IncRNA expression in the auditory forebrain during postnatal development

Yan Guo, Pan Zhang, Quanhu Sheng, Shilin Zhao, Troy A. Hackett

1. Introduction

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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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 (Cabrini et al., 2011; Harrow et al., 2012; Iliott 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; Perlke, 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 (Cabrini et al., 2011; Guttman et al., 2010; Liu et al., 2016), but transcript abundance is not known to be related to function (Ulltisy 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., 2008). With respect to nervous system development, the functions of most are unknown, but many are now observed for protein coding genes, lncRNA expression patterns in two different divisions of the auditory forebrain at key postnatal ages relative to the onset of hearing. 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; Gutmann 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. 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; Gutmann 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).

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
geniculate body), they were extracted using a sterile tissue punch or cuvette of a size appropriate to the brain region. A1 samples were obtained using a 0.5 mm diameter punch, with the ventral edge beginning approximately 1 mm dorsal to the rhinal fissure. MG samples were harvested with a cuvette after using a micro-dissecting scalpel to circumscribe its perimeter. Auditory cortex samples were centered on A1 but potentially included some tissue in the adjacent auditory field dorsal to A1. For the MG, the microdissection procedure was intended to exclude the lateral geniculate nucleus (LGN) and adjoining nuclei dorsal, medial, and ventral to the MG. The extreme rostral and caudal poles of the MG were largely excluded from these samples. Punches from homologous areas of both hemispheres were combined in a sterile tube containing 400 μl of Trizol, homogenized for 45 s using a mechanized sterile pestle, flash frozen on dry ice, then stored at −80 °C.

2.3. RNA extraction and sequencing

For each Trizol lysate, 100 μl of Reagent Grade Chloroform (Fisher Scientific, S25248) was added. The samples were centrifuged for 3 min on a desktop centrifuge to fractionate the aqueous and organic layers. After centrifugation, the resulting aqueous layer was carefully removed and transferred to 2.0 ml Sarstedt tubes (Sarstedt, 72.694), which were run on the QIAasympson by using the QIAasympson RNA Kit (Qiagen, 931636) and protocol RNA CT_400_V7, which incorporates DNase treatment. Prior to each run, the desk was uv-irradiated using the programmed cycle. The resulting RNA was eluted to 100 μl of RNase free water and stored at −80 °C in 2.0 ml Sarstedt tubes until use. Samples were initially quantitated using a Qubit RNA assay. Additional analyses of purity and the quantitation of total RNA were performed using a NanoDrop spectrophotometer (Thermo Scientific) and Agilent RNA 6000 Pico chip (Agilent) using the protocol, reagents, chips, and ladder provided in the kit. RNA Quality control data for the 48 samples sequenced are contained in Supplementary Table S1.

RNAseq was performed by the Vanderbilt Technologies for Advanced Genomics core (VANTAGE). Total RNA was isolated with the Aurum Total RNA Mini Kit. All samples were quantified on the Qubit RNA assay. RNA quality was verified using an Agilent Bioanalyzer. RNAseq data was obtained by first using the Ribo-Zero Magnetic Gold Kit (Human/Mouse/Rat) (Epicentre) to perform ribosomal reduction on 1 μg total RNA following the manufacturer’s protocol. After ribosomal RNA (rRNA) depletion, samples were then purified using the Agencourt RNAClean XP Kit (Beckman Coulter) according to Epicentre protocol specifications. After purification, samples were eluted in 11 μl RNase-free water. Next, 1 μl ribosomal depleted samples were run on the Agilent RNA 6000 Pico Chip to confirm rRNA removal. After confirmation of rRNA removal, 8.5 μl rRNA-depleted samples were put into the Illumina TruSeq Stranded RNA Sample Preparation kit (Illumina) for library preparation. Libraries were multiplexed six per lane and sequenced on the HiSeq 2500 to obtain at least 30 million paired end (2 × 50 bp) reads per sample.

The complete set of raw sequencing files is available from the National Center for Biotechnology Information (NCBI) database under accession number SRP053237 (http://www.ncbi.nlm.nih.gov/projects/geo/). All other supporting data are included in the Supplementary files.

2.4. RNAseq data processing

The RNAseq data went through multiple stages of thorough quality control as recommended by Guo et al. (2013c). Raw data and alignment quality control were performed using Q3 (Guo et al., 2014a), and gene quantification quality control was conducted using MultiRanKSeq (Guo et al., 2014b). Raw data were aligned with TopHat2 (Kim et al., 2013) against the mm10 mouse reference genome, and read counts per gene were obtained using HTSeq (Anders et al., 2014). Normalized read counts (used in all plots) were obtained by normalizing each gene’s read count against the sample’s total read count, then multiplied by a constant (1 × 10^6). pcRNA and IncRNA were annotated using references file MM10 v38.82 downloaded from Ensembl. Hierarchical clustering analysis and heatmaps were produced using the Heatmap3 (Zhao et al., 2014) package from R. For all samples, quality control data are contained in Table S2.

Differential expression analyses between all postnatal ages and brain regions were performed using MultiRankSeq (Guo et al., 2014b) with three methods for RNAseq analysis: DESeq (Anders and Huber, 2010); edgeR (Robinson et al., 2010); baySeq (Hardcastle and Kelly, 2010). These three methods were chosen based on results of several previous studies in which multiple RNAseq differential analysis methods were compared for accuracy and sensitivity of read count-based data (Dillies et al., 2013; Guo et al., 2013a; Kvam et al., 2012; Robles et al., 2012; Soneson and Delorenzi, 2013). In analyses of the same dataset, the methods typically differ in numbers of differentially expressed genes identified in a comparison of any two samples and also in the direction of expression (up- or down-regulation). False discovery rate (FDR < 0.05) was used to correct multiple testing. The differential expression datasets associated with each pairwise comparison (4 ages × 2 brain areas) are contained in Supplementary Tables S5–S10. Trend analysis of IncRNA expression across the four age points (P7 → P14 → P21 → Adult) was conducted using the Mann–Kendall trend test (Hirsch et al., 1982).

Potential interactions between IncRNAs and pcRNAs were identified using Spearman correlation analysis. To evaluate IncRNA coding potential, we employed the Coding-Potential Assessment Tool (CPAT) (Wang et al., 2013b) (Table S11). BEDTools (Quinlan and Hall, 2010) was used to extract the genomic sequences of IncRNA as input for CPAT. We also performed network analysis using Cytoscape (Saito et al., 2012) and function analysis using WebGestalt (Wang et al., 2013a) based on the correlation results (Table S12). To ensure high correlations were not due to static low expression values across all samples, we filtered out the lowest 25% of all RNAs based on standard deviation. For a subset of genes, IncPro (Lu et al., 2013) was applied to obtain interaction scores between IncRNAs and selected protein targets.

2.5. Database and Look-Up tool for generating IncRNA maturational profiles

Table S4 contains the raw read counts, differential analyses, and pcRNA correlations for all IncRNAs. Tables S5–10 contain the differential expression analyses for comparisons of postnatal age and brain region. To facilitate screening and extraction of maturational profiles from the database, a Look-Up tool was developed (Table S13). The tool automatically plots the maturational profiles and correlation matrices for any single IncRNA gene or list of genes (up to 25 at a time) by brain region. It also generates a listing of the normalized counts for all samples by age and brain region for custom applications.

3. Results

3.1. Data quality

RNAseq data were obtained from 48 samples and quality controlled. Sample information (sample ID, brain region, age, sex, and quality assessments) is contained in Table S1. On average, each sample was sequenced with 33.8 million reads (range: 27.6–45.1 million). Sample 10 failed sequencing with less than half million reads produced, and thus was removed from subsequent analyses. No other quality issue was observed. The raw data statistics are contained in Table S2. Alignment quality control was conducted, revealing an average of 77.19% of all reads (range: 51.86%–83.01%) were aligned to coding RNA regions (Table S3). The complete raw read count information can be found in Table S4.
3.2. Cluster analysis

To examine differences in lncRNA expression associated with age and brain region, we first performed an unsupervised cluster analysis using Heatmap3 (Zhao et al., 2014). This analysis revealed that lncRNA expression patterns are distinctively associated with postnatal age and brain region (Fig. 1). Of special interest were the stronger differences by brain region in lncRNA expression patterns for older mice. That is, P7 samples were clustered together first, then clustered by brain regions. P14 to adult samples were separated into two large clusters by brain region and then by age within each regional cluster. This suggests that lncRNA expression in the A1 and MG regions was relatively similar for younger mice before hearing onset. Then, with maturation, the lncRNA expression patterns became more regionally distinct. Gender, on the other hand, had no significant role in lncRNA expression in A1 or MG regions. The unsupervised cluster analysis showed that by using lncRNA expression information alone, we can distinguish brain regions and postnatal age.

3.3. Differential expression analyses

Differential expression analyses were carried out by comparing A1 and MG at different ages and collectively. Because we used three RNAseq expression analysis packages, differential expression for a gene was considered to be significant if all three methods identified it as significant. Summaries of various comparisons are contained in Tables 1 and 2. The detailed results (including fold change and raw and adjusted p-values of all genes) of the comparisons can be found in Tables S5–S10. The results reveal several trends. First, in comparing brain regions, there were substantial regional differences in expression at all ages. The total numbers were fairly stable from P7 to P14, decreased by nearly 60% from P14 to P21, and then by an additional 53% from P21 to adulthood (Table 1). Similarly, comparing successive ages within each region, the numbers of differentially expressed genes were greatest in the P7–P14 interval as compared to all other intervals (Table 2, top). These results indicate that regional differences in lncRNA expression are greatest during the earlier stages of postnatal
development, and before and shortly after the onset of hearing. Second, the total numbers of differentially expressed genes from P7 to adult were 70% greater in A1 (N = 554) as compared to MG (N = 388) (Table 2, top). This suggests a greater degree of genomic modification in A1 from P7 to maturity. Finally, of the differentially expressed genes in one age interval (e.g., P7–P14), a minority of the same genes was also differentially expressed at another age interval (e.g., P14–P21, P21–adult) (Table 2, middle and bottom). Only a handful of the same genes were that were differentially expressed between P7 and P14 exhibited significantly changed expression from P21 to adult (A1, N = 0; MG, N = 3). This indicates that IncRNA genes whose expression levels change with age tend to be different between age intervals.

Table 3 lists the top 50 up- and down-regulated genes in A1 and MG, ranked by log2 fold change (FC) in expression from P7 to adult. A minority of these genes were up-regulated (N = 11) or down-regulated (N = 9) in both regions. This indicates that the IncRNA genes with the greatest changes in expression during maturation tended to be preferentially expressed in A1 or MG. Only two genes (GM26524, GM15564) were up-regulated in one region (MG) and down-regulated in another (A1). These findings are evidence of strong regional specificity in IncRNA expression during maturation.

Table 4 lists the 50 genes at each age that were more highly expressed in MG than A1 (left) or A1 than MG (right). Several trends were noted. First, a relatively large number of genes exhibited regional dominance at only one age (MG, N = 47; A1, N = 56). This implies that many genes are differentially regulated in A1 or MG at a particular age. Second, a minority of genes was regionally dominant in A1 or MG at all ages (MG, N = 18; A1, N = 11) or three out of four ages (MG, N = 3; A1, N = 11). These genes have strong regional specificity, regardless of age. Third, only one gene (1700080N15Rik) was regionally dominant in A1 at one age and MG at another. This IncRNA was more highly expressed in MG in adults, but in A1 at P7. The rarity of genes with such patterns is further evidence of strong regional specificity among the majority of IncRNA genes.

### 3.4. Expression trend analysis

Expression trend analyses were carried out to identify genes with different expression growth patterns with age. We focused on three IncRNA expression patterns: monotonically increasing, monotonically decreasing and static. A gene was monotonically increasing if its expression continuously increased at each time point and the change between P7 and adult was statistically significant. Monotonically decreasing genes were defined in the same fashion, but had decreased expression at each time point. A gene was static if the absolute fold change of the expression value between any two time points was <1.5. IncRNA that had other patterns of expression between P7 and adult (e.g., increasing, then decreasing) were categorized as “other.” The numbers of significantly monotonically decreasing, increasing and static genes in A1, MG and A1 + MG are given in Table 5, along with the numbers of genes with a different trajectory (Other). In A1, about 7% IncRNAs had an increasing pattern, 10% had a decreasing trajectory, 32% had static profiles, and 51% had trajectories classified as “other.” For MG, about 5% of IncRNAs had an increasing pattern, 13% had a decreasing pattern, 32% were static, and 50% had “other” trajectories. A minority of the genes with increasing (N = 29) or decreasing (N = 96) profiles were common to both A1 and MG (A1 | MG). In contrast, many of the IncRNAs with static or other profiles were common to both regions. These findings indicate that expression levels for the majority of IncRNAs were changing during maturation, and also indicate strong regional specificity for genes with monotonically increasing or decreasing trajectories.

### 3.5. Analysis of protein coding potential

We evaluated all IncRNAs’ protein coding potential using CPAT (Wang et al., 2013b) (Table S11). CPAT summarizes each IncRNA’s coding potential with a coding probability. The majority of the IncRNAs have near zero coding probability, but a relatively small group has high coding probabilities (Fig. 2A). Of these, 190 IncRNAs had a coding probability > 0.90 (see Table S11), many of which are currently listed as provisional protein-coding genes in the NCBI drosophila gene database (source: flybase.org).

### 3.6. Analyses of potential interactions with pcRNAs and proteins

To learn more about potential interactions involving IncRNAs, we computed Spearman’s correlations (expression levels by age) between all possible IncRNA and pcRNA pairs and identified an abundance of highly correlated pairs. There are over 600 million possible IncRNA–pcRNA pairs, and the Spearman’s correlations followed a normal distribution (Fig. 2B). Only pairs with the highest correlations were selected for further functional analysis (Table S12). Using Spearman correlation [r] > 0.95 as the cutoff, there were 321 positively correlated pairs and 221 negatively correlated pairs. Putting these pairs into Cytoscape, we identified four major regulation network clusters in the mouse auditory forebrain (Fig. 3). These clusters were mainly associated with four sets of IncRNAs: Cluster 1: Gm13629, A33007422rik, 2900079g21rik, Miat, 530343g04rik; Cluster 2: 9530082p21rik, Sngh6, Neat1, B230217c12rik, A33002324rik, 2410018l13rik; Cluster 3: Rpo3–44271, Ccd4c41os1, C330006a16rik, Gms425; Cluster 4: Gm41290, Gm26794, 543041722rik.

Additional functional analysis was carried out for the pcRNA within each cluster using WebGestalt (Wang et al., 2013a) (Table 6). Functional categories differed between the four clusters. These functions included

### Table 1
Differential expression of IncRNAs between A1 and MG by age. The total numbers of IncRNAs that were differentially expressed between A1 and MG are listed by postnatal age.

<table>
<thead>
<tr>
<th>Age</th>
<th>A1 vs MG</th>
<th>Differentially expressed lncRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>P7</td>
<td></td>
<td>P7</td>
</tr>
<tr>
<td>P14</td>
<td></td>
<td>P14</td>
</tr>
<tr>
<td>P21</td>
<td></td>
<td>P21</td>
</tr>
<tr>
<td>Adult</td>
<td></td>
<td>Adult</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>A1 vs MG</th>
<th>Differentially expressed lncRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>P7</td>
<td></td>
<td>375</td>
</tr>
<tr>
<td>P14</td>
<td></td>
<td>430</td>
</tr>
<tr>
<td>P21</td>
<td></td>
<td>180</td>
</tr>
<tr>
<td>Adult</td>
<td></td>
<td>85</td>
</tr>
</tbody>
</table>

### Table 2
Differential expression of IncRNAs between age groups by brain region. In each panel, the totals re

### Table 3
Differential expression of lncRNAs between age groups by brain region. In each panel, the totals re

### Table 4
Differential expression of lncRNAs between A1 and MG by age. The total numbers of IncRNAs that were differentially expressed between A1 and MG are given in Table 5, along with the numbers of genes with a different trajectory (Other). In A1, about 7% IncRNAs had an increasing pattern, 10% had a decreasing trajectory, 32% had static profiles, and 51% had trajectories classified as “other.” For MG, about 5% of IncRNAs had an increasing pattern, 13% had a decreasing pattern, 32% were static, and 50% had “other” trajectories. A minority of the genes with increasing (N = 29) or decreasing (N = 96) profiles were common to both A1 and MG (A1 | MG). In contrast, many of the IncRNAs with static or other profiles were common to both regions. These findings indicate that expression levels for the majority of IncRNAs were changing during maturation, and also indicate strong regional specificity for genes with monotonically increasing or decreasing trajectories.

### Table 5
Differential expression of IncRNAs between age groups by brain region. In each panel, the totals re

### Table 6
Differential expression of IncRNAs between age groups by brain region. In each panel, the totals re
comparing P7 to adult (FC = average of DESeq2, EdgeR, BaySeq). Genes that were up- or down-regulated in both regions are in bold text.

As a group, lncRNAs tended to have lower overall expression levels, normalized read counts) varied within and sometimes between regions. Observed. First, the expression levels of pcRNAs and lncRNAs (mean Figs. 4 and 5, the expression levels of genes in both Clusters were plotted brain activity were selected as exemplars from Clusters 1 and 3. In coding transcripts with known involvement in brain development or pcRNAs that are essential for normal brain structure and function in the adult and developing brain.
pcRNAs were correlated with more than one IncRNA, yet the maturational trajectories of a given pcRNA was not always predictive of its correlations. For example, in Cluster 1, Kcn1 was positively correlated with both Gm13629 and A330074k22rik, Vamp1 was negatively correlated with Miat and 2900079g21Rik, while Palm was positively correlated with 5330434g0Rik and negatively correlated with Miat. Similarly, in Table 5.

Table 5

<table>
<thead>
<tr>
<th>Direction</th>
<th>A1</th>
<th>MG</th>
<th>A1</th>
<th>MG</th>
</tr>
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<tbody>
<tr>
<td>Increasing</td>
<td>216</td>
<td>143</td>
<td>29</td>
<td>15</td>
</tr>
<tr>
<td>Decreasing</td>
<td>325</td>
<td>427</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Static</td>
<td>1044</td>
<td>1034</td>
<td>1157</td>
<td>1157</td>
</tr>
<tr>
<td>Other</td>
<td>1628</td>
<td>1609</td>
<td>1931</td>
<td>1931</td>
</tr>
</tbody>
</table>

Cluster 3, Ncam1, Dyps13, and Srgap2 were positively correlated with Rrp3-4217.1 (1110015018Rik) and negatively correlated with Cdc41os1.

Tables 7 and 8 contain the Spearman correlations (r) between each pcRNA – IncRNA pair, along with the IncRNA-protein interaction scores (is) computed by the IncPro analysis. Several observations were notable. First, while the cutoff for the clustering of pcRNA – IncRNA pairs in Fig. 3 was set at |r| = 0.95, most pcRNAs also had significant correlations with the other IncRNAs in its Cluster. Second, the majority of pcRNA – IncRNA pairs with high correlations also had moderately-high to high predicted protein interaction scores, although some exceptions were also noted. For example, the pcRNA Kcn1 (voltage-gated potassium channel Kv3.1) was linked with two IncRNAs in Cluster 1 (Gm13629, A330074k22rik). The correlations with Kcn1 were high at r = 0.95, while the predicted protein interaction scores were moderate for Gm13629 and high for A330074k22rik (Table 7). Similarly, Vamp1 (vesicle-associated membrane protein 1) was highly correlated with...
Fig. 2. Protein coding probabilities and correlations. A: Histogram of the coding probabilities for all lncRNAs that were expressed in mouse brain. B: Histogram of Spearman's correlation coefficient between all lncRNA – pcRNA pairs. LncRNAs and pcRNAs with nominal expression levels were filtered out to avoid artificially high correlations.

Fig. 3. Cluster network clusters of correlated pairs. Cluster network built in Cytoscape from the correlation results in Fig. 2B. Four major network clusters were visible.
but the protein interaction score was rather low (i.s. = 46.82). Conversely, some pairs with relatively low correlations (e.g., Kcn2–Miat) had high interaction scores. Kcn2 (voltage-gated potassium channel Kv3.2) was modestly correlated with Miat (r = 0.50), but their interaction score was high (i.s. = 90.72). Finally, both positive and negative correlations were observed for some pairs with high protein interaction scores (e.g., Rehn, Kif5a), suggesting potential interactions between pairs with opposing maturational trajectories.

To further explore potential lncRNA–pcRNA interactions, we inspected the genomic loci of two highly-correlated pairs from Tables 7 and 8 with sequences located on the same chromosome, as sequences with overlapping or nearby domains tend to have higher correlations and greater interaction potential (see Discussion). Their genomic loci are illustrated in Fig. 6.

From chromosome 5, the pcRNA Rehn (Reelin) and lncRNA Miat had a strong correlation (r = 0.88) and protein interaction score (i.s. = 96.44). Miat is located nearly 90 Mb downstream of Rehn in a region occupied by several lncRNAs and other non-coding genes, and are transcribed in the same direction. Its distant location from Rehn on chromosome 5 is indicated in Fig. 6, but the sequence was not illustrated. By comparison, two antisense lncRNAs (Gm16110, Gm10475) overlapped regions of the Rehn sequence. Although, their correlations fell below the |r| = 0.80 cutoff, all three genes had significant downward trajectories in both A1 and MG (see Tables S9, S10).

On chromosome 10, the pcRNA Kif5a (Kinesin heavy chain isoform 5a) is located just downstream of the lincRNA F420014N23Rik and transcribed in opposite directions. Kif5a was strongly upregulated from P7 to adult in A1 and MG. F420014N23Rik expression was upregulated in A1 and static in MG. Of additional interest was that the F420014N23Rik locus overlaps another pcRNA, Pip4k2c (Phosphatidylinositol 4-phosphate 5-kinase), also strongly upregulated in both brain regions, which is in a family of proteins with roles in brain development and vesicular transport. Thus, potential interactions may be possible with one or both pcRNAs.

Overall, these results suggest that lncRNAs that are highly correlated with a given pcRNA may be more likely to interact with the associated protein, but the predictive value of correlated expression is uncertain.
4. Discussion

In the present study, we set out to achieve two goals. The first was to generate complete lncRNA transcriptome profiles of A1 and MG during postnatal development before and after the onset of hearing. As a foundation for future study, our second goal was to create a user-friendly searchable database and Look-Up Tool to facilitate the examination and exploration of trends in expression by brain region and postnatal age. Overall, the differential analyses of global lncRNA expression revealed significant differences between brain regions and changes in both regions with postnatal age. Based on lncRNA expression profiles alone, we could distinguish between brain regions and ages. Analyses of potential interactions between lncRNA and pcRNAs or proteins revealed how maturational changes may be manifested within functional categories.

4.1. Regional and temporal specificity in lncRNA expression

The expression of lncRNAs was regionally-specific. Globally, expression profiles in A1 were distinct from MG at every postnatal age examined. A relative minority was strongly up- or down-regulated in both regions. This is consistent with the expression patterns of pcRNA genes in the auditory forebrain (Hackett et al., 2015), and with studies of lncRNAs in other brain regions (Belgard et al., 2011; Liu et al., 2016). Indeed, numerous studies have found that lncRNA expression patterns vary by brain region (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). In addition, the distributions of many genes are specific to restricted loci, such as cortical layer, cell type, or subcellular compartment (Aprea et al., 2013; Belgard et al., 2011; 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; Spigoni et al., 2010; Tochitani and Hayashizaki, 2008). Thus, the functional roles of many pcRNAs and lncRNAs in the developing and mature brain are spatially-specific.

In addition to spatial specificity, several studies have reported that lncRNA expression is often temporally specific (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). These findings are consistent with the maturational trends that we observed in the auditory forebrain. Within each brain region (A1, MG), lncRNA expression profiles were distinct at each postnatal age. In both regions, the majority of lncRNAs had maturational trajectories that reflected a change in expression level between at least two consecutive age groups. Only about one-third of lncRNAs had static profiles from P7 through adult.

Table 7

lncRNA–pcRNA correlations and interactions. Correlations and predicted protein interaction scores between pcRNA and lncRNA genes involved in neurotransmission and plasticity in the brain (selected from Fig. 3, Cluster 1). Spearman correlations (r) and lncPro interaction scores (is; score range 1–100) are listed for each pcRNA-lncRNA pair. Bold type denotes pcRNA–lncRNA pairs illustrated in Cluster 1 with correlations of at least 0.95. Raw data in Table S12.

<table>
<thead>
<tr>
<th>Chr</th>
<th>Gm13629</th>
<th>A33007422rik</th>
<th>5330434604rik</th>
<th>Miat</th>
<th>2900079g21rik</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
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<td>62.56</td>
<td>0.89</td>
<td>82.90</td>
<td>0.76</td>
</tr>
<tr>
<td>8</td>
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<td>62.94</td>
<td>0.91</td>
<td>87.39</td>
<td>0.76</td>
</tr>
<tr>
<td>1</td>
<td>0.97</td>
<td>83.01</td>
<td>0.91</td>
<td>94.01</td>
<td>0.76</td>
</tr>
<tr>
<td>12</td>
<td>0.97</td>
<td>97.00</td>
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<td>0.76</td>
</tr>
<tr>
<td>14</td>
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<td>0.94</td>
<td>77.77</td>
<td>0.76</td>
</tr>
<tr>
<td>5</td>
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<td>-0.94</td>
<td>97.32</td>
<td>-0.82</td>
</tr>
<tr>
<td>10</td>
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<td>96.29</td>
<td>-0.70</td>
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</tr>
<tr>
<td>6</td>
<td>0.94</td>
<td>57.77</td>
<td>0.93</td>
<td>77.77</td>
<td>-0.88</td>
</tr>
</tbody>
</table>
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; Uilitsky et al., 2011; Vance et al., 2014). For example, the growth factors Bdnf (Brain-derived neurotrophic factor) and Fgfr2 (Fibroblast growth factor 2) are intensively-studied genes with key roles in brain development and plasticity. Both are regulated by their antisense sequences (Bdnf-AS, Fgfr2-AS) in a manner that impacts neuronal proliferation, neurite outgrowth, and maturation (MacFarlane et al., 2010; Modarresi et al., 2012). Similarly, the functions of other lncRNAs appear to be linked to neuronal activity, as suggested by altered expression with changes in activity (Barry et al., 2014; Kim et al., 2010; Lipovich et al., 2012).

Although functional characterization was beyond the scope of the present study, we made efforts to identify lncRNAs with the potential to interact with selected pcRNAs or their proteins in the auditory forebrain. One approach was to select lncRNA-pcRNA pairs with high correlations in the P7-adult expression trajectory. From these, several genes with known involvement in brain development or plasticity were selected for predictive analysis of lncRNA – protein interactions. This tactic was explored because the interactions of lncRNAs are not necessarily restricted to miRNA, as lncRNAs may also interact with proteins (Hacisuleyman et al., 2014) and can regulate post-transcriptional processes (Yoon et al., 2013). We found that most of the highly correlated genes also had strong predicted interaction scores, based on sequence matching and secondary structure (Lu et al., 2013). Naturally, predicted interactions require experimental validation. However, prior data suggest that the screening of correlated lncRNA-pcRNA pairs for potential interactions is an efficient way to identify genes that are promising for direct functional study.

For example, among the most highly expressed and upregulated lncRNAs in both auditory forebrain regions was the lincRNA, Malat1 (Metastasis associated lung adenocarcinoma transcript 1). Malat1 is strongly expressed in the brain, mainly by neurons (Bernard et al., 2010), and localized to nuclear speckles (Clemson et al., 2009; Hutchinson et al., 2007). In cultured hippocampal neurons, Bernard et al. (2010) found that Malat1 expression levels increased steadily from P0 through P28, consistent with our findings in A1 and MG. They also showed that overexpression of Malat1 increased presynaptic bouton density on dendrites, while knock-down reduced synaptic density. Accordingly, gene ontology categories linked to synaptic and dendritic formation were enriched after Malat1 overexpression, although not all genes in these categories were affected. Further, cells transfected with Malat1 oligonucleotides exhibited reduced expression of the post-synaptic proteins neurelin 1 (Ngl1) and synaptic cell adhesion molecule 1 (Cadm1), while the Eph receptor B2 (Ephb2) and neuronal

Table 8
lncRNA – pcRNA correlations and interactions. Correlations and predicted protein interaction scores between pcRNA and lncRNA genes involved in neurotransmission and plasticity in the brain (selected from Fig. 3, Cluster 3). Spearman correlations (r) and IncPro interaction scores (is; score range 1–100) are listed for each pcRNA-lncRNA pair. Bold type denotes pcRNA – lncRNA pairs illustrated in Cluster 3 with correlations of at least 0.95. Raw data is in Table S12.

<table>
<thead>
<tr>
<th>Chr</th>
<th>Gm4425</th>
<th>C339066A16Rik</th>
<th>Cdc41os1</th>
<th>RP23-442D7.1*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>is</td>
<td>r</td>
<td>is</td>
</tr>
<tr>
<td>Klf5a</td>
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<td>-0.95</td>
<td>95.05</td>
<td>0.87</td>
</tr>
<tr>
<td>Snap25</td>
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<td>-0.90</td>
<td>97.13</td>
<td>0.95</td>
</tr>
<tr>
<td>L1cam</td>
<td>X</td>
<td>0.93</td>
<td>60.77</td>
<td>-0.87</td>
</tr>
<tr>
<td>Ncam1</td>
<td>9</td>
<td>0.92</td>
<td>77.44</td>
<td>-0.86</td>
</tr>
<tr>
<td>Dpy313</td>
<td>18</td>
<td>0.94</td>
<td>59.98</td>
<td>-0.91</td>
</tr>
<tr>
<td>Srgap2</td>
<td>1</td>
<td>0.92</td>
<td>78.96</td>
<td>-0.90</td>
</tr>
</tbody>
</table>

* Alternative name: 1110015018Rik (ENSMUSG00000064659).
pentraxin 2 (Nptx2 or Narp) were relatively unchanged. This suggests that Malat1 regulates expression of a subset of genes involved in synapse formation in cultured hippocampal neurons. In the auditory forebrain, where Malat1 was strongly upregulated from P7 to adult, we found that Ngn1, Cadm1 and Ephb2 were significantly downregulated in one or both auditory regions, whereas Nptx2 was upregulated in A1 and static in MG (Hackett et al., 2015). The differences suggest that regulation of the same genes by Malat1 can vary by gene and brain region. Accordingly, the potential for interactions between lncRNAs and pcRNAs to be regionally and temporally specific suggests that the interactions identified in one brain region or cell population may not apply to others.

An additional approach that has been used to identify potential interactions between lncRNAs and pcRNAs involves inspection of the genomic loci. Generally, lncRNA expression tends to be positively correlated with the expression of neighboring or overlapping sequences (Cabilly et al., 2011; Dinger et al., 2008; Mercer et al., 2008; Ponjavic et al., 2009; Ulltsey et al., 2011). And, genes involved in the same biological pathways tend to have higher correlations when located in nearby genomic domains (Al-Shahrour et al., 2010). Although our inspection of such relationships was limited to a handful of genes, we found that some correlated pairs had overlapping or nearby genomic loci, while others were located at distant loci or on other chromosomes. Thus, in selecting lncRNA – pcRNA pairs that may interact for functional studies, it may be prudent to begin with pairs that have closely-associated loci, such as natural antisense transcripts (Carriere et al., 2012; Katayama et al., 2005; Smalheiser et al., 2008). The relationship between Bdnf and Bdnf-As mentioned above is a frequently-cited example. A second example provides additional context for discussion of such relationships. The expression of two lncRNAs (Dlx1os, Dlx6os1) are in flux during the generation and migration of GABAergic interneurons in early development (Mercer et al., 2010), where they appear to exert transcriptional control over interneuron specification in the hippocampus (Berghoff et al., 2013; Bond et al., 2009; Feng et al., 2006; Kraus et al., 2013). These studies indicated that loss of Dlx1os (aka Dlx1-AS), which partially overlaps the associated transcription factor, Dlx1, in the anti-sense direction (Dinger et al., 2008), resulted in increased Dlx1 transcript expression and increased interneuron number. Similarly, loss of Dlx6os1 (aka Ev2) resulted in increased expression of the transcription factor, Dlx6, but decreased interneuron number. Thus, loss of the antisense transcripts led to increased expression of the associated transcription factors, but disparate effects of interneuron number. In the auditory forebrain, two observations were notable with respect to the expression patterns of these genes. First, Dlx1os and Dlx6os1 expression was restricted to A1 (absent in MG), suggesting regional specificity in the auditory forebrain. Referring to in situ hybridization assays in the Allen Brain Atlas (http://mouse.brain-map.org), we noted that expression of Dlx1 and Dlx6 overlapped their antisense lncRNA counterparts in A1 and most other cortical areas, where signals are concentrated in sub-populations of putative GABAergic neurons in layers 1–3. The absence of expression in MG probably corresponds with the absence of GABAergic neurons in that structure (Hackett et al., 2016). Thus, these transcription factors and associated antisense lncRNAs have overlapping anatomical distributions in the auditory forebrain that are also restricted to a particular neuronal subclass in cortex. Second, the expression of Dlx1 and Dlx1os decreased significantly from P7 to adult in A1, mainly between P7 and P14. Dlx6 and Dlx6os1 were expressed at very low levels, but with downward trends that did not reach significance. Thus, we observed transient decreases in expression between these pcRNA-lncRNA pairs in A1 during maturation, while the loss of the same antisense lncRNAs led to increased pcRNA expression in developing hippocampus.

The reasons for these regional and temporal differences are not known, but could be informative with respect to the varied regulatory roles of lncRNAs in the adult and developing brain. However, based on the examples discussed above, we conclude that while prediction of pcRNA-lncRNA interactions based on proximity or correlated expression levels may be useful, those relationships could also be inaccurate and misleading. Carefully designed studies are needed to explore and better understand these relationships.

4.3. Species differences in lncRNA expression

In addition to cellular and regional differences in gene expression, species differences in pcRNA and protein expression evident in the central auditory pathway (Bush and Hyson, 2008; Wang et al., 2009a; Wang et al., 2009b), and are widespread throughout the brain (Bernard et al., 2012; Lin et al., 2014b; Mashiko et al., 2012; Nehme et al., 2012; Shukla et al., 2014; Van der Zee and Keijser, 2011; Watakabe et al., 2009; Zeng et al., 2012). Although not as extensively explored so far, species differences in lncRNA expression are notable, with several genes identified as rodent or primate-specific (e.g., Bdnf-As, Fmr-4, Har1f, Bac1-as, Disc2, Scant1) (Brandon et al., 2009; Faghihi et al., 2008; Khalil et al., 2009; Lipovich et al., 2012; Millar et al., 2004; Modarresi et al., 2012; Modarresi et al., 2011; Pollard et al., 2006; Pruunisild et al., 2007; Sopher et al., 2011; Tay et al., 2009; Washietl et al., 2014).

As an example, the pcRNA, brain-derived neurotrophic factor (Bdnf), has comparable expression patterns in mice, nonhuman primates, and humans, but the antisense lncRNA, Bdnf-As, is only expressed in primates. Thus, regulation of Bdnf by Bdnf-As is unique to primates (Lipovich et al., 2012), and therefore its regulation would appear to involve other mechanisms in mice or other species.

The proximity and genomic loci of interacting gene pairs may also be species dependent. One of the genes found by Bernard et al. (2010) to be regulated by Malat1 in cultured hippocampal neurons, Cadm1, is located downstream of Malat1 on human chromosome 19, whereas in mice, Malat1 is located on chromosome 19 and Cadm1 is on chromosome 9. Similarly, Malat1 (aka Neat2) is just downstream of the lncRNA, Neat1, on mouse chromosome 19. Malat1 and Neat1 were strongly upregulated in A1 and MG. Moreover, Neat1 has been linked to promotion of differentiation and maturation of neurons and oligodendrocytes (Ip and Nakagawa, 2012; Mercer et al., 2010), and is associated with neuroprotection in Huntington’s disease (Sunwoo et al., 2016). Interestingly, the genomic loci of Malat1 and Neat1 are flanked by several pcRNAs with no known functional roles in brain development or maturation (e.g., Fmrd8, Sk25a45, Dpp2, Tigd3, Pola2, Capn1, Scl1, Ltb3, Ehhbi11, Map3k1l, Sipai, Kat5). In fact, very few genes located on chromosome 19 are currently associated with these functions. One of these, Flrt1, was upregulated in A1 and MG from P7 through adult, is in a family of genes with varied roles in brain development (Haines et al., 2006; Wheldon et al., 2010; Yamagishi et al., 2011), but there is no known association with either Malat1 or Neat1.

Given these observations, we would argue that documentation of species differences is absolutely essential to make informed conclusions and predictions about the roles of particular genes, and we must be vigilant to consider such differences in the interpretation and application of profiling data.

4.4. Applications of the lncRNA database

RNAseq is a powerful tool for mRNA profiling and transcriptome analyses with broad potential applicability in neurobiology (Han et al., 2014). Relatively small amounts of starting material (<10 ng) are sufficient to conduct whole transcriptome sequencing of discrete brain areas or cell populations. The reduction in sequencing costs, development of bioinformatics tools, and availability of genomic libraries further add to the attractiveness of this approach (McGettigan, 2013; Sengupta et al., 2011). The dataset generated by this study comprises an extensive lncRNA reference library that indexes the expression of all known lncRNAs in A1 and MG from P7 to adult. In addition to information about these structures during postnatal development, the dataset is
also a rich source of information about mature animals. We envision several potential applications of this IncRNA dataset by those interested in the structure and function of the auditory forebrain.

4.4.1. Reference database and guide for functional studies of the auditory forebrain

At present, none of the known IncRNAs have demonstrated functional roles in the auditory system. A major goal of this study was to establish a database of IncRNA expression in the auditory forebrain that would provide a broad foundation to guide focused studies of IncRNA function and regulation of mechanisms that govern the maturation of auditory processing in the forebrain. By identifying the most highly expressed IncRNAs in each brain region, and those that were strongly up- and down-regulated with age, numerous IncRNAs in A1 and MG are candidates for additional study. Then, as illustrated for a subset of genes, examination of IncRNA – pcRNA correlations, shared genomic loci, and predictive analyses of protein interactions, genes with the highest potential for interaction may be identified. Thus, a potentially powerful application of this database is as a screening tool to explore novel roles and interactions. A second, and related, application is to provide a baseline for experimental studies of hearing (e.g., altered sound exposure during development, hearing loss, aging, other pathology) (Clarkson et al., 2012; Holt et al., 2005; Sharma et al., 2009; Sun et al., 2008). Transcriptomic analyses of global or targeted gene expression are powerful means to identify genes that are changing the most (or the least). The Look-Up Tool (Table S13) provides a convenient and user-friendly means to view expression levels and trends of any IncRNA and pcRNA by brain region and postnatal age.

4.4.2. Detailed anatomical profiling of IncRNA expression

In addition to regional differences in global IncRNA expression, insight into potential functional interactions may be gained by inspection of detailed anatomical distributions of IncRNA transcripts. Most IncRNA are expressed in the nucleus, but many have cytoplasmic expression, signaling potential regulation of gene expression in the cytoplasm (Batista and Chang, 2013). While only partially known, many IncRNA – pcRNA pairs in the developing brain may be co-localized within the same cells (Ponjavic et al., 2009). In addition, it is likely that IncRNAs are differentially expressed in distinct classes of neurons (e.g., glutamatergic, GABAergic, other) and glia (e.g., astrocytes, oligodendrocytes, microglia) (Cahey et al., 2008; Zeisel et al., 2015; Zhang et al., 2014). Thus, for IncRNAs that are candidates for functional studies, it would be prudent to conduct assays designed to reveal the cellular and subcellular localization of their transcripts. To some extent, resources such as the Allen Brain Atlas can be consulted to identify the regional and subcellular distributions of transcripts for some IncRNAs. Clearly, the inclusion of IncRNAs within such anatomical resources would be helpful for identification of genes expressed in target brain areas, and for validation of other detection tools (e.g., qRT-PCR, RNAseq).

5. Conclusions

The IncRNA transcriptome of the mouse auditory forebrain was profiled at four postnatal ages before and after the onset of hearing. Global, IncRNA expression was significantly different between brain regions (A1, MG) and at each postnatal age (P7, P14, P21, Adult). These patterns match trends observed for pcRNA in a prior study (Hackett et al., 2015), indicating that both RNA classes have spatial and temporal specificity in the developing (maturing) auditory forebrain. The results also identify IncRNAs with high expression levels and those with strong up- or down-regulation from P7 to Adult in both regions. Their expression levels are highly correlated with numerous pcRNAs, suggesting potential interactions. Additional analyses of selected highly-correlated pairs revealed that predicted interactions with associated proteins was often, but not always, strong, suggesting that the correlations may serve as an initial screen for potential interactions. Further study of these interactions may lead to new insights into the regulatory relationships between IncRNAs, pcRNAs, and proteins. A user-friendly database and Look-Up Tool were provided as supplementary files to facilitate inspection of the expression levels and maturational trajectories for any IncRNA or pcRNA in the database.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gene.2016.08.027.

References


Batista, P.P., Amal, Y., 2013. Transcriptome sequencing during mouse brain development identifies the least). The Look-Up Tool (Table S13) provides a convenient and user-friendly means to view expression levels and trends of any IncRNA and pcRNA by brain region and postnatal age.

References


