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lncRNA expression in the auditory forebrain during postnatal development

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ABSTRACT

The biological processes governing brain development and maturation depend on complex patterns of gene and protein expression, which can be influenced by many factors. One of the most overlooked is the long noncoding class of RNAs (lncRNAs), which are known to play important regulatory roles in an array of biological processes. Little is known about the distribution of lncRNAs in the sensory systems of the brain, and how lncRNAs interact with other mechanisms to guide the development of these systems. In this study, we profiled lncRNA expression in the mouse auditory forebrain during postnatal development at time points before and after the onset of hearing (P7, P14, P21, adult). First, we generated lncRNA profiles of the primary auditory cortex (A1) and medial geniculate body (MG) at each age. Then, we determined the differential patterns of expression by brain region and age. These analyses revealed that the lncRNA expression profile was distinct between both brain regions and between each postnatal age, indicating spatial and temporal specificity during maturation of the auditory forebrain. Next, we explored potential interactions between functionally-related lncRNAs, protein coding RNAs (pcRNAs), and associated proteins. The maturational trajectories (P7 to adult) of many lncRNA – pcRNA pairs were highly correlated, and predictive analyses revealed that lncRNA-protein interactions tended to be strong. A user-friendly database was constructed to facilitate inspection of the expression levels and maturational trajectories for any lncRNA or pcRNA in the database. Overall, this study provides an in-depth summary of lncRNA expression in the developing auditory forebrain and a broad-based foundation for future exploration of lncRNA function during brain development.

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1. Introduction

The biological processes governing the development and maturation of the brain depend on a complex network of gene and protein expression, which can be influenced by many factors. These expression patterns are incompletely understood and represent many interesting avenues of study in terms of temporospatial expression and regulation.

Especially in the sensory systems of the brain, the onset of sensory experience represents a dramatic shift in activity that could affect the mechanisms guiding their development and maturation. In altricial animals, for example, the marked increase of neuronal activity in the central auditory pathways upon opening of the ear canals in early postnatal life stimulates maturation of the brain's auditory circuitry. This window provides an opportunity to study the impact of hearing onset on neurophysiological response properties (Bao, 2015; Barkat et al., 2011; Chang et al., 2003; Chun et al., 2013; de Villers-Sidani and Merzenich, 2011; Froemke and Jones, 2011b; Hensch, 2005; Kral, 2013; Oswald and Reyes, 2011; Sanes and Bao, 2009; Sanes and Woolley, 2011; Yang et al., 2012) and critical periods for sound processing (Bao et al., 2001; Brown and Kaczmarek, 2011; Dorrn et al., 2010; Edeline et al., 2011; Froemke et al., 2013; Froemke and Jones, 2011a; Hurley and Sullivan, 2012; Kilgard and Merzenich, 1998; Metherate and Hsieh, 2003; O'Neil et al., 2011; Schachtele et al., 2011; Sun et al., 2010; Sutor and Hagerty, 2005; Venkataraman and Bartlett, 2013). Undoubtedly, these maturational events are supported by alterations in neuronal circuitry at the cellular and molecular levels, including changes in gene and protein expression; however, documentation of these properties is incomplete. To advance inquiry along these lines, we recently sequenced the transcriptome in divisions of the auditory forebrain of C57bl/6J mice from postnatal day 7 (P7) through adulthood, which spans the onset of hearing (~P11–P13) in this species (Hackett et al., 2015). In addition to generating a database of the entire transcriptome, approximately 5000 protein-coding RNAs (pcRNAs) were profiled in detail. Maturational changes in expression were observed in scores of gene families with important roles in brain structure and function.

Abbreviations: A1, primary auditory cortex, area 1; lncRNA, long non-coding RNA; MG, medial geniculate body, thalamus; pcRNA, protein-coding RNA.

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In addition to pcRNAs, non-coding RNA (ncRNA) expression is also essential to develop a complete understanding of the genomic landscape during brain development. Among the many ncRNA subtypes that could be explored, interest in long non-coding RNAs (lncRNAs) has increased considerably as awareness of their functional importance has grown. There are roughly 10,000 lncRNAs in mammalian genomes (Cabili et al., 2011; Harrow et al., 2012; Ilott and Ponting, 2013; Rinn and Chang, 2012). Traditionally believed to be non-functional, lncRNAs have recently been shown to possess functional roles (Dinger et al., 2009; Mercer et al., 2009), including roles in high-order chromosomal dynamics (Amaral and Mattick, 2008), embryonic stem cell differentiation (Dinger et al., 2008), telomere biology (Schoeftner and Blasco, 2008), subcellular structural organization (Mercer et al., 2008), and breast cancer (Bhan et al., 2014; Bhan et al., 2013). LncRNAs are usually defined as non-coding RNA with length >200 base pairs (Mercer et al., 2009; Perkel, 2013). Structurally, lncRNAs and mRNAs are very similar, as both can exhibit poly-adenylation (poly(A)). The number of definable lncRNAs varies by study. An early study in 2007 estimated that there are four times more lncRNAs than pcRNAs (Kapranov et al., 2007). Another study claims to have identified 35,000 lncRNAs (Carninci et al., 2005), and many of them have characteristics similar to mRNA, such as 5′ capping, splicing, and poly-adenylation, with the exception of open reading frames. In the latest effort to quantify human lncRNA, the Encyclopedia of DNA Elements (ENCODE) (Djebali et al., 2012) project identified 13,333 lncRNAs and further categorized them into four sub-classes: 1) antisense, 2) large intergenic non-coding RNAs (lincRNA), 3) sense intronic, and 4) processed transcripts. Compared to pcRNAs, lncRNAs tend to have much lower expression levels, often due to cell-type specific expression (Cabili et al., 2011; Guttman et al., 2010; Liu et al., 2016), but transcript abundance is not known to be related to function (Ulitsky and Bartel, 2013).

As a group, lncRNAs are relatively highly expressed the adult and developing brain (Derrien et al., 2012; Lin et al., 2011; Lipovich et al., 2012; Mercer et al., 2008; Ng et al., 2012; Smalheiser et al., 2008; Washietl et al., 2014). The functions of most are unknown, but many are now known to have regulatory influence over the expression of other genes and proteins (Carninci et al., 2005; Carrieri et al., 2012; Guttman et al., 2011; Guttman and Rinn, 2012; Halley et al., 2014; Katayama et al., 2005; Khalil et al., 2009; Kornienko et al., 2013; Kurokawa, 2011; Magistri et al., 2012; Mattick, 2007; Meng et al., 2012; Onoguchi et al., 2012; St Laurent and Wahlestedt, 2007; Tsai et al., 2010; Vance et al., 2014; Wu et al., 2013; Zhang et al., 2012; Zhao et al., 2013). With respect to nervous system development, lncRNAs may also influence maturational processes such as neurogenesis, synaptogenesis, cell migration, cell type specification, neurite outgrowth, and synaptic plasticity (Aprea et al., 2013; Berghoff et al., 2013; Bernard et al., 2010; Bond et al., 2009; Feng et al., 2006; Kraus et al., 2013; Lin et al., 2014a; Ling et al., 2011; Lipovich et al., 2012; Liu et al., 2016; Modarresi et al., 2012; Ng et al., 2012; Onoguchi et al., 2012; Tarabykin et al., 2001; Ulitsky et al., 2011; Vance et al., 2014).

As observed for protein coding genes, lncRNA expression patterns in the brain tend to be spatially and temporally restricted. Spatially, expression levels may vary substantially between major brain regions (e.g., hippocampus versus cerebral cortex) (Amaral et al., 2009; Kadakkuzha et al., 2015; Ling et al., 2009; Ling et al., 2011; Lv et al., 2013; Mercer et al., 2008; Ponjavic et al., 2009; Sauvageau et al., 2013; Spigoni et al., 2010; Ziats and Rennert, 2013), and also by subdivision or compartment within a region (e.g., cortical layer) (Aprea et al., 2013; Belgard et al., 2011; Kadakkuzha et al., 2015; Mercer et al., 2008; Sasaki et al., 2008; Sauvageau et al., 2013; Spigoni et al., 2010). Spatial specificity is also apparent at the cellular level, where expression may be restricted to subpopulations of neurons, glia, or even subcellular compartments (e.g., nuclei, cytoplasm) (Aprea et al., 2013; Kadakkuzha et al., 2015; Korneev et al., 2008; Liu et al., 2016; Mercer et al., 2008; Mercer et al., 2010; Pollard et al., 2006; Sasaki et al., 2008; Sauvageau et al., 2013; Sone et al., 2007; Tochitani and Hayashizaki, 2008). Temporally, lncRNA expression in a given locus (e.g., region, subregion, cell type) often changes over the long course of nervous system development, most notably between key developmental stages or significant events (e.g., the onset of sensory experience) (Amaral et al., 2009; Aprea et al., 2013; Lin et al., 2011; Ling et al., 2009; Ling et al., 2011; Lipovich et al., 2012; Liu et al., 2016; Mercer et al., 2010; Ponjavic et al., 2009; Spigoni et al., 2010; Tarabykin et al., 2001). Accordingly, the roles played by lncRNAs in brain development may well depend on the precise timing and location of a given event. A number of outstanding reviews of this rapidly growing literature are available (Aprea and Calegari, 2015; Clark and Blackshaw, 2014; Geisler and Coller, 2013; Guttman and Rinn, 2012; Knauss and Sun, 2013; Mattick, 2007; Mehler and Mattick, 2007; Ng et al., 2013; Qureshi et al., 2010; Qureshi and Mehler, 2012; St Laurent and Wahlestedt, 2007; Wu et al., 2013).

The specificity in temporal and spatial expression patterns among lncRNAs suggest that profiles differ between brain region and cellular subtype, as well as developmental stage. Informed by knowledge of those patterns, subsequent studies may be implemented to identify regulatory relationships, interactions, and functional pathways greater specificity. In the present study, we used high throughput sequencing of total RNA (RNAseq) to profile lncRNA expression patterns in two different divisions of the auditory forebrain at key postnatal ages relative to the onset of hearing. RNAseq has been traditionally used as a replacement for microarray technology to profile pcRNAs (Asmann et al., 2009; Cloonan et al., 2008; Guo et al., 2013b; Marioni et al., 2008; Wang et al., 2009c). While the majority of studies focus solely on pcRNAs, high throughput sequencing allows us to perform advanced data mining (Han et al., 2014; Samuels et al., 2013; Vickers et al., 2015; Ye et al., 2014). One of the primary minable yet underutilized products of RNAseq data is lncRNA. Based on previous findings (Guo et al., 2015), total RNAseq produces data better suited for studying lncRNA than RNAseq data produced from a poly(A) RNA library. Taking advantage of the unique properties of total RNAseq, we profiled lncRNA expression in the mouse auditory forebrain at four postnatal time points (postnatal days P7, P14, P21, and adult), spanning the period before and after the onset of hearing (i.e., ~P11). The primary goal was to characterize the development of lncRNA over time by employing differential expression analyses of the transcriptome between time points and brain regions. The findings augment our previous characterization of pcRNA in the auditory forebrain acquired from the same animal subjects (Hackett et al., 2015).

2. Materials and methods

2.1. Tissue acquisition

All procedures were approved by the Animal Care and Use Committee at Massachusetts Eye and Ear Infirmary and adhered to the guidelines established by the National Institutes of Health for the care and use of laboratory animals. The morning that a new litter of pups was first observed was designated P0. Brains were collected from 24 adult (8–10 weeks) and juvenile (P7, P14, and P21) male and female C57BL/6J mice (Jackson Labs 000664) ($N = 6$ per age, equal numbers of males and females, total = 24). Animals were euthanized intraperitoneally with a lethal dose of ketamine and xylazine (200/50 mg/kg, respectively). Brains were removed immediately, flash frozen on dry ice, and stored at −80 °C.

2.2. Sample acquisition

Frozen brains from 6 animals in each age group (3 male, 3 female) were sectioned in the coronal plane (rostral to caudal) on a sliding microtome and viewed through a surgical microscope. As areas targeted for sampling became visible (A1, primary auditory cortex; MG, medial

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