Alzheimer's patients³⁰. In this case, *BACE1-AS* functions through a post-transcriptional feed-forward mechanism. Anti-sense nitric oxide synthetase (anti-NOS) negatively regulates neuronal NOS, implicating anti-sense regulation as a modulator of long term memory formation³¹. In schizophrenia and affective disorders, *DISC2*, an anti-sense RNA to *DISC1*, is implicated in regulating these neural disorders³².

The BC1 ncRNA and its primate form BC200 are not transcribed as anti-sense to their targets, but function as translational regulators. Both BC200 and BC1^{33,34} are targeted to somatodendritic domains of neurons, and thought to be involved in synaptic plasticity. In support of this hypothesis, mice lacking BC1 RNA show decreased exploratory behavior and increased anxiety³⁵. BC200 RNA is upregulated in Alzheimer's disease³⁶, whereas BC1 RNA has been shown to directly bind the fragile X syndrome protein (FMRP) affecting translational repression^{37,38}. However, binding of BC1 RNA to FMRP is controversial and has recently been challenged³⁹. Evidence from the challenging group suggests that BC1 represses translation by inhibiting the RNA unwinding activity of eukaryotic initiation factor 4A (eIF4A)⁴⁰.

The SZ-1 RNA has been proposed to be an anti-sense regulator of *DLG-2*, controlling functional assembly of N-methyl-D-aspartate receptors⁴¹. In autism, a patient with a breakpoint in 7q31 raises the possibility of the involvement of another opposite strand RNA, *ST7OT*, a suppressor of tumorigenicity (*ST7*)⁴².

While many of these ncRNAs function at the post-transcriptional level, very few mechanisms utilized by anti-sense or opposite-strand ncRNAs at the transcriptional or epigenetic level have been defined. Only a small group of long-polyadenylated RNAs with known transcriptional or epigenetic regulatory activity has been identified (Tables 2 and 3), recently reviewed in Khalil et al. 2009. How such aberrant regulation might cause complex mental disorders is a topic of intense investigation.

In this symposium, evidence was presented supporting that developmental control of *Dlx* genes by *Evf2*, a transcription-regulating ultraconserved ncRNA (trucRNA⁴³), affects the number of GABAergic interneurons in the hippocampus (summarized in Figure 1)⁴⁴. It was also shown that the *Evf2* ncRNA recruits both positive (*Dlx*) and negative (*MECP2*) transcription factors to key DNA regulatory elements that control balanced *Dlx* 5 and 6 gene expression during embryonic brain development⁴⁴. Further, data showed that *Evf2* mouse mutants have reduced numbers of GABAergic interneurons in the early postnatal hippocampus and dentate gyrus. This is the first functional evidence linking a noncoding RNA transcriptional mechanism to *MECP2* and GABAergic interneuron development, with possible relevance to an etiology for an ASD.

Given that GABAergic interneuron activity in the brain controls multiple higher functions, and altered GABA activity, as discussed above, has been linked to complex mental disorders³, it will be important to determine how commonly anti-sense or opposite-strand RNAs throughout the genome are responsible for recruitment of specific and/or general transcription factors. The ability to demonstrate that aberrant non-coding RNA-dependent epigenetic regulation can cause complex mental disorders would have important consequences to future investigations of these disorders. Specifically, any potential RNA regulators of genes involved in neuronal function including development, plasticity, dendritic branching, axonal transport, and signal transduction, would be studied and considered potential candidates in causing complex mental disorders. This demonstration would broadly impact studies on normal gene regulation in neuronal and non-neuronal cells and would also directly influence how genetic studies of complex mental disorders are investigated. Specifically, geneticists would not only focus on potential disease-causing candidates in protein-coding regions, but also on the expression and sequence of non-coding RNA transcripts, as well as DNA methylation profiles. As a result, RNAs like *Evf2* that subtly regulate genes important to specific neural activities may be identified as the cause of some subset of complex mental disorders, yielding specific therapeutic targets.

Development of greater target specificity is critical to replacing present drugs that prevent or activate neural activity affecting multiple targets with detrimental side effects. Ultimately, our goal in discovering the exact mechanism responsible for a specific neurological disease or mental disorder is to develop specific drug targets that would rescue these disorders. Therefore, a major impact on drug development for long-term treatment and cures for complex mental disorders could potentially arise from developing novel RNA regulators.

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wt Forebrain

Evf2 TS/TS Forebrain

Figure 1. *Evf-2* ncRNA dependent gene regulation and GABAergic interneuron development
 Modified from Bond et al. (2009, Fig S3) showing SHH (sonic hedgehog protein), MECP2 (DNA methyl-binding protein 2), GAD67 (glutamate decarboxylase, also GAD1), Dlx (vertebrate homologues of *distalless*, Drosophila homeodomain-containing transcription factor), *Evf1* and *2*, embryonic ventral forebrain ncRNAs, LGE (lateral ganglionic eminence), MGE (medial ganglionic eminence), CGE (caudal ganglionic eminence) embryonic structures that are the sources of adult GABAergic interneurons in the cortex, hippocampus, dentate gyrus and olfactory bulbs.

Table 1

Long non-coding RNAs and neurological disorders

Long Non-coding RNA	Disease	Significance	Function	Refs
BACE1-AS (anti-sense BACE1)	Alzheimer's disease	increased expression in Alzheimer's disease	enhances beta-secretase-1 (BACE1) mRNA stability, an important enzyme in Alzheimer's disease	30
Evf-2 (anti-sense Dlx6)	GABA neuropathies	potentially implied in GABA neuropathies	transcriptional regulator of Dlx 5 and 6 expression, required for GABAergic interneuron development	43,44
SCA8 (or ATXN8OS)	Spinocerebellar Ataxia 8 (SCA8)	induced expression associated with neurodegenerative disease Spinocerebellar Ataxia 8	may contribute to neurodegeneration through the alteration of RNA binding protein associations	45
anti-NOS (anti-sense nNOS)	long-term memory disorders	expression associated with improper long-term memory formation	negatively regulates the enzyme neuronal nitric oxide synthase (nNOS), crucial for the formation of long-term memory	31
DISC2 (anti-sense DISC1)	schizophrenia	expression is disrupted in schizophrenia	may be an anti-sense regulator of DISC1, essential for neuronal development	32,46
BC200	Alzheimer's disease	increased expression in Alzheimer's brains	translational regulator targeted to somatodendritic domains of neurons, may affect long-term synaptic plasticity	36
BC1	Fragile X Syndrome	associated with fragile X syndrome	binds fragile X protein (FMRP), required for FMRP-mediated inhibition of translation at the synapse	37,38
Tmevpg1	Theiler's virus induced neurological disease	positionally cloned candidate associated with susceptibility to Theiler's virus induced neurological disease	may control the cytokine interferon gamma expression	47
PSZA11q14 (anti-sense DLG2)	schizophrenia	decreased expression in schizophrenia	anti-sense regulation of DLG2, involved in the assembly of NMDA receptors	41
ST7OT (anti-sense to ST7)	autism	associated with autism in one patient	possible regulator of ST7 gene	42
LIT1 (anti-sense KvLQT1)	Beckwith-Wiedemann Syndrome (BWS)	disrupted expression in BWS	anti-sense negative regulation of KvLQT1, a gene implicated in BWS	48,49
Peg8	BWS	increased expression in BWS	regulates the expression of IGF2, associated with BWS	50
IPW	Prader-Willi Syndrome (PWS)	not expressed in PWS	regulates imprinted, paternally expressed genes found at location 15q11-q13, which is altered in PWS	51

Long Non-coding RNA	Disease	Significance	Function	Refs
Prion-associated RNAs	Prion Disease	expression may be associated with Prion Disease	may stimulate prion protein conversion, the infectious agent of prion disease	52,53
H19	BWS	disrupted expression in BWS	possible regulator of the imprinting of chromosome 11p15.5	54
ZNF127AS (anti-sense ZNF127)	PWS	disrupted expression in PWS	may regulate the imprinting of ZNF127, a gene altered in PWS	55
UBE3A antisense (antisense UBE3A)	Angelman Syndrome (AS)	increased or decreased expression levels in AS	regulates the imprinting of UBE3A, a gene implicated in AS	56

Table 2

Transcription-regulating long non-coding RNAs

	Long noncoding RNA	Function	Refs
Specific Transcription Factors	SRA	forms a ribonucleoprotein complex with steroid hormone receptors (SHRs) to co-activate transcription	57,58
	Evf-2	recruits Dlx, a homeodomain transcription factor, and MECP2 to key intergenic DNA regulatory elements, regulating Dlx5 and Dlx6 expression	43,44
	HSR-1	in response to heat shock, allows for the trimerization of the heat shock factor-1 (HSF-1), which then binds to the translation- elongation factor 1A (eIF1A) to initiate heat shock protein expression	59
	RNA upstream of CCND1	forms a complex with the RNA-binding protein, TLS, facilitating the repression of CCND1 by the chromatin binding protein (CBP) and p300	60
	LXRBSV	acts as a transcriptional co-activator with liver X receptor (LXR)- β to enhance receptor- mediated transactivation	61
General Transcription Factors	7SK	forms a complex with hexamethylenes bisacetamide-induced protein-1 (HEXIM1), which then binds to PTEFb, thereby preventing transcriptional elongation by RNA polymerase II	62–64
	RNA upstream of DHFR	creates a triplex structure in the core promoter of DHFR, blocking the binding of TFIID and repressing transcription	65
RNAP II	Alu Elements	binds to RNA polymerase II, blocking transcription	66

Table 3

Long non-coding RNA and chromatin modification

Chromatin Modifying Complex	Long Non-coding RNA	Function	Refs
Polycomb chromatin remodeling complex	HOTAIR	recruits to the HoxD locus to silence gene expression	67
	Tsix/RepA	RepA recruits the Polycomb complex to the X chromosome to induce heterochromatin formation and repress gene expression; Tsix inhibits this interaction	68
	Kcnq1ot1	recruits the Polycomb complex and the G9a histone modifying complex to the Kcnq1 domain to silence gene expression	69
Ash1	TRE transcripts	possible recruitment to Ultrabithorax (Ubx), a Drosophila homeotic gene, or cis- acting repression of transcription	70,71
Histone methyltransferase MLL1	Hoxb5/6as, Evx1as	associate with MLL1 and trimethylated H3K4 histones, suggesting a role in epigenetic regulation	72