Role of Long Non-coding RNAs in Sculpting Neurons

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The morphology of neurons, a collective measure of features like the size, number, length, density and branching patterns of dendrites, as well as axonal features like length and diameter of the axon, is critical to its function. At a finer level of detail, dendrites bear membranous bulb-like projections on their surfaces called dendritic spines. Dendritic spines are the sites of neurotransmitter signalling and undergo activity dependent dynamic changes in shape associated with a regulated rearrangement of the actin cytoskeleton. Here, we provide an overview of four lncRNAs: MALAT1, BC200, 7H4 and Durga, which are implicated in altering neuronal architecture. We have recently discovered the lncRNA named Durga, in the zebrafish genome, in close proximity to Kalirin gene, which modulates expression of Kalirin and dendritogenesis. Kalirin is a Rho-GEF kinase that is involved in the cytoskeletal rearrangements that occur during dendritogenesis and dendrite spine morphologenesis. It is closely associated with proteins of post-synaptic density, a structure found at dendritic spines, and through sequential activation of other RhoGTPases, brings about cytoskeletal changes in response to developmental signals and neurotransmitters. Here, we provide an overview of dendritogenesis, the molecular basis of dendrite and dendritic spine formation and discuss the involvement of lncRNA in the process.

Keywords: Non-coding RNAs; Long Non-coding RNAs; Dendritogenesis; Dendritic Spines; Synapticplasticity; Neuronal Architecture

Introduction

Neurons are the basic information processing units of nervous system composed of three major structures: soma, dendrites and axons. Dendrites, the branched projections of neurons, receive and integrate synaptic inputs through small protrusions called dendritic spines. The soma subsequently processes the synaptic inputs and passes it to other neurons via elongated projections, the axons. A single axon makes synapses with dendritic spines from multiple neurons. A synapse is formed by three ultramicroscopic structural components: presynaptic terminal, postsynaptic terminal and synaptic cleft. At the presynaptic terminal on dendritic spines, neurotransmitters are packaged into synaptic vesicles and upon calcium influx released into the space of the synaptic cleft (Pitman, 1984). Receptors at postsynaptic terminals receive and interpret messages from presynaptic terminals. At the postsynaptic plasma membrane many receptors, effectors, adhesion proteins, kinases, phosphatases and cytoskeletal elements get assembled into an electron dense region called postsynaptic density (PSD) of synapse (Okabe, 2007). Compared to our knowledge of the proteins localized to such specialized compartments of neurons, we know little about the RNA species present in these regions. Messenger RNA of several proteins, notably Actin and Fmrp, are enriched and locally translated at the synapse (Bramham and Wells, 2007). Various classes of non-coding RNAs account for the bulk of eukaryotic cellular RNAs. Recently, a large number of less abundant non-coding RNAs - the long non-coding RNAs (lncRNAs), have been discovered, many of them are regulatory in function. lncRNAs are transcripts without any protein coding capability, they have now been located in every compartment of the cell, including common locations such as the nucleus and the cytoplasm; or specialized areas, like axons.

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and dendrites of neurons. Some of these lncRNAs regulate gene expression and signaling events at various stages of brain development, including neural stem cell differentiation (Wu et al., 2013; Ramos et al., 2015).

Here, we briefly review neuronal architecture and then focus on lncRNAs which reside within dendrites and axons. They may be highly enriched near synapses, modulated by neuronal electrical activity and may participate in plasticity responses, such as learning and memory. Based on the available evidence, we also point out gaps in the current understanding of lncRNA at the synapse and suggest promising lines of investigation that will help in future to elucidate the purpose of lncRNAs that are docked at such specialized compartments and their relevance in human disease.

Nuts and Bolts of Neuronal Architecture

Neurons are specialized cells of the brain involved in rapidly transmitting signals- electrical and chemical - from cell to cell. Neurons receive signals from neighboring cells, integrate these inputs to determine if they will be transmitted further and finally communicate it to target cells. The target cells may be other neurons in a network or other cell types like muscle cells. Ramon y Cajal’s early description of the neurons in brain sections based on the staining of the Golgi bodies is replete with references to the shapes of the cells. His beautiful and intricate drawings of neurons made by meticulously following the course of each neuron in brain sections give us our earliest insights into the complexity of cell shape and its relation to the function of the neuron (Cajal, 1906). The main cell body or soma of the neuron contains the nucleus and is also a site for the central synthesis of protein. The cell body is surrounded by protrusions of varying length and branching pattern. Typically, a single, relatively long axon and a number of shorter highly branched dendrites together specify the morphology of neuron. Besides the shape, absence of the myelin sheath and presence of tiny bulb like membranous protrusions called dendritic spines decorated on the surface differentiate dendrites from the axon. The dendritic spines are critical features of neurons with strong functional implications. Dendritic spines can be thought of as sub-cellular locations containing neurotransmitter receptors, organelles and molecules involved in synaptic functions. Since each dendritic spine connects to a single excitatory axon, it is thought to be the unit of synaptic activity. The endoplasmic reticulum of the neuron may penetrate into the spine and facilitate local and strong calcium flux following neurotransmitter binding to the receptor (Kovalchuk et al., 2000).

Dendrites receive the excitatory and inhibitory signals coming into the neuron, the sum of which determines its response. A neuron uses its dendrites to sample an area around it called the dendritic field. During development, the number of dendrites increase and are subsequently pruned (Riccomagno and Kolodkin, 2015). Mature neurons usually have a stable dendrite arborization pattern. At the molecular level, a large number of proteins are implicated in the determination of dendrite numbers, density and branching patterns. Mutations in transcription factors like *Abrupt* and *Cut* and cell surface receptors like *Robo* show altered dendrite genesis (Jan and Jan, 2010). The small RhoGTPaseRhoA and RAC1 have opposite effects on dendrite length and branching. PSD95 a protein present in the postsynaptic density is also involved in regulation of dendritogenesis and dendrite spine morphogenesis (Penzes et al., 2011). In young hippocampal neurons, PSD95 is readily detected outside the synapses. Over-expression of PSD95 reduces secondary branching of dendrites. Besides, its role in activity independent regulation of dendrite branching, PSD95 is known to modulate dendrite spine morphology in an activity dependent manner. PSD95 is connected to the cytoskeleton, indirectly, by intermediate proteins and can act as a scaffolding protein in the dendritic spine (Hu et al., 2011). Proteins like Cypin and Kalirin 7 that interact with PSD95 are also important in dendritogenesis and dendrite spine morphogenesis (Xie et al., 2007).

Diversity of Neuronal Architectures

Neurons adopt diverse shapes and sizes depending on their specific functions. For instance, a motor neuron that connects the spinal cord to limbs can be about a meter long while most other neurons are just a few microns in length. Some model organisms, for
instance the sea slug, Aplysia, are chosen for their gigantic neurons that are especially amenable to electrophysiological studies (Moroz, 2011). The giant axon of the squid can be upto 1mm in diameter, allowing rapid transmission of signals and triggering powerful contractions of the muscular body to eject a stream of water that propels the squid away from danger. The morphology of a typical neuron with short branching dendrites surrounding the soma and long axon extending away from the cell body is found in motor neurons. Bipolar neurons may have a single basal dendrite and axon of similar length, emerging from the central cell body. Interneurons that connect between neurons may be bipolar and depending on whether they go between local or distal neurons they may have short or long axons and dendrites. True unipolar neurons found in invertebrates have a denuded appearance with the dendrites being absent and a single long axon that connects to the target cell. A pseudounipolar cell arises from a bipolar cell when the cell body is located a small distance away from the dendrite and axon. In such cells, the axon and dendrite appear to be connected directly - a morphology commonly found in sensory neurons. In pseudounipolar cells, a single process emerging from the cell body is split into equally long processes: the dendrite and the axon (Kandel et al., 2013). Thus, it is clear that the nervous system consists of morphologically diverse neurons (Fig. 1).

The shapes of neurons are directly linked to their functions (Vetter et al., 2001). Presumably, inappropriate changes in the shapes of neurons may lead to dysfunctional neural networks and disease. Until recently, the relevance of neuronal form was not studied directly in neurodegenerative diseases and altered neuronal shape was hardly explored as an underlying cause for functional deficits (Šišková et al., 2014). From studies using labeling on sections of postmortem tissue of Huntington’s disease patient’s brain it is now clear that an overall reduction in dendrite length and abnormal patterns of branching are seen in neurodegenerative diseases (Graveland et al., 1985). In the early stages of the disease, striatal spiny neurons show an abnormal bending of distal dendrites and increased branching. While a number of cortical, prefrontal pyramidal neurons degenerate, some pyramidal neurons may show increased dendritic spine length, density and volume (Sotrel et al., 1993). Besides Huntington’s disease, dendritic morphology is affected in a variety of neurodegenerative diseases of the brain including taupathies, prion diseases and alpha-synucleopathies (Kweon et al., 2017).

**Long Non-coding RNAs: Active Players at Dendrites and Synapses**

On the evolutionary scale, biological complexity has steadily increased, without a corresponding change in a number of protein coding genes, whereas non-coding portion of the genome has undergone substantial change. It is now a well accepted fact that in higher organisms, majority of the genome gives rise to non-coding RNAs (ncRNAs) that lead to increased biological complexity. By virtue of their ability to modulate, the flow of information from DNA to RNA to protein, ncRNAs have gained unprecedented attention (Morris and Mattick, 2014). Recent advancement in the next generation sequencing (NGS) technology has also allowed discovery of several classes of ncRNAs. lncRNAs, defined by convention as longer than 200 nucleotides, form the largest class of ncRNAs.
IncRNAs are involved in a variety of functions in the nervous system, by targeting multiple processes through regulation of genes and proteins. The IncRNA may be an antisense to a messenger RNA (mRNA) thus regulating its stability or translation. Alternatively, they may recruit protein factors on the gene promoter or on the mRNA, switching on or off expression from the locus. They may also drive localization of the mRNA to specific sub-cellular compartments. IncRNA can also exert epigenetic effects by recruiting chromatin modifiers to target gene loci. Presence of IncRNAs in multi-protein complexes suggests that they can also be a scaffold for binding of other RNA and protein components (Batista and Chang, 2013).

A remarkably large number of ncRNAs especially microRNAs (miRNAs) of 19 to 24 nucleotide length are present in dendrites leading to the suggestion that they are “pervasively” dendritic (Smalheiser, 2014). Many ncRNAs act through their membership in ribonucleoprotein complexes. In keeping with the view that ncRNAs play a key function in dendrites, several RNA binding proteins are also localized to the same sub-cellular location (Weiler et al., 1997). These include RNAi pathway proteins, members of the splicing machinery and miRNA biogenesis proteins. There is ample evidence suggesting irreplaceable role of miRNAs in dendritogenesis, spine morphogenesis, maturation, synaptic development and/or plasticity (Reviewed in detail Iyengar et al., 2014; Schratt, 2009; Smalheiser and Lugli, 2009; Weiß et al, 2015; Ye et al., 2016). Certain specific miRNAs, for instance, miR-134, has been found in the dendritic spines of cultured hippocampal neurons (Schratt et al., 2006). It appears that these miRNAs are engaged in activity dependent, local modulation of translation that can in turn lead to stimulus dependent remodeling of dendritic spines. It is becoming increasingly obvious that many steps of gene expression, especially miRNA biogenesis and splicing, conventionally thought to be performed in the nucleus also occur in the dendritic spine. The presence of mRNAs that contain retained introns and precursors of miRNAs in the dendritic spines (Lugli et al., 2008) suggest that RNA is held in an inactive state which can be rapidly processed following external stimuli. Apart from miRNAs, IncRNA being multi-functional molecules have emerged as important components of specialized neuronal compartments. Here, we describe four IncRNAs that are known to modulate neuronal architecture (Table 1).

In the pre-NGS era, BC1 RNA was identified by in situ hybridization of RNA probes in the dendrites of rat neurons (Tiedge et al., 1991). This rodent specific BC1 RNA and its primate specific functional analog BC200 is a typical RNA polymerase III transcribed gene almost exclusively expressed in nervous system. These cytoplasmic RNAs are present in the somatic and dendritic compartments of the central and peripheral nervous system (Tiedge et al., 1993, 1991). Expression of BC1 RNA coincides with the functional maturation of dendrites and synaptogenesis in hippocampal neurons, which can be further modulated by electrical activity of neurons (Muslimov et al., 1998). BC1 RNA appears to be a part of a high molecular mass ribonucleoprotein complex in neurons. Although these RNAs do not show strong sequence conservation, their specific location in post-synaptic compartments of rodent and primate neurons may indicate conserved functions related to pre- or post-translational events (Martignetti and Brosius, 1995; Tiedge et al., 1993). An attempt to study the function of BC1 RNA using knockout

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**Table 1: Summary of IncRNAs modulating neuronal architecture**

<table>
<thead>
<tr>
<th>ncRNA</th>
<th>Functions</th>
<th>Interacting partners</th>
<th>Associate disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC1, BC200</td>
<td>Part of ribonucleoprotein complex, modulate exploration and anxiety related behaviour</td>
<td>FMRP, Pur α and β, SRP9/14, HnRNP A2, PABP</td>
<td>Alzheimer’s disease</td>
<td>(Sosińska et al., 2015; Smalheiser, 2014; Tiedge et al., 1991, 1993)</td>
</tr>
<tr>
<td>Malat1</td>
<td>Synaptogenesis</td>
<td>miR-124</td>
<td>Parkinson’s disease</td>
<td>(Bernard et al., 2010; Liu et al., 2017)</td>
</tr>
<tr>
<td>7H4</td>
<td>Precursor of miR-206 and miR-133b at dendrites</td>
<td>Not yet known</td>
<td>Not yet known</td>
<td>(Rodriguez et al., 2004; Valdez et al., 2014)</td>
</tr>
<tr>
<td>Durga</td>
<td>Modulator of dendritic length and number</td>
<td>Not yet known</td>
<td>Not yet known</td>
<td>(Sarangdhar et al., 2017)</td>
</tr>
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Non-coding RNAs Modulate Neuronal Architecture

(KO) mice revealed no visible change in brain morphology. However, the KO mice showed subtle behavioral changes such as reduced exploration and increased anxiety without change in spatial memory (Lewejohann et al., 2004). At the molecular level, the knockout of BC1 alters efficiency of D2-mediated transmission but not the sensitivity of cannabinoid CB1 receptors. This specific effect is mediated by increase in Dopamine D2 receptor (D2DR) protein levels in the striatum of BC1-KO mice affecting the functional pool of receptors (Centonze et al., 2007).

Another lncRNA abundantly expressed in neurons is Metastasis-associated lung adenocarcinoma transcript 1, Malat1 (Neat2). In cultured hippocampal neurons, loss of this lncRNA has been shown to decrease synaptic density. Gene expression analysis after knockdown of Malat1 suggests its role in synapse formation in neurons, executed by regulating expression of genes involved in dendrite and synapse development(Bernard et al., 2010). Interestingly, Malat1 has several miRNA binding sites, for example, Malat1 directly interacts with and mutually represses brain enriched miR-124. The expression of Malat1 is increased while miR-124 is decreased in a model of Parkinson’s disease (PD), a neurodegenerative disorder characterized by loss of dopaminergic neurons (DA) and motor control with cognitive deficits (Liu et al., 2017). In an attempt to identify differentiation regulating lncRNAs, Malat1 found to be significantly induced in a model of in vitro differentiation of mouse neuroblastoma (N2a) cells by serum withdrawal and Retinoic Acid treatment. Loss of Malat1 in this model led to the inhibition of MAPK/ERK signalling pathway and reduction in average neurite length (Chen et al., 2016).

Apart from regulation of gene expression at cell body of neurons, IncRNAs can act as precursor or sponge for miRNAs at synapse. For example, synapse-associated lncRNA 7H4 have precursor sequence for miR-206 and miR-133b embedded within its transcript. 7H4 RNA was identified as an RNA enriched at the motor endplate of skeletal neuromuscular junction (NMJ) (Velleca et al., 1994). Interestingly, like 7H4, miR-206 and miR-133b are also enriched in the synapse and gets up-regulated at postnatal stages simultaneously, hinting at the possibility of active transport and local processing of long non-coding miRNA host transcripts to sub-cellular compartments away from the nucleus (Rodriguez et al., 2004; Valdez et al., 2014). Such local processing of host IncRNAs in specialized compartments may protect processed transcript products from unwanted interactions.

Recently, we discovered a lncRNA that regulates dendritogenesis during development in zebrafish. This lncRNA partially overlaps with the 5’ end of the Kalirin gene (Sarangdhar et al., 2017), a RhoGEF kinase present in dendrites and synapse of neurons. Ectopic expression of this lncRNA by injection in the single cell stage of zebrafish embryos led to an increase in Kalirin mRNA expression (Fig. 2) (Sarangdhar et al., 2017).

Kalirin, an essential player in dendritic outgrowth and branching derives its curious name from its multi-domain nature resembling the multi handedness of the Indian goddess Kali (Alam et al., 1997, Yan, 2015). lncRNA antisense to Kalirin was named Durga after the Indian goddess who is presented in mythology as the alter ego of “Kali” (Sarangdhar et al., 2017). Cultured neurons derived from the 48 hour old...
zebrafish embryos which received Durga transcripts by microinjection at the single celled stage showed a severe reduction in the number of dendrites (Sarangdhar et al., 2017). Using total ribosome fractionation, we see that Durga over-expression results in increased localization of Kalirin transcript to the ribosomes fraction (Beena Pillai, unpublished observation). This implies, IncRNA Durga may localize Kalirin mRNA to the ribosome and protect it from degradation factors. Further investigation is required to find possible interactions of Durga IncRNA with potential mRNA stability or degradation factors. Although IncRNAs are poorly conserved, the human and mouse genomes also harbor an IncRNA at the 5' end of the Kalirin gene (Sarangdhar et al., 2017).

Looking at the close association of Kalirin with PSD95 in the post synaptic density (Xie et al., 2007) and role of Kalirin in microfilament re-organization of dendritic spines, the ability of Durga to modulate local Kalirin translation needs to be investigated with respect to neuronal activity. Further, differential regulation of Kalirin transcript isoforms in the developing brain and the potential involvement of Durga in this process are exciting prospects. Lastly, the relevance of the syntenically conserved IncRNA in the mammalian genome will be revealed through studies in the primary neurons of mouse which also provide suitable models for in vitro dendritogenesis.

**Conclusion and Future Perspectives**

The current understanding of IncRNAs in dendritogenesis, axogenesis, synaptogenesis and spine modulation is in its infancy. However, looking at the multi-functional nature of IncRNAs and evidence of a few IncRNAs localized in specialized compartments of neurons promises widespread role in advanced functions of the brain such as memory and cognition. Further, the loss of function of these IncRNAs is also likely to be linked to the neuropsychiatric and neurodegenerative disorders because of the inherent role of dendritogenesis, axogenesis, synaptogenesis and spine modulation in such pathological conditions.

In future, studies may focus on presence of IncRNAs in synaptic vesicles and neuronal exosomes that have received little attention and remain poorly understood. How IncRNAs contribute to synaptic plasticity in the mammalian brain is still an open question (Fig. 3). The physiological and pathological functions of synaptic IncRNAs need to be explored further. The classification of IncRNAs based on their localization in specialized neuronal compartments is under-explored perhaps because of continuity of cytoplasm of neuronal cell body and axons/dendrites, sometimes blurring their boundaries. Transfer of synaptic IncRNAs from the synapse to the nucleus and vice versa to participate in the signaling has not yet been demonstrated. The innate potential of IncRNAs to be transported as cargo during vesicular transfer and their ability to act by sponging miRNAs or scaffolding protein factors in the central nervous system is not explored.

We hope that detailed investigations on questions posed above will reveal the importance of IncRNA in Cajal’s century old neuroscience puzzle. Sculpting of neurons in general and more specifically, the role of novel IncRNAs like Durga in this dynamic process that underlies learning and memory promise an exciting period of discovery.
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