Published in final edited form as:
Child Dev. 2013 January ; 84(1): 76–88. doi:10.1111/cdev.12014.

Gene Expression in the Human Brain: The Current State of the Study of Specificity and Spatio-temporal Dynamics

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Abstract

Gene expression is one of the main molecular processes regulating the differentiation, development, and functioning of cells and tissues. In this review we introduce relevant concepts and describe selected techniques used in studies of gene expression/expression profiling (i.e., studies of the transcriptome or transcriptomics). The main foci of this review are the advancements in studies of the transcriptome in the human brain, the transcriptome's variability across different its structures, and systematic changes through different lifespan stages in general and childhood in particular. In conclusion, we discuss how the accumulating data on the spatial and temporal dynamics of the transcriptome may shed light on the molecular mechanisms of the typical and atypical development of the central nervous system (CNS).

Keywords

Human Brain; Gene Expression; Gene Expression Profiling; Transcriptome Variability and Dynamics

Gene Expression, Concepts and Terms

Gene expression is the most fundamental process by which the genotype gives rise to the phenotype. Step by step, information from the genes is used to create functional products through 1) the generation of RNA copies of the DNA via a process called transcription, which results in pre-mRNA (pre-messenger RNA) molecules; 2) posttranscriptional RNA modifications that produce different forms of protein-coding mRNAs (messenger RNA) from the same template pre-mRNA molecule, through the action of RNA splicing; 3) protein synthesis, called translation; and 4) posttranslational modifications of the synthesized proteins. Each of these steps is a complex set of interrelated and highly regulated events. In this article we focus on the first step—transcription. At any given moment, a cell is producing a set of RNA molecules (or transcripts), including protein-coding mRNA, ribosomal RNA (rRNA), transfer RNA (tRNA), and non-coding RNA (ncRNA). The specific subset of transcripts present in particular cell types and involved in the cell's life

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support, development and specific functioning, is called the *transcriptome*. The term *transcriptome* might be applied to the total set of transcripts or to a special type of RNA (such as mRNA) in a given cell population, tissue or organism.

Thus, the *transcriptome* is a complex and dynamic structure that is determined by and sensitive to the stages of the cell cycle and organism development, cell and tissue types, and the effects of external signals during both transcriptional and post-transcriptional processes. The study of the transcriptome is the main subject of *transcriptomics*, also referred to as *gene expression profiling* or the measuring of the activity (at the expression level) of thousands of genes at once by means of high-throughput techniques based on microarray technology or direct sequencing of the RNA. The main purpose of transcriptomics is to investigate transcripts and their differential temporal and spatial distribution in different cell types and tissues with the aim of understanding the molecular mechanisms of their differentiation, development, and functioning. Whole transcriptome profiling (or whole genome expression profiling) provides information about multiple differences in the expression of a set of genes under various conditions, with the purpose of, for example, distinguishing different type of cells or investigating cell/tissue response to treatment. Whole transcriptome profiling has particular value in studies of the genome functioning in different conditions (developmental, environmental, health), especially when large-scale gene expression changes in many genes are expected *a priori*. Correspondingly, when different conditions are compared to each other (e.g., health vs. disease or young vs. old), genome/gene expression profiling enables researchers to formulate hypotheses regarding the involvement of particular genes whose expression is different in different groups, such as candidate genes for processes that differentiate the groups (e.g., getting ill vs. being healthy or getting old vs. staying young).

There is a substantial amount of evidence that multiple changes in gene expression occur throughout the process of cell differentiation. That is, as an organism develops from the undifferentiated ball of cells that result from a fertilized egg, to a developing embryo, to a multitude of cell and tissue types present in an adult, a single gene may express itself in multiple ways depending upon its developmental context, i.e. the particular stage of development of the cell it is in as well as millions of events external and internal to the cell. Similarly, many changes in gene expression have been associated with complex somatic diseases (Cookson, Liang, Abecasis, Moffatt, & Lathrop, 2009; Dermitzakis, 2008; Emilsson, et al., 2008), developmental and behavioral disorders (Buechel, et al., 2011; Gregg, et al., 2008; Mudge, et al., 2008), and age-related changes (e.g., Lu, Pan, Kao, Li, Kohane, Chan, et al., 2004; Somel, Franz, Yan, Lorenc, Guo, Giger, et al., 2009) in different tissue types and in the organism as a whole.

Common Techniques of Gene Expression Profiling

DNA microarrays

There are basic methodological requirements for any analysis of transcript structures and their differential distribution. The method should be able to measure the absolute and relative content of transcripts of a single gene and the abundance of transcripts of a large set of genes with different expression levels simultaneously. The most frequently used method of studying whole genome expression is DNA microarrays. This technique is based on measuring differential gene expression using two-color fluorescent hybridization of target fragments with specific DNA probes attached to the solid surface of a biochip. In different microarray platforms, such as Applied Biosystems, Affymetrix, Agilent, Illumina and others, oligonucleotides that are 25-70 bases in length representing specific gene coding regions are usually used as hybridization probes. Yet, there are serious limitations in using microarray technologies for transcriptome analysis (Irizarry, et al., 2005; Kawasaki, 2006).

First, microarrays measure only the relative quantities of transcripts. Specifically, this technology suffers from fluorescence background and cross-hybridization problems that might lead to underestimating low-abundance transcripts or the expression levels of infrequently expressed genes. Second, the technique measures the activity of previously identified genes—array probes represent a predefined set of well-annotated genes. Correspondingly, this leads to underestimating new transcripts and splicing forms of a transcript, which are produced by ~95% of the multiexonic genes in the human genome (Pan, Shai, Lee, Frey, & Blencowe, 2008). Third, there is a lack of universal standards among the different platforms (e.g., differences in numbers of probes, probe designs and concentrations, the usage of fluorescent dyes to characterize different stabilities, and quantum efficiency in response to light) that result in some inconsistencies between the data obtained via different platforms. Even within the same array type there are problems with the low reproducibility of results among different experiments and different labs due to differences in RNA target preparation for hybridization and data analytic methods (Irizarry, et al., 2005; Kawasaki, 2006; Pedotti, et al., 2008).

Due to all these complexities and to concerns regarding the reliability and concordance of microarray data, especially when samples with only subtle differences in gene expression are analyzed, it has been recommended (Pedotti, et al., 2008) that two different platforms be used simultaneously. Also, international consortia and technical groups have been formed to standardize microarray technology, specifically those related to data validation and presentation (Kawasaki, 2006). Yet, despite all of its disadvantages, microarray analysis has enabled the measurement of thousands of genes in a single RNA sample simultaneously, and this has made the technology especially valuable for studies of global patterns of gene expression. Correspondingly, since 1995, there has been an exponential increase of the number of gene expression studies using microarrays. As a result of this interest, our current knowledge of transcriptomes in the human brain is based on reports of studies that used microarray techniques for transcriptome profiling.

Sequencing techniques

Along with microarray technology, sequencing techniques such as serial analyses of gene expression (SAGE, Velculescu, Zhang, Vogelstein, & Kinzler, 1995) and its subsequent modifications (RL-SAGE, SuperSAGE, and LongSAGE) have also been used for gene expression profiling. The principle of the method is based on the detection of sequences of short cDNA fragments (10-26 base pairs depending on the version of the method) from established positions of each RNA molecule. The technique includes consecutive fragmentation of the DNA template, ligation of these fragments to form a long chain, cloning the concatemer into a bacterial vector, and sequencing this chain. The output of SAGE is a list of short sequence tags and the number of times it is observed. After being annotated into the reference genome (the digital DNA sequence database assembled by scientists as a representative example of a set of genes of *Homo sapience*—the human genome), these tags provide qualitative and quantitative characteristics of transcripts in the given transcriptome. So, the serial analysis of gene expression is a method for the comprehensive analysis of gene expression patterns. In contrast to microarray techniques, this method is able to measure not only the relative but the absolute content of the transcripts of a gene, as well as detect any active gene, not just a predefined set placed on a chip. However, laborious and costly cloning and sequencing steps have greatly limited the use of SAGE techniques for large-scale studies of gene expression.

High-throughput sequencing or RNA-Seq

The development of high-throughput sequencing technologies that parallelize the sequencing process by making it possible to produce millions of sequences at once (i.e., in

parallel) has made sequence-based expression analysis increasingly popular. Since the first publication on using massively parallel signature sequencing (i.e., one particular method of high-throughput sequencing, among many) in gene expression analysis in 2000 (Brenner, et al., 2000), different manufacturers have developed various technologies for such sequencing (Ansorge, 2009). They are all based on common principles of massively parallel shotgun sequencing (a method used for sequencing long DNA strands named by analogy with the quasi-random rapidly-expanding firing pattern of a shotgun) of cDNA (complimentary DNA) libraries derived from total RNA or special RNAs depending on the objective of the study. Each of the cDNA fragments is flanked on one (single-end sequencing) or both (paired-end sequencing) ends by special oligonucleotides or adapters to be attached to the surface of the appropriate sequencing platform. Then clonal amplification produces ~1,000 copies of a single member of the template library (i.e., copies, produced from a specific cDNA fragment); this set of copies is named a cluster. After cluster generation, the templates are sequenced by synthesis to obtain short sequences named targets or reads, which are typically 30-400 bp, depending on the technology used. As output, massively parallel sequencing produces tens of millions of reads, which describe the qualitative and quantitative composition of transcripts in the transcriptome. Following sequencing, the reads are either aligned to a reference genome or transcriptome to produce a large-scale transcription map that reveals the structure and abundance of the transcripts.

RNA-Seq offers several advancements that make it particularly valuable for studies of global gene expression. By excluding cloning steps and related sequence errors, RNA-Seq produces more accurate data on transcript sequences and requires less RNA than microarray techniques (Z. Wang, Gerstein, & Snyder, 2009). Unlike hybridization-based technologies, RNA-Seq is not limited to detecting transcripts that correspond to predefined sets of well-annotated genes, and it allows the detection of alternative splice variants, the alternatively transcribed versions of a single gene resulting in different mRNAs that, in turn, are translated into different protein isoforms, so that a single gene may code for multiple proteins. RNA-Seq also allows the detection of novel transcripts (Mortazavi, Williams, McCue, Schaeffer, & Wold, 2008; E. T. Wang, et al., 2008). This makes RNA-Seq especially useful for studying complex transcriptomes. Moreover, RNA-Seq quantifies expression levels with high accuracy (Asmann, et al., 2009). The absence of significant background signal, more relaxed limits for quantification, and the greater range of expression levels that might be detected compared to microarrays allow the detection of genes expressed either at very low or high levels (’t Hoen, et al., 2008; Asmann, et al., 2009; Marioni, Mason, Mane, Stephens, & Gilad, 2008). Additionally, the results of high-throughput sequencing of the transcriptome are characterized by a high level of reproducibility both for technical and biological replicates (Marioni, et al., 2008). Furthermore, RNA-Seq produces information on the level of gene expression as either a raw number of reads (i.e., relative) or the number of reads normalized by the transcript length (i.e., adjusted for the length of the sequence whose transcript is being sequenced) and total count of reads (i.e., absolute); this capability increases the comparability of data obtained by different laboratories and in different experiments in the same laboratory.

At present, RNA-Seq, dubbed “a revolutionary tool for transcriptomics” (Z. Wang, Gerstein, & Snyder, 2009), is the most promising and increasingly used technique in global gene expression studies. Although still few, transcriptome studies of the human brain have been featured in a recent review that discussed how high resolution high-throughput sequencing techniques might increase our knowledge of gene expression in such complex organs as the brain (van der Brug, Nalls, & Cookson, 2010).

Postmortem brain tissue as a source of RNA for gene expression profiling

The source of RNA is a special issue for studies of gene expression in the brain. Commonly, postmortem brain tissue is used for the transcriptome profiling. In contrast to the relative stability of the carrier of the genetic information, DNA, RNA molecules, as temporal functional products of the realization of genetic information, are characterized by a short life-span. A special set of enzymes--ribonucleases, or RNases--are responsible for the fast degradation of RNA in vivo. This enzymatic utilization of RNAs might continue for a time after death, also postmortem chemical and thermal processes might lead to the rapid postmortem degeneration of RNAs. RNA degradation leading to qualitative and quantitative changes in the composition of the transcriptome is one of the main problems in the reliable detection of whole-genome expression profiles. Thus the nature and effects of pre- and postmortem conditions on RNA in brain tissue – such as the condition of the patient prior to death and the postmortem interval (the time that has elapsed since a person has died, PMI) – are the special subject of investigations.

Several studies report the observation that RNA degradation does not correlate with PMI in tissue frozen 36 hours or more after death (Barton, Pearson, Najlerahim, & Harrison, 1993; Heinrich, Matt, Lutz-Bonengel, & Schmidt, 2007). At the same time, there is evidence of the selective reduction in mRNA at PMIs of 48 hrs or more (Catts, et al., 2005). It was shown that increasing PMI is associated with the increase of RNA degradation (accordingly the index 28S/18S ribosomal RNA ratio). By the PMI of 48 hrs, about 12% of mRNAs show more than a two-fold decrease in abundance; transcription factors with a specific structure (carrying the AUUUA polynucleotide motif in the 3′UTR–3′ untranslated region of RNA, the, sequences on the 3′ end of an mRNA that are not translated into protein) prevail among RNAs that are susceptible to PMI-related degradation. This finding suggests that PMI-related RNA degradation might affect the spectrum of mRNA transcripts in postmortem tissue that ultimately might lead to the selective elimination of certain populations of RNA-molecules and significantly impact the original transcriptional profile in studied tissue (Catts, et al., 2005).

Similarly, the premortem period can affect the number of certain messenger RNAs (Barton, et al., 1993). A number of studies report that RNA degradation correlates with such parameters as the environment and the circumstances of death. Thus, in typically developing/functioning organisms such factors as trauma, alcohol, and drug abuse, along with others, may cause changes in gene expression patterns (Heinrich, et al., 2007). Premortem hypoxia, coma and prolonged agony are also known to be linked to significant RNA degradation in postmortem brain tissue (Barton, et al., 1993; Mexal, et al., 2006). One of the mechanisms found to affect the pre- and postmortem factors listed above on RNA degradation is the reduction in the pH (an indicator of acidity) of the brain tissue (Barton, et al., 1993; Mexal, et al., 2006).

Taken together, the published evidence indicates that considerable attention must be paid to the influences of pre- and postmortem factors in each individual case when using postmortem brain tissue, especially when quantitative analyses such as whole transcriptome profiling are performed.

The Specificity of Gene Expression in Brain Tissue

Comparison with others tissues

Brain tissue is characterized by a high level of gene expression; at least 30–50% of ~25,000 known protein coding genes (International Human Genome Sequencing Consortium, 2004) are expressed across all parts of the brain (Colantuoni, Purcell, Bouton, & Pevsner, 2000; Myers, et al., 2007). Moreover, the human brain has the highest level of gene expression

compared with other mammals such as the mouse (Enard, et al., 2002; Lockhart & Barlow, 2001) and *Homo sapiens'* closest primate relative (Caceres, et al., 2003; Enard, et al., 2002; Khaitovich, Muetzel, She, Lachmann, Hellmann, Dietzsch, et al., 2004). The results of several studies comparing transcriptomes in human and chimpanzee brains suggest that most genes differentially expressed in these species are up-regulated, or more highly expressed, in the human brain than vice versa (Caceres, et al., 2003; Khaitovich, Muetzel, She, Lachmann, Hellmann, Dietzsch, et al., 2004). In contrast, gene expression differences in other human and chimpanzee tissues, such as the heart and liver, are nearly identical in their numbers of up-(increased expression of one or more genes) and down-(decreased expression of one or more genes) regulated genes (Caceres, et al., 2003). It has been suggested (Caceres, et al., 2003) that this increased gene expression in the brain might allow for a higher level of neuronal activity and extensive changes in the physiology and function of the human brain.

The gene expression profile of the brain is clearly distinct from other tissues, as shown by a comparison of global expression patterns across 45 different human tissues, including the CNS; in this comparison, many genes have been identified that distinguish the CNS from all other tissues (Roth, et al., 2006). Several computational analyses of gene expression variability across different human tissues using exon array (de la Grange, Gratadou, Delord, Dutertre, & Auboeuf, 2010) and RNA-Seq (Ramskold, Wang, Burge, & Sandberg, 2009) data suggest that the brain (together with the kidney and testes) has higher gene expression levels and transcriptome complexity compared to other tissues. That is, brain tissue has a high number of expressed genes – 13,298 genes were found to be expressed in the human brain (the range of the number of genes expressed across different human tissues is from 11,199 to 15,518), and the brain transcriptome has more diverse populations of RNA (de la Grange, Gratadou, Delord, Dutertre, & Auboeuf, 2010; Ramskold, Wang, Burge, & Sandberg, 2009) than other tissues and cell types. There is evidence that the brain transcriptome is especially enriched with mRNAs with longer 3′UTR sequences compared with other tissues; these might be required for transporting mRNA far away from the nuclei or for specific protein functions in the brain tissue (Ramskold, Wang, Burge, & Sandberg, 2009).

Notably, some studies of gene expression in the brain based on sequencing technologies reported a high proportion of transcripts from introns and intergenic repeats— portions of the gene not coding for protein—including a subfamily of Alu-elements (a short stretch of biochemically distinctly recognizable DNA), in the transcriptome of brain tissue (Faulkner, Kimura, Daub, Wani, & Plessy, 2009; Xu, et al., 2010). Thus, we would expect an especially high level of enrichment of regulatory elements, such as transcriptional and translational factors, microRNAs and so forth. It was shown that the set of non-coding long and short RNAs (lncRNA and miRNA, respectively) is also highly enriched in the transcriptome of the brain (Chodroff, et al., 2010; Kuss & Chen, 2008; Ponjavic, Oliver, Lunter, & Ponting, 2009; Schonrock, et al., 2010; St Laurent, Faghihi, & Wahlestedt, 2009). A complex orchestra of these non-coding RNAs, which have important structural and regulatory functions as transcriptional and posttranscriptional regulators of gene expression (Amaral, Clark, Gascoigne, Dinger, & Mattick, 2011; Landgraf, et al., 2007), is expressed in the brain, where ncRNA play key roles in neuronal differentiation, development, and synaptic plasticity. Additionally, it was found that a particular set of miRNAs in the brain transcriptome seems to be structurally quite different from those found in other tissues (Landgraf, et al., 2007).

The enrichment and complexity of the transcriptome in the human brain are accompanied by high—compared to others tissues—magnitudes and levels of alternative splicing, the process by which the exons (the portions of the gene coding for protein) of a gene are reconnected in multiple ways producing different forms of mature RNA (de la Grange, et al., 2010;

Mortazavi, et al., 2008; E. T. Wang, et al., 2008; Yeo, Holste, Kreiman, & Burge, 2004). Using data from public transcript databases corresponding to 800 cDNA library sources, Yeo and colleagues (Yeo, et al., 2004) assessed the frequencies of genes containing skipped exons, i.e., genes containing alternative 3′ or 5′ splice site exons, which indicate which version of the gene needs to be transcribed (or not), that are shortened or lengthened due to alternative splicing, across a sample of 16 human tissues. Unusually high levels of all these alternative splicing events were found in the human liver, testes and brain. Additionally these particular human tissues have highly distinct splicing patterns (or sets of spliced isoforms) that differ from most other tissues. Another study (de la Grange, et al., 2010) also demonstrated that alternative splicing is especially prevalent in the brain and testes compared to other tissues and organs. It has been shown, while comparing splicing factors and exon expression profiling across 11 human tissues, that the higher prevalence of alternative splicing might be determined by a larger number of genes, including splicing factors, which are expressed in a subset of tissues and in the brain in particular.

Variability among the brain regions and anatomical structures

The brain is a complex organ that is comprised of several anatomical substructures. The literature contains evidence indicating the high variability of gene expression in distinct sites in the brain tissue. Studies that present data on transcriptome variation across brain regions suggest that the cerebellum has the most distinguishable gene expression pattern compared with other brain substructures (Lockhart & Barlow, 2001; Roth, et al., 2006; Strand, et al., 2007). Variation in gene expression across brain regions is related to both functional and anatomical differences in its substructures. Moreover, studies involving animal models show that gene expression appears to correlate with performance (e.g., on motor tasks) in some brain substructures, such as the cerebellum, amygdala, and hippocampus (Nadler, et al., 2006). Significant differences in the cell composition of the various anatomical brain substructures result in cell-specific differences in gene expression (Colantuoni, et al., 2000). For example, it has been shown that genes involved in the regulation of glutamate receptor signaling pathways are especially enriched in their expression in the cerebellum, which contains a large number of glutamatergic granule cells— small cells with axons projecting glutamate (Roth, et al., 2006). Even between cortical layers there is a difference in gene expression due to distinct populations of projection neurons that are located in different cortical layers and areas of the neocortex (Molyneaux, Arlotta, Menezes, & Macklis, 2007).

A large-scale study on gene expression profiling 20 anatomically distinct sites in the human CNS using the Affymetrix microarray suggested that different sites in the CNS fall into recognizable clusters corresponding to the CNS's functional and anatomical groups, such as the cerebral cortex, basal ganglia, limbic system, forebrain, midbrain, hindbrain and spinal cord (Roth, et al., 2006). It was shown that the global transcriptome profile reflects the anatomy/function of the CNS and cluster-specific genes were detected, which ranged in number from 8 (in the hypothalamus) to ~2,000 (in the cerebellum). Another important finding of this study is that within groups—with the exception of the cerebellum and the spinal cord—very few substructures of the CNS, such as the putamen and the corpus callosum, contain transcriptions of region-specific genes, and no genes show differential expression among the tested cortical sites of the brain tissue from the occipital, parietal, frontal, and temporal lobes of the cortex (Roth, et al., 2006).

The high variability of the transcriptome among distinct brain regions has been confirmed in another study (Strand, et al., 2007) comparing the global transcriptome analyses in the motor cortex, caudate nucleus, and cerebellum, using the same Affymetrix microarray technique. In this study approximately 30 genes were detected that showed region-specific expression of the "on/off" type. Comparing these three distinct substructures of the brain across 12 individuals (eight men and four women, whose ages ranged from 36 to 77), the researchers

showed that human individual variability in gene expression neither obscures nor significantly contributes to regional differences (Strand, et al., 2007). In contrast, there were many fewer differences found between cortical areas within the individual brain (i.e., between areas within the same individuals) than in regional differences between individuals (i.e., within one area between different individuals), despite their substantial differences in function and the cellular architecture of the neurons in the cerebral cortex of the brain (Khaitovich, Muetzel, She, Lachmann, Hellmann, & Dietzsch, 2004; Naumova, et al., unpublished data). For example, only one of the 4,998 genes with detectable expression differences between Broca's area (inferior frontal cortex) and the left prefrontal cortex, was identified in three human individuals analyzed in a study comparing the dorsolateral prefrontal cortex, anterior cingulate cortex, primary visual cortex, and Broca's area (Khaitovich, Muetzel, She, Lachmann, Hellmann, Dietzsch, et al., 2004). The high variability and low concordance of gene expression within the cortical regions between individuals might reflect genetic heterogeneity, differential responses of different individuals to the environment, brain plasticity in the realization of higher cognitive functions throughout the lifetime and in different environments, among many other "unknowns." Only the systematic study of the gene expression variation among distinct functional cortical areas will be able to resolve these intriguing questions on the interindividual differentiation of the cerebral cortex in terms of gene expression, as well as to provide a better understanding of the molecular mechanisms underlying the distinct behavioral functions controlled by the CNS and the typical and atypical (pathological) development of these functions.

Dynamics of Gene Expression in the Brain through the Life-Span

Development, maturing and aging of the brain

Most of the brain's cells are formed before birth, but the weight of a newborn's brain is about 25% of its approximate adult weight. For a few years after birth the brain continues to grow through glia dividing and multiplying; these are the non-neuronal cells that maintain homeostasis, form myelin, and provide support and protection for neurons in the brain. By the age of 2 years, the brain is about 80% of the adult size (Dekaban & Sadowsky, 1978). The neurons in the brain make many new connections after birth, but most of the connections among cells are made during infancy and early childhood, as responses to new experiences and learning, which continue throughout life but occur most intensively during early childhood, especially in the first three years of life. By the age of 20-25 years the brain has matured, but some developmental processes, such as axon myelinization, extend into adulthood and continue until 40 years of age (Sowell, Thompson, & Toga, 2004). Then after middle adult age, multiple aging-related changes occur in the brain, which are clearly detectable by the age of 50-60: the weight and the volume of the brain decreases (Dekaban & Sadowsky, 1978); the number of neurons diminishes and their morphology changes (Masliah, Mallory, Hansen, DeTeresa, & Terry, 1993); the number of synapses decreases (Peters, Sethares, & Luebke, 2008); the synthesis of neurotransmitters and the density of their receptors decrease (Amenta, Zaccheo, & Collier, 1991; Ota, et al., 2006; Wong, et al., 1984). As a result of those aging-related changes the brain loses some of its plasticity (Burke & Barnes, 2006), which is clearly observed in the decline of certain cognitive functions and a rise in neurological disorders (Salthouse, 2009).

These changes are related to dynamic changes on the molecular level, which involve gene expression (Hof & Morrison, 2004; Hong, Myers, Magnusson, & Prince, 2008; Lu, Pan, Kao, Li, Kohane, Chan, et al., 2004; Masliah, et al., 1993; Somel, Franz, Yan, Lorenc, Guo, Giger, et al., 2009; Somel, Khaitovich, Bahn, Paabo, & Lachmann, 2006). The transcriptome of the brain, as well as of other tissues, is a dynamic system that changes throughout life, through the development of the organism and the brain in particular. Some studies have

found an association between transcription and the development of the brain, and have shown the important role of gene expression in the formation of synapses and various other aspects of higher mental functioning (Diaz, 2009; Diaz, et al., 2002; Flavell & Greenberg, 2008). The changes in gene expression in the brain throughout the life-span correspond closely to the major stages of prenatal and neonatal development, the maturing and aging of the brain.

Gene expression in the developing brain

Numerous anatomical changes of the brain during fetal and infancy development (Courchesne, et al., 2000) are accompanied by dynamic changes in gene expression in the brain tissue. A number of studies (Colantuoni, et al., 2011; Johnson, et al., 2009; Kang, et al., 2011; Lambert, et al., 2011; Somel, et al., 2010) of whole-genome expression analysis in the developing brain report that spatial organization and temporal dynamics of the transcriptome in the fetal brain are more robust and complex than those presented in the postnatal developmental stages. It was found that the number of genes differentially expressed among distinct regions of the neocortex crucially decrease across developmental stages – from 57.7% of genes temporally differentially expressed in the fetal stage to 9.1% and 0.7% of genes, which show spatial differences of expression during postnatal development (from birth to adolescence–20 years of age) and adulthood (from 20 to 60 years of age and older), consequently (Kang, et al., 2011). In another study, two orders of magnitude more gene expression differences (or the number of significantly differentially expressed genes) were detected across cortical areas of fetal brain compared with the adult human cortex; a subset of those genes are known to be involved in the control and development of higher cognitive functions (Johnson, et al., 2009; Lambert, et al., 2011). For instance, the genes that are considered to be candidates for studies of speech and language abilities and disorders (such as *FOXP2*, *CNTNAP2*, and *CNTNAP5*) were found to be especially enriched in the perisylvian cerebral area – the main cortical region that contains the functional domains controlling speech and language – of the fetal brain (Abrahams, et al., 2007; Johnson, et al., 2009).

An example of the temporal dynamics of the transcriptome in the fetal human brain is a reduction of interhemispheric differences in gene expression during the prenatal stages of development. Significant asymmetry of gene expression between the right and left hemispheres, which is detected at 12 and 14 post-conception weeks (pcw) (T. Sun, et al., 2005), is reduced at 16-17, not detectable by 19 pcw (Johnson, et al., 2009; Lambert, et al., 2011), and is almost undetectable later in adult life (Khaitovich, Muetzel, She, Lachmann, Hellmann, & Dietzsch, 2004).

The rapid change of gene expression patterns in the brain during the prenatal and neonatal stages of its development is associated with major neurodevelopmental trajectories (Colantuoni, et al., 2011; Kang, et al., 2011). The functional annotation of sets of co-expressed genes (i.e., genes expressed simultaneously or in concert), which show temporal dynamics in their expression levels during the prenatal development of the brain, suggests that genes related to neuronal differentiation, cell proliferation and migration show the highest expression levels in the early fetal cortex (at 10-13 pcw), which decrease after the mid-fetal period (16-19 pcw) until early childhood. By contrast, genes associated with dendrite and synapse development (such as genes controlling ionic channels and neuroactive ligand–receptor interaction) dramatically increase their expression between the late mid-fetal period (19-24 pcw) and late infancy (Kang, et al., 2011).

Although the rate of expression changes during fetal development is faster than at any other stage of life and reduces considerably after birth, this rate remains relatively high during infancy (especially the first half year of postnatal life) and childhood (Colantuoni, et al.,

2011). During early postnatal life (in infancy and childhood) the gene expression changes of the developing brain have substantially larger amplitudes than later age-related changes (Somel, et al., 2010). Most of these changes occur during the first years of life (Somel, Franz, Yan, Lorenc, Guo, & Giger, 2009). At the same time, the formation of the transcription status of some genes is complete by adolescence, and is maintained throughout the rest of the life, as shown in a study of transcriptional dynamics of excitatory and inhibitory vesicular neurotransmitter transporter mRNAs (VGluT1 to VGAT) during normal human development (Fung, Webster, Weickert, 2011). Specifically, it was found that the ratio of VGluT1/ VGAT increased during early postnatal development to reach a peak at age 5–12 years, after which the balance between those mRNAs remains constant into adulthood.

In addition to findings that report continual spatio-temporal dynamics of gene expression across prenatal and postnatal developmental stages, there is evidence of fundamental differences between transcriptional programs at these distinct stages of development (Colantuoni, et al., 2011). It was shown that select fetal gene expression changes are negatively correlated with those occurring at postnatal stages of life. Thus, approximately 75% of the genes, that show a significant change in their expression in both the fetal and infant stages, reverse the direction of intra-stage expression dynamics between fetal and early postnatal life. And most of these reversals go from an increase of expression level in the prenatal period to a decrease in infancy. A second significant change in global gene expression in the developing brain, when a number of genes change the trajectory of their expression, occurs at the end of teenage stage of development–close to 20 years of age (Colantuoni, et al., 2011).

It is necessary to note that there is a shortage of studies specially focused on the specificity of gene expression status and its dynamics throughout late childhood and adolescence that might be partly explained by an inaccessibility of the biological material for such research, namely brain tissue, from young individuals. Most of the research on the developmental dynamics of the transcriptome in the CNS have been carried out on the fetal brain. Only a few studies consider postnatal developmental stages (Colantuoni, et al., 2011; Kang, et al., 2011; Somel, et al., 2009; 2010), which provide data on general trends of transcriptional changes in the brain during its postnatal development. Taken together, the published evidence indicate that most of the spatio-temporal differences of gene expression in the brain, which are detectable before birth, subsequently decrease during postnatal development; the similarity of transcriptomes among the brain regions in contrast increases.

Gene expression in the mature and aging brain

High levels of gene expression are registered during brain maturation before the age of 20-25 (Somel, Franz, Yan, Lorenc, Guo, Giger, et al., 2009). As shown in two studies on transcriptome profiling in the brain from individuals of different ages, the gene expression rate reduces over time, reaching a plateau by the age of 30 years. After middle age (approximately 40 years) multiple changes in gene expression occur, which are clearly detectable at the age of 60-65 (Lu, Pan, Kao, Li, Kohane, Chan, et al., 2004; Somel, Franz, Yan, Lorenc, Guo, Giger, et al., 2009).

Globally, age-related decreases in expression levels (i.e., the expression of genes goes down) and increases in the variation of the profile of gene expression (i.e., the patterns of expressed genes diversify) have been observed. Regarding the latter, with minor effects on specific genes, this age-correlated heterogeneity of expression is widespread throughout the transcriptome. This effect was explained as an outcome of accumulating stochastic changes in the somatic cells, such as mutations, cellular and DNA damage, especially in gene promoters (Lu, Pan, Kao, Li, Kohane, & Chan, 2004; Somel, et al., 2010; Somel, et al., 2006). In general, stochastic age-related changes in gene expression have a systematic

directed component: a set of age-related genes has been detected, which have shown a special trajectory of age-related changes of their expression levels.

Partly, the heterogeneity in age-related changes in gene expression across the genome have been explained by differences in their compositional properties. Thus, a study based on publicly available microarray data on gene expression in the cortex of individuals 26–106 years of age, revealed that gene localization in different chromatin structures (the combination of DNA and proteins that constitutes the contents of a cell nucleus), which correlates with the GC level (the level of guanine-cytosine dinucleotide content in the DNA-molecule), might be a case of opposite age-related expression patterns of genes. Namely, the results of the study revealed an induction of the GC-poor pivotal genes (such as housekeeping genes, which are involved in the control of basic cellular functions and are expressed in all cells of an organism) and a repression of the GC-rich non-pivotal genes in the cortex of individuals at adult stages of development (Arhondakis & Kossida, 2011).

At the same time it was shown that different functional groups of genes have opposite directions of age-related changes of their expression levels. Two independent studies on the relationships between gene expression in the brain and aging using global transcriptome profiling by microarray techniques (Colantuoni, et al., 2011; Kang, et al., 2011; Lu, Pan, Kao, Li, Kohane, Chan, et al., 2004; Somel, Franz, Yan, Lorenc, Guo, Giger, et al., 2009) provide evidence that genes involved in nervous system development, synaptic plasticity, vesicular transport and mitochondrial functions correlate negatively (reduce their expression) with age. In contrast, a set of genes that correlate with age positively is significantly enriched by genes involved in DNA repair and binding, the regulation of transcription, inflammatory processes, myelinization (i.e., the construction of the myelin sheath around a nerve fiber) and lipid metabolism. Altogether these findings suggest significant changes in the transcriptome of the aging brain, which are related to a number of crucial functions of the brain, such as brain plasticity, learning and memory among others.

Program of developmental changes of gene expression in the brain through the life-span

A global view on transcriptional dynamics across the human lifespan (Colantuoni, et al., 2011) suggests that there are several redirections of transcriptional age-related change that are most pronounced after birth, then reoccur at the end of the teenage years; after that, relatively constant transcriptional status is observed through adulthood until the age of 60 years. It was found that, while initially reversed in early postnatal life compared to the fetal stage, the transcriptional pattern of the brain is mirrored a half century later in further reversals of the transcriptome in aging (Colantuoni, et al., 2011). The study of mRNA microRNA (miRNA) and protein expression in the prefrontal cortex of humans from birth to more than 100 years of age have also provided evidence that most gene expression changes occurring in aging represent extensions or reversals of patterns that are observed in the early developing brain: approximately 10-15% of genes exhibit opposite trends of expression changes with aging (Somel, et al., 2010).

In a number of investigations into the temporal dynamics of the transcriptome in the brain, some of the age-related changes in gene expression tend to be interpreted in terms of regulatory patterns of brain development through the lifespan (Colantuoni, et al., 2011; de Magalhaes & Church, 2005; Somel, et al., 2010). One of the main arguments in support of this conclusion is the fact that most of the changes in gene expression during aging are regulated by the same factors as during the postnatal period, in the early stages of brain development. Specifically, miRNA and transcription factors are involved in the regulation of both developmental and postdevelopmental gene expression changes; those regulatory processes continue throughout the life-span (Somel, et al., 2010). Additionally it has been found that even those associated with aging gene expression changes, such as the down-

regulation of genes involved in neural functions, are initiated in childhood (Erraji-Benchekroun, et al., 2005).

Since gene expression dynamics are a characteristic of regular molecular mechanisms of development during the entire life span, studies of gene expression patterns at different stages of the lifespan are needed to understand the main molecular processes underlying development and its disorders. During the last decade, those studies have become an issue of special interest to a number of scientific teams whose efforts are aimed at understanding gene expression and its age-related dynamics in the brain, as well as creating special resources combining those data, such as Atlases representing the temporal and spatial variability of gene expression in the brain.

The Atlases and Databases on Gene Expression in the Human Brain

At the present time a number of databases and atlases containing data on gene expression in the central nervous system exist; these databases represent outcomes of commercial and/or academic scientific efforts. Thus, information on the specificity of the expression of a particular gene in the brain tissue, in normal and pathological development, is available from searchable databases such as GeneCards, which provides gene-related transcriptomic, genetic/genomic, proteomic, functional and disease information. Some databases contain information on genome-wide expression profiling in a number of human tissues, including the CNS. Besides providing large-scale gene expression data in the brain tissue, these databases generally contain some analytical domains. For instance, they allow the performance of large-scale analyses of tissue-specific gene regulation in human tissues, including the brain (http://bioinfo.wilmer.jhu.edu/tiger), analyses of gene expression patterns among different tissues (http://home.ccr.cancer.gov), and the comparison of gene expression patterns in the brain in normal and pathological development, such as Alzheimer's and Parkinson's diseases, major depression, and schizophrenia (BioExpress® Atlas Reference Data Suite (http://www.genelogic.com).

Of particular value to studying the transcriptional mechanisms involved in human brain functioning and development are such foundational resources as Atlases, which present data on gene expression in different anatomical structures of the brain at its different developmental stages; examples are the HUDSEN and BrainSpan Atlases. Both were created as ongoing systems to combine data on the anatomical regions of the brain and gene expression in those regions. The HUDSEN atlas particularly focuses on representing human embryonic brain development and currently provides information on the expression of 104 genes in different subdivisions of the brain through the12 Carnegie stages (CS 12–23, corresponding to 26–56 days post conception) of prenatal development (http://www.hudsen.org). The BrainSpan atlas (http://www.brainspan.org) represents spatiotemporal gene expression patterns in different anatomical structures of the human brain across all of the main stages of its development. The Atlas provides data on gene expression profiles by RNA sequencing and exon microarray in 8-16 cortical and subcortical structures of the brain across 13 developmental stages – from embryonic (5–7 post-conception weeks) to adult (20-40 years of age and older).

An example of research on the transcriptional mechanisms of CNS development and its developmental disorders is a recent study (Ziats & Rennert, 2011) of the genomic etiology of Autism Spectrum Disorders (ASD), which was carried out based on data from the transcriptional atlas of human brain development (http://www.brainspan.org). The research was based on the hypothesis that the main molecular mechanisms underlying such a heterogenic developmental disorder as autism might be more clearly detectible if focused on the interaction networks of the genes known to be implicated in ASD and expressed in the

developing brain. The authors identified such genes and described the gene ontologies, canonical pathways, and interactome networks for these genes. A particular focus on a subset of the candidate-genes that are highly expressed in the normal developing brain have shown that immune signaling, particularly the classical cytokine signaling pathway, which occurs via small cell-signaling protein molecules and is prevalent in intracellular communication, is central to ASD networks. Additionally the correlating ASD-implicated transcriptomes with cell-type specific protein expression provided evidence on the significance of considering cell-type specific expression in studies of the transcriptional mechanisms of ASD. Thus, many highly expressed ASD genes are mainly detected in glia but not in neurons, as well as in specific layers of the cerebellum (Ziats & Rennert, 2011).

In summary, here we have briefly reviewed the growing, but still relatively small, literature on transcriptomics with regard to pertinent issue of life-span development in general and child development in particular. The ever-changing technology has made studies of transcriptomes affordable to a large community of scientists; the existing public and private databases have provided/will provide wonderful sources of reference to the typical development of the brain during different stages of human life. The accumulation of data on the spatial and temporal structures of the transcriptome in the brain tissue—notably the data obtained using methods of transcriptome profiling with high-resolution RNA-Seq—and the organization of the data into the resources described above, provide a needed spring board for the development of new types of studies of the transcriptome—studies that correlate its structure and dynamics with behavioral development across different stages of human life, in its variable environments, and in its typical and atypical manifestations.

Acknowledgments

This research was supported by NIH grant R01 DC007665 to Elena L Grigorenko. Grantees undertaking such projects are encouraged to express freely their professional judgment. This article, therefore, does not necessarily reflect the position or policies of the National Institutes of Health, and no official endorsement should be inferred. We are grateful to Ms. Mei Tan for her editorial assistance.

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