

Genomic Perspectives of Transcriptional Regulation in Forebrain Development

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The forebrain is the seat of higher-order brain functions, and many human neuropsychiatric disorders are due to genetic defects affecting forebrain development, making it imperative to understand the underlying genetic circuitry. Recent progress now makes it possible to begin fully elucidating the genomic regulatory mechanisms that control forebrain gene expression. Herein, we discuss the current knowledge of how transcription factors drive gene expression programs through their interactions with *cis*-acting genomic elements, such as enhancers; how analyses of chromatin and DNA modifications provide insights into gene expression states; and how these approaches yield insights into the evolution of the human brain.

Overview

Working at the turn of the twentieth century, Santiago Ramón y Cajal demonstrated the variety of neuronal cell types and provided insights into the network of connections within the brain using simple histological stains and light microscopes (Cajal, 1899). Over 100 years later, despite the availability of advanced imaging, and molecular and functional analysis tools, much remains unknown about the genetic factors controlling the development, structure, and function of the intricate features that, in their entirety, form the human central nervous system. The forebrain houses the neural structures that control higher-order brain functions, including the pallium (cortex and hippocampus), subpallium (striatum, pallidum, preoptic area, and septum), hypothalamus, and thalamus. Understanding the development, evolution, function, and dysfunction of the forebrain requires a deep understanding of the genetic control of how its components are assembled and interconnected.

At the core of the processes that regulate forebrain development and function is the transcriptional circuitry. Over the past 25 years, numerous regional and cell type-specific transcription factors (TFs) have been identified and characterized. We are now aware of some components of TF networks that control regionalization within the embryonic brain. Although general paradigms for identifying TFs and defining their cellular functions have been established, studies are now needed to explore and understand the molecular and genomic mechanisms through which networks of such TFs function during development. Of paramount importance is elucidating the *cis*-regulatory genomic elements where sets of TFs interact to control forebrain gene expression.

Recent technological advances, highlighted by the invention and application of chromatin immunoprecipitation (ChIP)-based and high-throughput sequencing assays, have enabled large-scale efforts to functionally annotate the genome. The results from studies that have applied these technologies to the developing brain reveal the essential role of neuronal TFs, the dynamic

gene expression landscapes in brain development, and the extensive role of noncoding regulatory elements. These findings point to complex regulatory systems underlying the diversification of neuronal cell types and structural connectivity that Cajal drew in his formative illustrations. While many of the details of the emerging regulatory landscape of the brain remain to be explored, recent advances highlight the role of transcriptional control in normal development and in neurological disorders and disease. In this review, we describe the interplay between TFs, distal transcriptional enhancers, chromatin structure and epigenomic features, and DNA-binding and chromatin remodeling proteins in establishing the regulatory circuitry underlying transcriptional control and development of the forebrain. In addition, we describe approaches for the identification and characterization of enhancers and other regulatory elements and provide a perspective on emerging and exciting research on the genomic and regulatory control of forebrain development, evolution, and disease.

Annotating Regulatory Elements Active in Forebrain Development

Metazoan Gene Regulation via *cis*-Regulatory Elements

Cis-regulatory control of gene expression during development is a complex process, dependent on distal sequences, spatial organization of the chromosome, and chromatin or epigenetic state (Figure 1A). For some genes, notably housekeeping genes, the proximal regulatory sequence is sufficient for correctly activating transcription (Lenhard et al., 2012). In contrast, genes with complex expression patterns can be acted on by many distal transcriptional enhancers located in intronic, intergenic, and even exonic sequence, with enhancers potentially located far from target genes. Enhancers appear to be the most numerous regulatory elements in mammalian genomes and exhibit extensive tissue and stage specificity, suggesting that distal enhancers are required for the precise control of gene expression

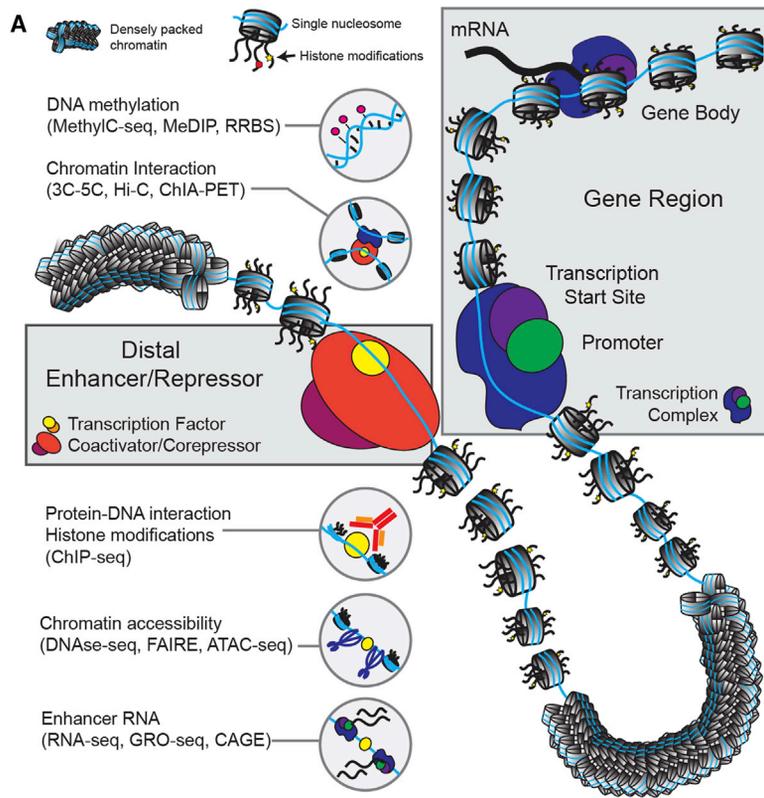


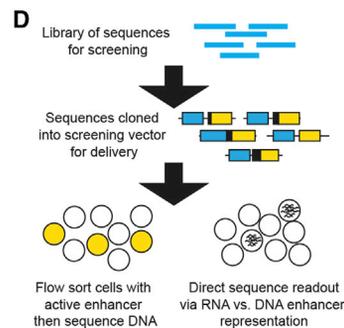
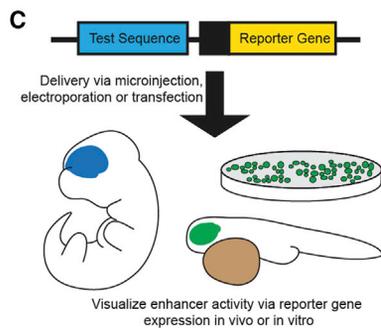
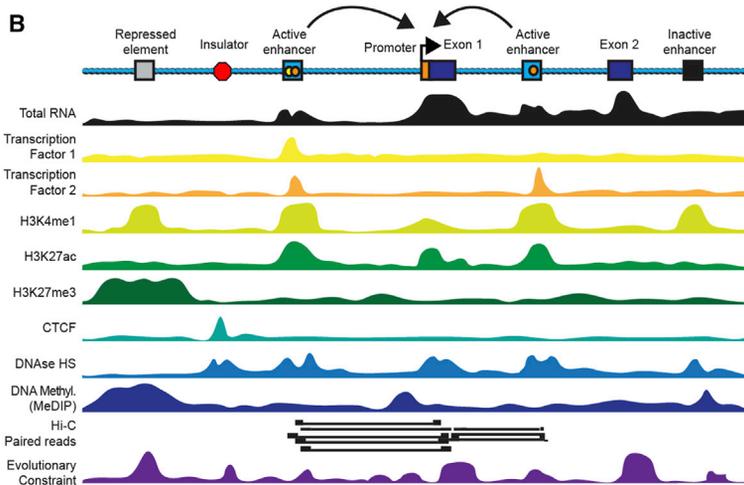
Figure 1. Types of Gene Regulatory Sequences and Methods for Their Discovery and Characterization

(A) Schematic view of different chromatin states in genic and intergenic regions, with characteristic classes of epigenomic features and an overview of selected methods for their genome-wide mapping.

(B) Track-style view of features commonly associated with different types of regulatory sequences.

(C) Transgenic reporter assays enable the validation and detailed characterization of enhancer activity patterns in vitro and in vivo.

(D) Massive parallel reporter assays enable medium- and large-scale function-based enhancer discovery screens.



(ENCODE Project Consortium, 2012; Stamatoyannopoulos et al., 2012; Nord et al., 2013; Shen et al., 2012). With the binding of activating TFs, enhancers are brought within spatial proximity of target promoters through the formation of loops in DNA (Kulaeva et al., 2012), with structural proteins such as mediator and cohesin involved in this process (Kagey et al., 2010). It is now clear that enhancers may interact with multiple promoters, and clusters of coregulated genes may exhibit promoter interactions, with the spatial aspects of gene regulation just now beginning to be characterized (Zhang et al., 2013). Many of the details regarding the mechanisms and timing of transcriptional control via regulatory sequences remain uncertain. There is evidence that enhancer-promoter looping may actually be very stable and not reflect activation (DeMare et al., 2013; Kieffer-Kwon et al., 2013). Additional classes of regulatory sequences are also important in gene regulation, such as insulators and silencers (Cuddapah et al., 2009). Significant progress in characterizing the complexity of noncoding regulatory circuitry has been made in model systems (Lagha et al., 2012), and in specific lineages and developmental loci (Montavon et al., 2011; Stamatoyannopoulos, 2005). It is increasingly recognized that transcriptional regulation is achieved endogenously through a complex interplay of cues that involve the binding of activating and repressive TFs, chromatin state and structure, epigenomic modifications and chromatin remodeling proteins, the activity of long noncoding RNAs, and *cis*-regulatory elements that can have activating or repressing function depending on developmental context. Despite this complexity, new technologies have enabled the identification and characterization of hundreds of thousands of candidate regulatory elements in the human and mouse genomes.

Early Approaches to Enhancer Identification in the Brain

Before the publication of the human and mouse reference genome sequences, regulatory regions were typically found via trial and error, such as through deconstructing BACs or other large sequence fragments to determine subregions that controlled expression patterns of target genes. The first genome-wide predictions of regulatory elements were based on the presence of evolutionary sequence conservation (homology across species) or constraint (relative local sequence conservation across evolution) (Cooper et al., 2005; Frazer et al., 2004; Ovcharenko et al., 2004; Prabhakar et al., 2006b; Schwartz et al., 2000; Siepel et al., 2005) coupled to functional screening via reporter assays (Kothary et al., 1989; Nobrega et al., 2003; Pennacchio et al., 2006). The combination of trial and error and comparative genomics-guided screens, when applied to individual loci of interest, led to the identification of regulatory elements with activity in the developing forebrain near genes including *Arx*, *Dach1*, *Dlx1/2*, *Dlx5/6*, *Emx2*, *Fezf2*, *Meis1*, *Otx1/2*, *Pax6*, and *Sox2* (Ahituv et al., 2007; Colasante et al., 2008; Ghanem et al., 2007; Kammandel et al., 1999; Kurokawa et al., 2004, 2014; Machon et al., 2002; Mariani et al., 2012; Royo et al., 2012; Shim et al., 2012; Suda et al., 2010; Theil et al., 2002; Zerucha et al., 2000). These elegant and labor-intensive studies provided first insights into the regulatory architecture of these key developmental loci. However, due to the required effort they were limited in the scope of genomic regions covered and likely missed additional regulatory elements, particularly those far from the genes of interest or lack-

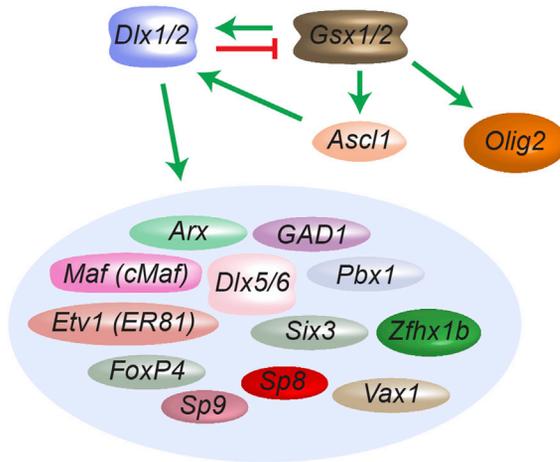
ing strong cross-species conservation. Many additional embryonic brain enhancers have been identified via large-scale unguided genome-wide screens of extremely conserved non-coding regions for sequences that drive reporter gene expression at specific embryonic time points, with the results available in the VISTA enhancer database (Visel et al., 2007). This database contains over 2,100 tested human and mouse sequences, over 1,100 of which function as enhancers *in vivo* in embryonic mouse tissues with whole-mount staining images available. The VISTA enhancer set includes over 350 annotated to drive expression in the forebrain at embryonic day 11.5 (E11.5), and 147 of these enhancers additionally include high-resolution images of developmental brain sections that can be used to map the spatial activity of forebrain enhancers (Visel et al., 2013).

Epigenomic Approaches to Study Gene Regulation

Two parallel developments have resulted in rapid expansion of the catalog of regulatory elements in mammalian genomes and in annotation of their function. The first is the availability of next-generation sequencing technologies that cost-effectively generate enough sequence coverage to enable genome-wide enrichment maps in a single experiment. The second is the knowledge about interpreting epigenomic marks that emerged from early studies in the area of cellular and chromatin biology, with additional traction from ENCODE pilot studies (Birney et al., 2007). Current proxy signatures of regulatory element activity and chromatin state include coactivator binding (e.g., p300), histone modifications, binding of TFs or other DNA-associated proteins, chromatin accessibility, DNA methylation, and nongenic RNA transcription (Figures 1A and 1B). Using approaches to assay these signals, it is possible to identify and differentiate classes of regulatory elements and thus to identify enhancers that are active in particular cell lines or tissues. There are also emerging genome-scale tools to map interactions between regulatory sequences and their target genes (e.g., ChIA-PET; Fullwood et al., 2009) and for generating genome-wide interaction maps (e.g., chromosome capture assays such as HiC; Lieberman-Aiden et al., 2009). There is support for sequential chromatin modifications that are associated with repressed, poised, and active enhancers (Creighton et al., 2010; Rada-Iglesias et al., 2011). For example, the histone modification H3K27me₃, shown in Figure 2B, can be indicative of a repressed region whereas H3K27ac can indicate active enhancers (Creighton et al., 2010; Rada-Iglesias et al., 2011). Despite the general correlation between specific chromatin modification patterns and activity states, no specific signatures that have been reported appear to capture function exactly (Cotney et al., 2012; Visel et al., 2009b).

Although the resources generated by large-scale centralized efforts, such as the ENCODE and Epigenomics Roadmap initiatives, have significantly advanced our understanding of *cis*-regulatory elements, there are limitations in using these data sets to characterize regulatory elements active in the developing brain due to the incomplete representation of representative cell lines and tissue types. Nonetheless, application of functional genomics to identify regulatory circuits controlling brain function has already produced major insights. There are now many publicly available genomic data sets relevant to the developing brain (see Table 1 for a partial list). These resources can be of tremendous value to researchers. For example, the Brainspan

A CGE/LGE/MGE pathways



B MGE pathways

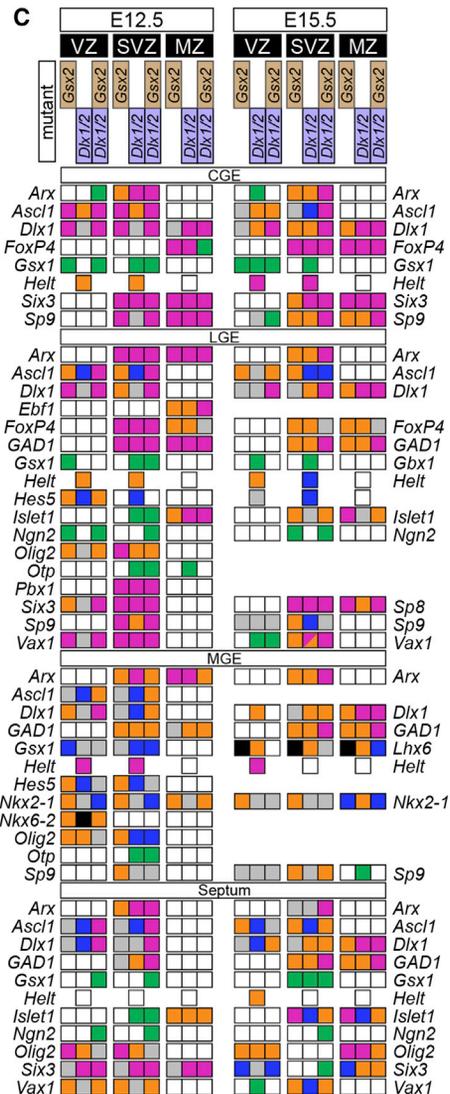
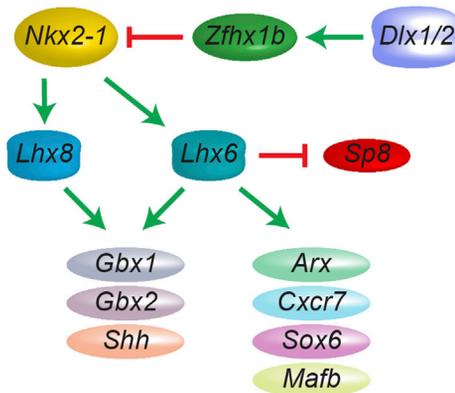


Figure 2. TFs with Known Roles in Forebrain Development

(A and B) Models of transcriptional pathways in the developing mouse basal ganglia based on RNA expression analyses in the embryonic brain of loss-of-function TF mutants. Green arrows, activation; red stop signal, repression. (A) Pathways in the caudal, lateral, and medial ganglionic eminences (CGE, LGE, MGE) based on data in references in main text. (B) Pathways in the medial ganglionic eminence (MGE) based on data in references in main text. (C) Expression of TFs in the basal ganglia of *Gsx2*^{-/-}, *Dlx1*^{-/-}*Dlx2*^{-/-}, and *Gsx2*^{-/-}*Dlx1*^{-/-}*Dlx2*^{-/-} mutant mice. Expression changes are reported separately for two different developmental stages (E12.5 and E15.5) in the ventricular zone (VZ), subventricular zone (SVZ), and mantle zone (MZ) of the CGE, LGE, MGE, and Septum. Colors indicate the effect of each mutation on TF expression: black or no square, not analyzed; gray, no obvious expression change in mutant; white, no detectable expression; magenta, severe reduction in expression; orange, moderate/mild reduction in expression; green, ectopic expression; blue, increased expression. In diagonally divided boxes, the top part represents the dorsal region and the bottom the ventral region. Modified from Wang et al. (2013).

expression data sets have been instrumental in mapping the expression networks of genes perturbed in autism and schizophrenia (Gulsuner et al., 2013; Parikhshak et al., 2013; Willsey et al., 2013).

Functional Characterization of Enhancer Activity in the Brain

Although a number of epigenomic markers, signatures, and assays are now available to predict the genomic location of enhancers, a continued major challenge is validation of functional predictions and determination of the exact activity of noncoding

sequences. Reporter-based enhancer assays (Kothary et al., 1989) have been used extensively to map enhancer activity of both human and mouse regulatory sequences in vivo, and have been extended via library-based screening to examine hundreds to thousands of sequences in parallel. In comparison to assays performed in cell lines, in vivo functional enhancer analyses reveal specific cell types, tissue subregions, and developmental stages at which an enhancer drives expression (Pennacchio et al., 2006; Visel et al., 2013). Enhancer assays further differ in how the DNA is delivered, the size of fragment that is introduced,

Table 1. Functional Genomics Data Sets Relevant to Gene Regulation in the Developing Brain

Resource Name	URL	Description
Genomic Data Sets Focusing on the Brain		
Allen brain atlas	http://www.brain-map.org	developmental transcriptomes, microarray, and in situ hybridization
Brainspan	http://www.brainspan.org	mouse and human developmental brain gene expression
MethylomeDB	http://www.neuroepigenomics.org/methylomedb	mouse and human brain methylome data sets
Nord et al., 2013	http://enhancer.lbl.gov/mouse_time_course	mouse forebrain developmental H3K27ac data sets
Brain Expression Resources (In Situ, Enhancer, BAC)		
VISTA	http://enhancer.lbl.gov	whole-mount E11.5 mouse activity data for human/mouse enhancers
Gensat	http://www.gensat.org/index.html	BAC-driven reporter assays/mice and in situ data
Large-Scale Centralized Functional Genomics Initiatives		
ENCODE	http://www.genome.gov/encode	many data types, expanding to developmental brain tissues
Roadmap epigenomics	http://www.roadmapepigenomics.org	many data types, numerous brain samples
Blueprint epigenome	http://www.blueprint-epigenome.eu	population level epigenomic data, no brain relevant samples to date
FANTOM	http://fantom.gsc.riken.jp	CAGE expression data for limited human brain/neuronal cell lines
Shen et al., 2012	http://chromosome.ucsd.edu/mouse/download.html	mouse ENCODE project as described in Shen et al., 2012

the number of copies or constructs per cell, stable or transient integration, and whether the readout is image or sequence based, as reviewed recently (Shlyueva et al., 2014). Beyond assaying in vivo activity, reporter assays have been used to document the functional autonomy of distal enhancers (Visel et al., 2009a), changes in enhancer activity due to sequence variation associated with evolution and human disease (Poitras et al., 2010; Prabhakar et al., 2008), and to label cells in fate mapping experiments (Chen et al., 2013; Pattabiraman et al., 2014; Visel et al., 2013). Reporter-based approaches have been further adapted to discover new enhancers in the genome (e.g., enhancer trapping methods (Hill and Wurst, 1993)), generate molecular reagents for regional/cell-type specific expression in the brain (Gong et al., 2003), and used in high-throughput function-based identification and characterization of enhancers (Arnold et al., 2013; Dickel et al., 2014; Kheradpour et al., 2013; Murtha et al., 2014; Patwardhan et al., 2012; White et al., 2013). Similar methods have been used in simpler and more distant model organisms, which offer the trade-off of lower cost but larger differences in brain anatomy and function compared to humans (Ariza-Cosano et al., 2012). The combination of single-enhancer studies that produce detailed spatial and temporal maps of in vivo enhancer activity complemented by sequence-based assays of function have the potential to greatly expand understanding of regulatory element activity and the effects of regulatory sequence variation on brain development. Considering the results from traditional single gene studies alongside genome-wide or high-throughput approaches, we next attempt to synthesize current understanding of how transcriptional regulation orchestrates forebrain development, evolution, and function.

TFs Controlling Forebrain Development

Elucidating the transcriptional networks in the developing forebrain requires the marriage of defining the cellular and develop-

mental functions of individual TFs with their genome-wide molecular functions. In the telencephalic region of the forebrain (cerebral hemispheres), this has begun through the identification of TFs with region and cell-type specific expression patterns. For instance, telencephalic expression of TFs such as *Emx1*, *Emx2*, *Fezf2*, *Ngn1*, *Ngn2*, *Pax6*, *Satb2*, *Tbr1*, and *Tbr2* are largely restricted to cortically derived glutamatergic progenitors and neurons (Lai et al., 2013; MacDonald et al., 2013), whereas *Dlx1*, *Dlx2*, *Dlx5*, *Dlx6*, *Gsx1*, *Gsx2*, *Lhx6*, *Lhx8*, and *Nkx2-1* are largely restricted to subcortically derived GABAergic and cholinergic progenitors and neurons (Rubenstein and Campbell, 2013). Note that interneurons of cortical structures (neocortex, hippocampus, and olfactory bulb) are believed to be largely generated by subcortical progenitors (Batista-Brito and Fishell, 2013; Rubenstein and Campbell, 2013). Other telencephalic TFs are jointly expressed by cortical and subcortical regions, such as *Arx*, *Ascl1* (*Mash1*), *Brm1*, *Brm2*, *COUPTFI*, *COUPTFII*, *CTIP1*, *CTIP2*, *Cux1*, *Cux2*, *Foxg1*, *Lhx2*, *Pbx1*, *Pbx2*, *Satb1*, *Sox5*, *Sox6*, *Sp8*, and *Zfhx1b* (*Sip1*; *Zeb2*) (Lai et al., 2013; MacDonald et al., 2013; Rubenstein and Campbell, 2013; Rubenstein and Rakic, 2013; Thompson et al., 2014), suggesting that they may share similar functions in both cortical and basal ganglia development. Many other TFs are likely to be centrally involved in forebrain development and defining these factors and their roles is an active area of research. Below we highlight two signaling pathways active in the developing subcortical forebrain as examples of how understanding these transcription factor networks reveals the transcriptional control of neurodevelopment.

Analysis of Forebrain Development Using TFs Mutants

Many of the TFs involved in forebrain development have been studied in loss-of-function mouse mutants with the goal to define their individual and combined in vivo functions (Figure 2). For instance, analysis of *Dlx1*^{-/-}, *Dlx2*^{-/-}, *Dlx5*^{-/-}, and *Dlx6*^{-/-}

single mutants demonstrated distinct selective phenotypes in subsets of neurons in lineages that express the *Dlx* genes (Cobos et al., 2005; Long et al., 2009a, 2009b; Qiu et al., 1995; Rubenstein and Campbell, 2013; Wang et al., 2010, 2011), such as defects in dendrite innervating cortical interneurons (Cobos et al., 2005; Howard et al., 2014; Mao et al., 2009; Seybold et al., 2012). On the other hand, the double mutants have earlier and more pervasive phenotypes that affect regional and cell type identity, differentiation, and cell migration (Anderson et al., 1997a, 1997b; Cobos et al., 2007; Wang et al., 2010; Yun et al., 2002).

Dlx1&2-Associated Network

Systematic analysis by RNA expression arrays and RNA in situ hybridization showed that *Dlx1*^{-/-}*Dlx2*^{-/-} mice have altered expression of tens of TFs in the embryonic subpallium (Cobos et al., 2007; Long et al., 2009a, 2009b). *Dlx1*^{-/-}*Dlx2*^{-/-} mice have overexpression of *Ascl1*, *Gsx1*, *Gsx2*, and *Olig2*, suggesting that some of the *Dlx1&2* mutant phenotype is due to overexpression of these TFs (Figures 2A and 2C). This hypothesis has been tested by generating *Dlx1&2* triple mutants with *Ascl1* (*Mash1*), *Gsx1*, *Gsx2*, and *Olig2*. For instance, *Dlx1&2* promote neurogenesis and repress oligodendrogenesis; *Dlx1*^{-/-}*Dlx2*^{-/-} mutants generate excessive oligodendrocytes; this phenotype is reversed in the *Dlx1*^{-/-}*Dlx2*^{-/-}*Olig2*^{-/-} triple mutant, and exacerbated in the *Dlx1*^{-/-}*Dlx2*^{-/-}*Ascl1*^{-/-} mutant (Petryniak et al., 2007). Like *Olig2*, *Olig1* represses *Dlx*-mediated neurogenesis (Silbereis et al., 2014). Triple mutant analyses also provided evidence that *Dlx1/2*, together with *Ascl1* and *Gsx2*, promote neurogenesis and fundamental subcortical properties, such as expression of *GAD1*, the gene encoding the GABA synthesizing enzyme (Long et al., 2009a, 2009b; Wang et al., 2013) (see Figure 2C for an approach to annotate TF expression changes in *Dlx1/2*, *Gsx2*, and *Dlx1/2;Gsx2* mutants).

Dlx1^{-/-}*Dlx2*^{-/-} mutants have reduced expression of a distinct set of TFs, including *Arx*, *Sp8*, and *Zfhx1b* (Figures 2A and 2C) (Long et al., 2009b; McKinsey et al., 2013). Analysis of *Arx*, *Sp8*, and *Zfhx1b* single mutants has given insights into their individual contributions to the *Dlx1*^{-/-}*Dlx2*^{-/-} phenotype. *Arx* mutants have a time-dependent block in the maturation and migration of neurons from basal ganglia progenitor zones (Colombo et al., 2007), similar to *Dlx1*^{-/-}*Dlx2*^{-/-} mutants, which have a progressive accumulation of nonmigrated immature cells in the subventricular zone (Anderson et al., 1997b; Yun et al., 2002). *Sp8* mutants (Waclaw et al., 2006), as well as *Dlx* single and compound mutants (Long et al., 2003, 2007; Qiu et al., 1995), have olfactory bulb interneuron differentiation defects. *Zfhx1b* mutants have abnormal migration of interneurons generated by the MGE subcortical progenitor region. *Zfhx1b* and *Dlx1*^{-/-}*Dlx2*^{-/-} mutants both fail to repress expression of *Nkx2-1* (Figure 2B) (McKinsey et al., 2013; van den Berghe et al., 2013), a TF that is essential for MGE identity (Sussel et al., 1999). *Nkx2-1* expression needs to be turned off during maturation of interneurons that migrate to the cortex (Nóbrega-Pereira et al., 2008). In *Zfhx1b* mutants, *Nkx2-1* expression persists, leading to an accumulation of MGE-derived interneurons in the striatum, suggesting that *Zfhx1b* regulates the switch between the generation of cortical and striatal interneurons, and is required for the expression of *Maf* (*cMaf*) in migrating cortical

interneurons (Figure 2A) (McKinsey et al., 2013). Furthermore, there is evidence that *Zfhx1b* regulates the expression of the *Unc5b* receptor, which contributes to the migration defect (van den Berghe et al., 2013).

Nkx2-1-Associated Network

Nkx2-1 functions in part parallel to the *Dlx*-driven TF hierarchy (Figure 2B). *Nkx2-1* has a fundamental role in specification of MGE progenitor cell identity—in its absence, the MGE changes fate, taking on the more dorsal properties of the LGE and CGE (Butt et al., 2008; Flandin et al., 2010; Sussel et al., 1999). *Nkx2-1* drives the expression of *Lhx6* and *Lhx8* (Du et al., 2008; Sussel et al., 1999), which together with their cofactor *Ldb1* (Zhao et al., 2014) are required for the differentiation of GABAergic (*Lhx6*) and cholinergic (*Lhx8*) neurons (Fragkouli et al., 2005, 2009; Liodis et al., 2007; Zhao et al., 2003, 2008). Interneurons lacking *Lhx6*, have reduced expression of the *Arx*, *MafB*, *Npas1*, and *Sox6* TFs (Figure 2B) (Denaxa et al., 2012; Zhao et al., 2008). *Satb1* function has been linked to activity-dependent differentiation of cortical interneurons (Close et al., 2012; Denaxa et al., 2012). *Arx* function was noted above; *Npas1* represses the generation of a specific set of cortical interneurons (Stanco et al., 2014); *Sox6* is required in the MGE to repress pallial proneural gene expression and to promote interneuron development (Azim et al., 2009; Batista-Brito and Fishell, 2013; Batista-Brito et al., 2009). *MafB* function in the telencephalon is currently under study. To identify the role of individual molecules that are downregulated in the *Lhx6* mutant, we used a novel complementation approach (Vogt et al., 2014). For instance, to test whether reduced *Arx* expression contributes to the *Lhx6* mutant phenotype, lentiviral transduction introduces *Arx* downstream of a *Dlx1&2* enhancer into dissociated MGE cells in vitro; these are then transplanted into a wild-type cortex, and the derived interneurons are phenotyped. In this case, restoring *Arx* expression partially restored interneuron expression of parvalbumin (Vogt et al., 2014).

Mapping TF Function, Combinatorial Activity, and Genomic Binding in the Forebrain

Thus, even examining these two networks, loss of function analyses of over 20 TFs have enabled the field to perform detailed histological, cellular, and molecular analyses of the mutant developing subpallium and its derivatives, including cortical and olfactory bulb interneurons. Similar progress has been made in defining TF function during pallial development, including its regionalization and generation of projection neuron subtypes, although due to space constraints, we will not amplify upon this important subject (MacDonald et al., 2013; O'Leary et al., 2013). However, very little is known about how these TFs fit into the transcriptional circuitry orchestrating such processes, including the combinatorial activity of TFs. Models based on co-transcription (Ravasi et al., 2010) and DNase I footprinting (Neph et al., 2012) to predict co-occurrence of transcription factor binding sites show that there are robust combinatorial sets of transcription factors that are associated with specific lineages and tissues, including in the brain. Understanding this combinatorial functionality of TFs will likely be essential to understanding neurodevelopment. In a functional study of odorant receptor (OR) regulation in sensory neurons in *Drosophila*, it was shown via systematic RNAi-based TF knockdown that combinations of

seven TFs accomplish OR regulation via transcriptional activation and repression to prevent ectopic expression (Jafari et al., 2012). This study highlights how combinatorial TF activity via differential expression and binding can drive neurodevelopmental processes and suggests that integrative models of TF activity will be necessary to understand the complex developmental architecture of the forebrain. Several additional critical gaps of knowledge remain to be filled, including identifying all of the TFs that may participate, the gene regulatory elements (enhancers), and the genomic regions where TFs bind.

As noted above, there are now expression databases that define the spatial and temporal expression patterns of most TFs (Table 1). Careful annotation of this information, and additional analyses using RNA expression arrays and RNA-seq in specific cell types, will greatly facilitate defining the sets of TFs that are candidates for contributing to TF circuitries. What remains perhaps the largest unmet gap, is the mapping of TFs to their *in vivo* genomic binding sites. One method to obtain this information is ChIP-seq; however, performing TF ChIP-seq on embryonic brain tissue has lagged, in part because the identification of high-affinity antibodies that specifically bind to a given TF that is bound to fixed chromatin has been problematic. Nonetheless, there are examples where inroads have been made. For instance, there is ChIP-qPCR evidence that DLX2 directly binds to and regulates the *Dlx1&2*, *Dlx5&6*, and *Zfhx1b* loci (Colasante et al., 2008; McKinsey et al., 2013; Potter et al., 2009; Zhou et al., 2004), that NKX2-1 directly binds to and regulates the *Lhx6* locus (Du et al., 2008), and that LHX6 directly binds to and regulates the *Arx* locus (Vogt et al., 2014). Genome-wide analyses of ASCL1 promoter binding in the embryonic brain and in neural stem cell cultures demonstrated that ASCL1 is bound to promoters of genes regulating cell cycle progression (Castro et al., 2011). Ongoing studies are now making progress using ChIP-seq to define the genome-wide landscape of TF binding in the developing forebrain.

The Regulatory Circuitry Underlying Forebrain Development

Forebrain Development Is Characterized by Dynamic Transcription

Analysis of TF expression and effects of loss-of-function in neuronal stem cell proliferation, differentiation, and migration, reviewed elsewhere (Hébert and Fishell, 2008; Kohwi and Doe, 2013; Molyneaux et al., 2007), have led to models of sequential or combinatorial expression of TFs in the regulation of these processes. The dynamic expression profiles of key TFs are mirrored by genome-wide transcription patterns generated from microarrays and more recently, RNA-seq approaches. Transcriptome analysis of multiple brain regions across multiple developmental stages revealed extensive regional and temporal differences (Kang et al., 2011; Miller et al., 2014; Pletikos et al., 2014). These expression differences coalesce into transcriptional modules of coexpressed genes that are involved in stage-specific processes in specific structures, such as opposing expression patterns of genes associated with neuronal differentiation (at early stages) and ion channels (at later stages) in the developing neocortex and hippocampus. To achieve tightly regulated dynamic expression profiles, it is likely that *cis*-regulatory elements

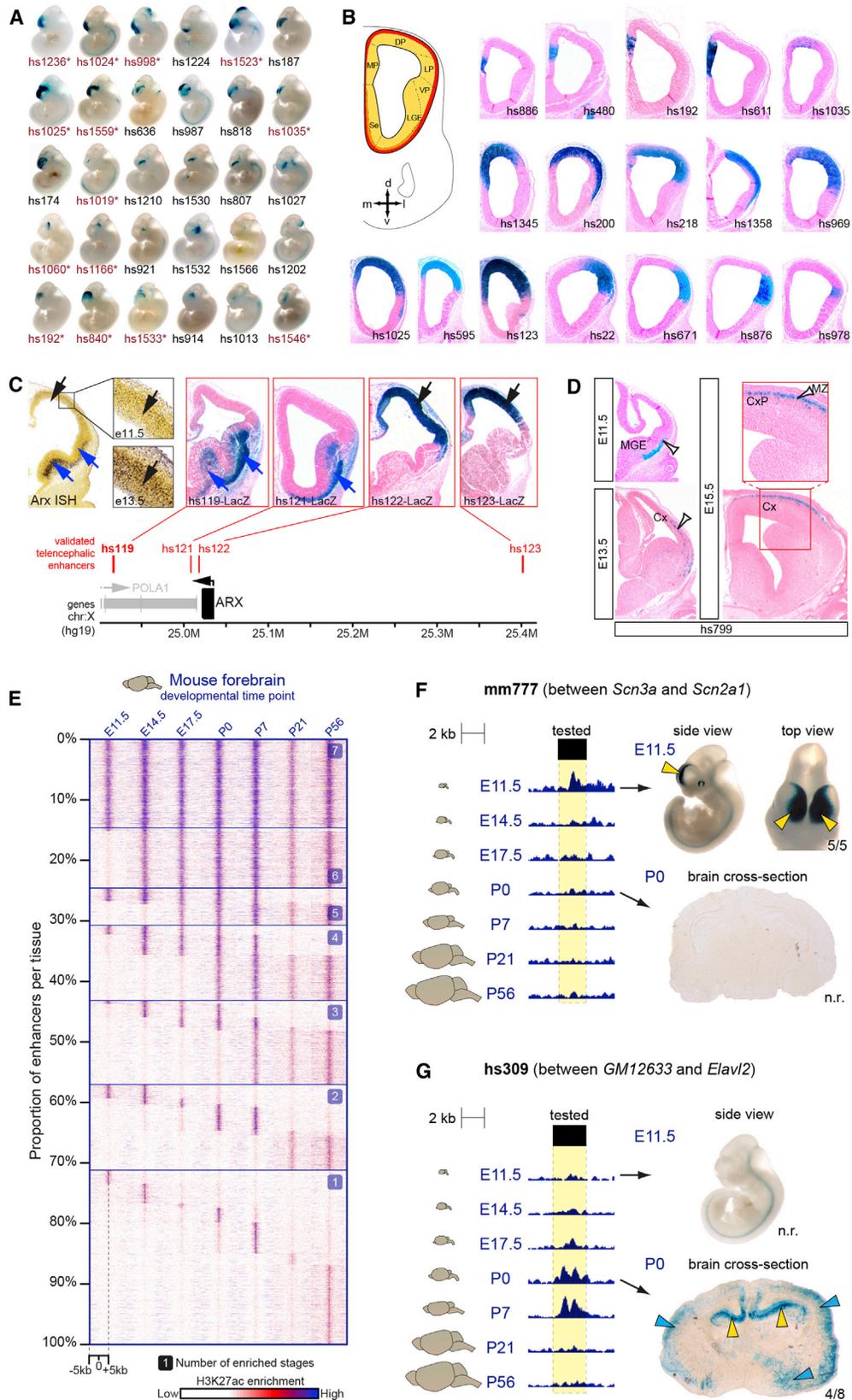
represent a substrate for TF binding, enabling precise control via localization of transcriptional machinery to target promoters, activate transcription, and recruit chromatin-remodeling proteins that together direct specific spatiotemporal expression of target genes.

Enhancers Drive Subregional Expression Patterns in the Developing Forebrain

Gene-centric studies demonstrated that some genes, such as *Arx* (Ahituv et al., 2007; Colasante et al., 2008), *Dlx1&2*, and *Dlx5&6* (Ghanem et al., 2007; Zerucha et al., 2000), have multiple enhancers with similar activity patterns, raising the possibility of enhancer redundancy. In addition, some loci (e.g., in the *Dlx* family) had multiple enhancers with overlapping, yet distinct activity patterns, suggesting that a given gene may be regulated by many enhancers with distinct temporal and spatial activities. A recent study examined *in vivo* activity patterns of 145 human enhancer sequences at high spatial resolution in the mouse telencephalon at E11.5 using reporter assays and serial histological sectioning to generate a digital atlas of enhancer function in the developing brain (Figures 3A–3D) (Visel et al., 2013).

In addition to generating activity maps for enhancers that are located nearby genes with key functions in brain development and neurological disorders, this analysis identified several recurrent characteristic features of enhancers active in the developmental brain. First, individual enhancers drove highly reproducible, spatially restricted enhancer patterns, with large variation in patterns of expression observed across the sampled enhancers that represent all the major subregions of the E11.5 telencephalon (Figures 3A, 3B, and 3D). Generation of stable transgenic lines for 15 pallial enhancers enabled fate mapping analysis, showing that enhancers with activity in distinct pallial progenitor domains generate distinct cortical regions (Pattabiraman et al., 2014), consistent with the protomap hypothesis (Rakic, 1988).

Second, when combined to form a composite pattern, the expression patterns of individual enhancers recapitulate gene expression patterns as mapped via *in situ* hybridization, consistent with observations in other organ systems (e.g., Schwartz and Olson, 1999) that the combined activity of discrete enhancer sequences drives complex endogenous gene expression patterns. For example, four distant-acting enhancer sequences were described located in the extended locus containing *Arx*, a gene that regulates pallial and subpallial development and is associated with mental deficiency and epilepsy (Colasante et al., 2008; Friocourt and Parnavelas, 2010; Kitamura et al., 2002, 2009; Marsh et al., 2009; Olivetti and Noebels, 2012). Together, these enhancers recapitulate endogenous *Arx* expression (Figure 3C). Third, enhancers that activated gene expression in the same anatomical structures were enriched for shared sequence motifs, indicating that highly specific enhancer activity is, at least in part, due to the presence of binding sites for particular transcriptional regulators active in the brain. This and other *in vivo* studies of enhancer activity in the developing forebrain establish that the *cis*-regulatory landscapes guiding tissue-specific expression patterns are complex, with the combined action of multiple, sometimes redundant, enhancers likely involved in regulation of specific loci.



(legend on next page)

Functional Genomics Reveal Regulatory Circuits in the Brain

Experiments that characterize neuronal *cis*-regulation one element at a time have revealed many aspects of the regulatory control of forebrain development, yet these studies fail to capture global patterns of enhancer use and chromatin state during forebrain development. Early attempts to fill this gap involved ChIP-seq experiments targeting p300-binding sites in the developing mouse brain and targeting various histone modifications in neuronal cell lineages (Creyghton et al., 2010; Visel et al., 2009b). More recent genome-wide studies in human and mouse tissues, including across brain regions, assaying p300 and histone modifications, DNase hypersensitivity, enhancer [e]RNA, and DNA hypomethylation are in line with results from *in vivo* transgenic assays showing that enhancers are highly tissue-specific (Andersson et al., 2014; Shen et al., 2012; Stergachis et al., 2013; Visel et al., 2007; Zhu et al., 2013).

Two recent studies have directly interrogated temporal changes in enhancer and epigenomic landscapes by profiling mouse and human forebrain tissues across developmental stages. In one approach, ChIP-seq targeting H3K27ac, a histone modification strongly associated with active enhancers, was performed on mouse forebrain collected across fetal and postnatal development (Nord et al., 2013) (Figures 3E–3G). This study generated in depth maps of enhancer activity *in vivo* across development in the forebrain, identifying over 50,000 candidate enhancers active across development in the forebrain, with the majority of enhancers predicted to be transiently active. The predicted activity of a set of enhancers was validated *in vivo* using transgenic assays (Figures 3F and 3G). Dynamic enhancer activity was associated with genes expressed in the control of stage-specific biological processes such as neuronal proliferation at early embryonic time points and synapse development and plasticity later. In another time course study, Methyl-seq was performed on cortical tissues from the human and mouse at three time points to profile dynamic methylation at cytosine residues, with additional examination of 5-hydroxymethylcytosine in selected samples (Lister et al., 2013). Hypomethylation was indicative of enhancer activity, and differentially methylated regions were detected that indicate extensive turnover in enhancer activity consistent with the H3K27ac signatures observed in the developing mouse brain. As further evidence of the dynamic activity of enhancers in the developing brain, comparison of embryonic stem cells, neural stem cells, and neural progenitors showed that enhancer-promoter interactions are specific to each cell population (Zhang et al., 2013). In a finding suggestive

of how some noncoding regions act to control lineage specification, it was recently observed that there are large domains exhibiting chromatin modifications consistent with regulatory sequence that are particularly responsive to essential lineage specification or pluripotency factors. These loci, referred to as “super-enhancers” (Chapuy et al., 2013; Hnisz et al., 2013; Whyte et al., 2013) or “stretch enhancers” (Parker et al., 2013) have been predicted to exist in brain tissues as well, with initial analysis suggesting that TFs such as NKX2-2, OLIG1, BRN2, SOX10, and SOX2 are master regulators that interact with these regulatory regions in the brain (Hnisz et al., 2013). It is unclear whether these larger domains represent regulatory elements that act in a cooperative way that is qualitatively different from enhancers as previously described, or if they instead are simply regions that are densely packed with regulatory sequences.

In parallel to gene activation, *cis*-regulatory sequences and chromatin state are also involved in the repression of transcription. Specific TFs can have activating or repressing effects depending on context. For example, REST/NRSF is a master regulator of neurogenesis that acts to repress transcription of neuronal genes in nonneuronal lineages by recruiting chromatin remodeling proteins (Ballas et al., 2005). Major markers of repressive remodeling in the brain include H3K27me3 and DNA hypermethylation at gene bodies and distal enhancers (Lister et al., 2013; Zhu et al., 2013). Likely due to extensive repressive chromatin in intergenic regions and nonexpressed genes in mature neuronal lineages, the majority of enhancers active in the adult brain were found within gene bodies of neuronal genes or near the transcription start site in two independent studies (Nord et al., 2013; Zhu et al., 2013). These studies indicate that chromatin state dynamics are an important factor in brain development, a finding paralleled by recent studies of neurodevelopmental disorders discussed below.

Enhancers as Tools for Analysis of Forebrain Development

Enhancer elements generally maintain correct temporal-spatial control when ectopically positioned in the genome (e.g., (Ghanem et al., 2007; Visel et al., 2013; Zerucha et al., 2000)). Thus, these elements can be used experimentally to drive spatiotemporally restricted gene expression in specific brain regions and cell types. For instance, enhancers driving Cre expression have been used for fate mapping experiments of the subpallium (Potter et al., 2009; Waclaw et al., 2010) or pallium (Pattabiraman et al., 2014). Figure 4 illustrates the use of an enhancer (*hs636*) with activity in ventral parts of the pallial primordium (GFP expression in Figure 4D, schematically summarized in Figure 4C) to fate

Figure 3. Spatial and Temporal Specificity of Enhancers Active in the Developing Forebrain

- (A) Subset of forebrain enhancers with a spectrum of subregional specificities at whole-mount resolution.
(B) Examples of enhancers with restricted pallial activity in the mouse telencephalon at E11.5.
(C) Multiple enhancers in the larger region surrounding the *Arx* gene show subregional forebrain activity patterns that recapitulate endogenous *Arx* mRNA expression in the mouse forebrain. Notably, enhancer activities show partial spatial redundancy.
(D) Example of an enhancer with activity across multiple developmental stages, labeling cell populations whose location is consistent with migration from the MGE, through the LGE, to the cortex (white arrows).
(E) Developmental dynamics of enhancer-associated histone mark H3K27ac at candidate forebrain enhancers analyzed by ChIP-seq across seven stages of brain development. Most sites show temporally restricted H3K27ac marks.
(F and G) Examples of *in vivo* validated temporally dynamic enhancer activity in the forebrain, as predicted by temporally dynamic H3K27ac signatures. CP, choroid plexus; Cx, cortex; CxP, cortical plate; DP, dorsal pallium; LGE, lateral ganglionic eminence; LP, lateral pallium; MGE, medial ganglionic eminence; MP, medial pallium; MZ, marginal zone; VP, ventral pallium.
(A)–(D) modified from Visel et al. (2013); (E)–(G) modified from Nord et al. (2013).

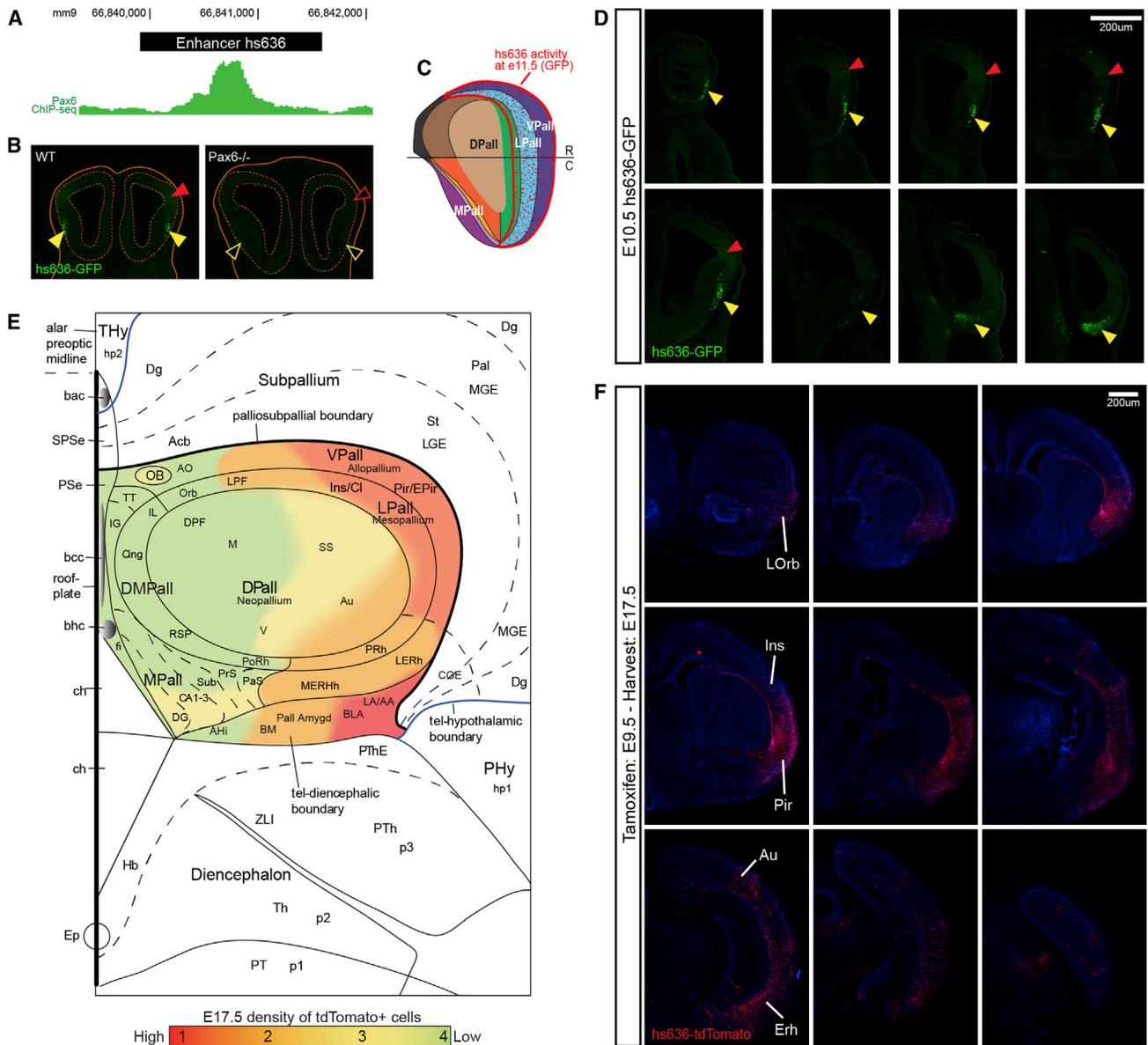


Figure 4. Enhancers as Tools for Analyzing Forebrain Development

(A) PAX6 ChIP-seq analysis from E12.5 cortex showing a peak directly over endogenous enhancer 636 (black bar).

(B) GFP pallial expression driven by enhancer hs636 in E11 cortex and reduced pallial GFP expression in Pax6^{-/-}.

(C) Schema showing approximate position of GFP expression (red) within flattened view of E11.5 pallial progenitor zones.

(D) Enhancer hs636 activity in E10.5 telencephalon in stable transgenics (yellow arrowheads: ventrolateral pallial neurons; red arrowheads: ventrolateral pallial progenitors 24127591).

(E and F) Fate mapping using enhancer hs636. Cre recombination was tamoxifen-induced at E9.5 and brains were analyzed at E17.5 for tdTomato staining (F). Results are summarized in a schematic map showing dtTomato expression within a flattened view of E17.5 pallial subdivisions, color-coded according to approximate density of tdTomato+ cells (E).

(A)–(F) modified from [Pattabiraman et al. \(2014\)](#). Abbreviations according to region: ventral pallium (VPall, allo pallium): AO, anterior olfactory nuclei; OB, olfactory bulb; Pir/EPir, piriform and ectopiriform; LERh, lateral entorhinal; MERh, medial entorhinal. Lateral pallium (LPall, mesopallium): Ins/Cl, insula/claustrum; LO, lateral orbital; PRh, perirhinal; Orb, orbitofrontal. Dorsal pallium (DPall; neopallium): AU (A), auditory; DPPF, dorsal prefrontal; F, frontal; LPF, lateral prefrontal; M, motor; SS, somatosensory; V, visual. Dorsomedial pallium (DMPall): Cing (C), cingulate gyrus; IL, infralimbic (and PrL, prelimbic); MOrb, medial orbital; RSP, retrosplenial; PoRh, postrhinal. Medial pallium (MPall): CA1-3, CA fields 1–3; DG, dentate gyrus; fi (F), fimbria; IG, indusium griseum; Sub (S), subiculum; PaS, parasubiculum; PrS, presubiculum; TT, tenia tecta. Dorsal midline: bac, brachium of the anterior commissure; bcc, brachium of the corpus callosum; bhc, brachium of the hippocampal commissure; ch, choroid plexus; PSe (PS), pallial septum. Pallial amygdala (Pall Amygd): AA, anterior amygdala; Ahi, amygdalohippocampal area; BM, basomedial; BLA, basolateral; LA, lateral. Subpallium: Acb, accumbens; CGE, caudal ganglionic eminence; Dg, Diagonal area; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; Pal, pallidum; SPSe, subpallial septum; St, striatum. Hypothalamus: hp1, 2, hypothalamic prosomere 1 and 2; PHy, peduncular; Thy, hypothalamus. Diencephalon: Hb, habenula; p2, p3, prosomeres 2 and 3; Thy, terminal hypothalamus; PThE, prethalamic eminence; Th, thalamus.

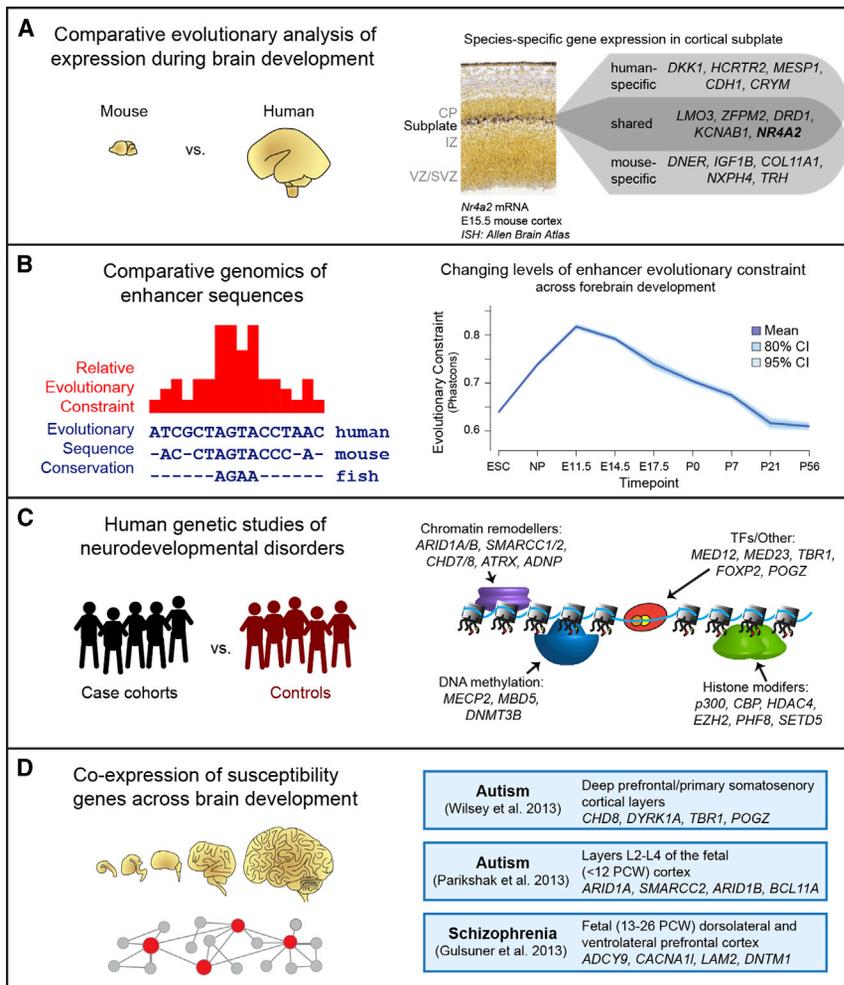


Figure 5. Insights and Challenges in Deciphering the Regulatory Architecture of Forebrain Development

(A) Comparative functional genomic studies, such as large-scale studies of gene expression patterns in the developing brain by RNA in situ hybridization, provide insight into general and human-specific aspects of molecular pathways involved in brain development.

(B) Comparative genomic studies reveal a deeply conserved regulatory framework associated with brain development, but can also identify specific changes in regulatory sequences that underlie structural and functional innovations in the brain observed in vertebrate evolution. Remarkably, regulatory sequences active during early stages of brain development (midgestation in mouse) tend to be under higher evolutionary constraint than those active later in development and in the adult brain.

(C) Genome-wide association, exome sequencing, copy number variation, and whole-genome sequencing studies of patient cohorts are powerful tools for identifying genes and noncoding sequences associated with neurodevelopmental disorders. These studies have revealed a major role for proteins involved in chromatin remodeling, DNA methylation processes, histone modification, and other transcriptional regulatory pathways and processes, with individual genes reported in human genetic studies offered as examples.

(D) Systems-level analysis integrating expression, genetic, epigenomic, and functional data has the potential to elucidate genetic and functional networks required for normal brain development and function, which are thought to be disrupted in neurodevelopmental and neuropsychiatric disorders.

et al., 2014) (Figure 5A). Evolutionary differences in the structure and representative cell types and connectivity of the forebrain are observed across vertebrates,

map its derivatives (Cre-mediated induction of tdTomato expression in Figure 4F, schematically summarized in Figure 4E). Enhancer *hs636* is bound by PAX6 in vivo (Figure 4A), and depends on *Pax6* function for its expression (Figure 4B). Enhancers driving markers such as GFP can be used in stem cell differentiation experiments to indicate when particular telencephalic cell states differentiate and for cell purification using FACS (Chen et al., 2013). Given their small size, they can also be used in viral vectors to confer cell type-specific gene expression, and have recently been used to drive expression in cortical interneurons (Lee et al., 2014; Vogt et al., 2014).

Evolutionary Conservation and Novelty of Gene Regulation in the Brain

Ongoing studies of various vertebrate species, including human, are defining the transcription factors and enhancers that control cortical development (Miller et al., 2014; Shim et al., 2012). These analyses are expected to shed light on the genetic mechanisms that have contributed to cortical evolution and disease (Willsey et al., 2013). Comparative transcriptomic analyses have demonstrated differences in expression of genes in the developing brain across primates (Khaitovich et al., 2004; Konopka et al., 2009; Nowick et al., 2009) and between mice and humans (Miller

et al., 2014) (Figure 5A). Evolutionary differences in the structure and representative cell types and connectivity of the forebrain are observed across vertebrates, with the six-layered laminar structure arising in mammals and the expansion in cortical surface area producing complex involutions observed in a number of species, including humans. These differences may be associated with changes in the expression patterns of and the interactions between specific transcription factors in the developing brain. Studies on the evolution of regulatory control systems and *cis*-regulatory elements have produced paradoxical findings highlighting both extreme evolutionary constraint and human-specific regulatory changes as strong forces in shaping the forebrain. King and Wilson demonstrated high levels of coding sequence conservation between human and chimpanzee and postulated that noncoding changes account for the majority of sequence level differences between the species (King and Wilson, 1975). The availability of sequenced genomes has enabled comparisons of noncoding sequence homology across the vertebrate evolutionary tree and recently, between humans and extinct hominins (Green et al., 2010). Perhaps surprisingly, very high levels of sequence conservation have been observed at regulatory sequences active in the developing forebrain across the vertebrate lineage, with ultra-conserved regulatory elements enriched for forebrain activity in transgenic assays compared to other tissues in E11.5 mice

and increased constraint levels observed genome-wide for forebrain enhancers relative to other tissues (Blow et al., 2010; Nord et al., 2013; Pennacchio et al., 2006). Interestingly, the level of constraint on stage-specific forebrain enhancers decreases significantly from midgestation to adult (Nord et al., 2013) (Figure 5B), a pattern also observed across various developmental lineages (Bogdanović et al., 2012; Stergachis et al., 2013). This pattern is observable in the constraint of gene expression patterns and general morphology (Domazet-Lošo and Tautz, 2010; Kalinka et al., 2010; Quint et al., 2012), in line with the hourglass model of evolutionary constraint on development (Casici, 2011). Further supporting the conservation of neuronal regulatory networks, a study of transcription factor co-expression and interaction comparing human and mouse identified a number of transcription factor networks that appear conserved between mouse and human in the developing brain (Ravasi et al., 2010). These findings suggest that *cis*-regulatory landscapes controlling early forebrain development are generally relatively ancient.

Despite the observation of strong evolutionary constraint at brain enhancers, other studies examining sequence and functional conservation of enhancers suggest that human regulatory elements exhibit high levels of evolutionary innovation both in sequence and function. One locus that has been examined in detail is *AUTS2*, a gene implicated in human evolution and in neurological disorders. Sequence comparisons at this locus between human and Neanderthal genomes identified one of the strongest signals for human-specific noncoding sequence changes (Green et al., 2010), and noncoding sequences at this locus exhibiting human-specific changes drove expression in the developing brain (Oksenberg et al., 2013). A second approach has been to look for regulatory regions that have high levels of evolutionary constraint but where there are human-specific changes, also known as human-accelerated regions (HARs) or accelerated conserved noncoding sequences (aCNSs), or where there is no mappable human homolog (McLean et al., 2011; Pollard et al., 2006; Prabhakar et al., 2006a). HARs and aCNSs are enriched near TFs and near genes associated with neuronal cell adhesion and human-specific deletions are similarly associated with neural functions, findings suggestive of sequence-level changes that drive human-specific aspects of brain development. Genome-wide comparison of functional conservation of enhancers as assayed by p300 interaction revealed evidence that sequences exhibiting significant homology between human and mouse can show functional differences in the developing brain. This comparison only included a single developmental time point at which mouse and human brains should be relatively stage matched (Clancy et al., 2007) and candidate enhancers identified using only a single epigenomic mark (Visel et al., 2013), and further experiments are necessary to elucidate functional conservation across tissues and developmental stages. While not in a neuronal tissue, recent work comparing functional conservation using epigenomic assays in hepatocytes (Odom et al., 2007) and developmental limb tissue (Cotney et al., 2013) demonstrate significant lack of functional conservation between humans and other vertebrate lineages. These findings are supported by functional enhancer assays comparing activity in *Drosophila* lineages (Arnold et al.,

2013). Finding of limited functional conservation of noncoding elements in the brain is in line with studies directly comparing TF binding and H3K27ac across evolution in other tissues, which suggest substantial turnover in TF binding sites and evolution of new enhancers via functional modification or co-option of enhancers active in other tissues (Lettice et al., 2011; Schmidt et al., 2010; Stefflova et al., 2013). Annotated maps of human forebrain enhancers will be required to determine the balance between evolutionary conservation and innovation in *cis*-regulatory control of human forebrain development.

Gene Regulation and Neurodevelopmental Disorders *Loss of Function of Regulatory Genes in Forebrain Development*

Mouse knockout models are now available for thousands of genes, including many genes known or proposed to be involved in transcriptional regulation during brain development. Studies using these models have revealed severe neuroanatomical consequences and frequently embryonic lethal phenotypes associated with loss of function of neuronal transcriptional regulators, as described above. In parallel, some of the first successful efforts to map human monogenic neurodevelopmental disorders implicate transcriptional regulation and chromatin remodeling in synapse development and plasticity, such as *MECP2* in Rett syndrome and *FMR1* in fragile X syndrome (Amir et al., 1999; Verkerk et al., 1991). A growing body of evidence supports that multiple human neurodevelopmental disorders are associated with mutations in TFs, including *Arx*, *Pax6*, *Six3*, and *Tbr1* (Bhatia et al., 2013; Hehr et al., 2010; Olivetti and Noebels, 2012; van Heyningen and Williamson, 2002; Willsey et al., 2013).

Human Genetic Studies of Forebrain Transcriptional Regulation

There has been a leap forward in understanding the genetic components of complex neurodevelopmental disorders in recent years (McCarroll and Hyman, 2013). While the relative contribution to these traits across the spectrum of allele frequency remains an active discussion (McClellan and King, 2010), replicated findings at specific loci have established a role for both common and rare variants via genome-wide association studies (GWAS), rare or de novo CNV screening, and exome sequencing, as reviewed recently (Krystal and State, 2014; McCarroll and Hyman, 2013). The findings from human genetics studies point to polygenicity and a significant role for genes involved in transcriptional regulation and chromatin remodeling in the developing forebrain in the etiology of disorders such as autism, schizophrenia, and intellectual disability (Figure 5C). Studies of autism and schizophrenia have highlighted pathways required for transcriptional regulation and chromatin remodeling, as well as other functional classes, such as calcium channels and synaptic plasticity genes (Krystal and State, 2014; McCarroll and Hyman, 2013). Evidence is mounting for dosage effects or haploinsufficiency of developmental TFs, such as *TBR1*, and chromatin remodeling proteins, such as *CHD8*, as a mechanism in these disorders (O'Roak et al., 2011, 2012a, 2012b). It is also likely that regulatory sequence variation contributes to these traits because regions around many of the replicated GWAS signals as well as rare disease-associated CNVs do not contain coding sequence variants. Furthermore, disease-linked variants have

been identified within enhancers active in the developing brain that result in changes in enhancer activity (Poitras et al., 2010).

Transcriptional Regulation and Chromatin Remodeling in Autism and Schizophrenia

While there are now many genes identified via rare and de novo CNV screening and exome sequencing, it will take larger screening efforts and functional follow-up to establish the causality and pathophysiology of these mutations. An approach to learn more about these networks that has yielded success for autism and schizophrenia is to identify brain subregions and developmental stages at which the implicated gene networks are coexpressed using publicly available brain transcriptome data (Kang et al., 2011) (Figure 5D). In autism, coexpression networks present in midfetal layer 5/6 cortical projection neurons were identified that were organized around high-confidence “seed” genes that are mutated in autism (Willsey et al., 2013). A similar study linked rare de novo coding mutations in autism to coregulated highly expressed genes during early neuronal fate determination, migration, and establishment of the cortical layers, providing suggestive evidence of a gene set that contributes to autism via haploinsufficiency (Parikshak et al., 2013). The same study identified glutamatergic neurons in superficial cortical layers (layer 2–4) as a potential cell type where autism-associated expression networks may be critical. These two studies describe different specific cortical layers as relevant, but the developmental regulatory processes affected by both sets of gene network analyses are highly convergent. An earlier study of gene expression in autism-associated cortical regions from autistic subjects versus controls identified differentially expressed genes and expression networks in autism, with a number of genes that overlap the two recent studies (Voineagu et al., 2011). A study examining de novo mutations in schizophrenia linked genes with predicted deleterious coding mutations in cases to expression networks in the fetal dorsolateral and ventrolateral prefrontal cortex (Gulsuner et al., 2013). While a complex combination of genetic and environmental factors is likely to influence these phenotypes, these studies illustrate how human genetic studies in tandem with functional genomics data can reveal the pathophysiology of neurodevelopmental disorders and the causal role that loss of endogenous gene expression and transcriptional regulatory circuits during brain development plays.

Knockout Models of Enhancer Function

Noncoding sequence variation is predicted to represent a substantial proportion of disease-associated variants (ENCODE Project Consortium, 2012), yet the requirement for specific enhancers during development remains largely unclear. There are examples of sequence variation at enhancers that are linked to developmental phenotypes, with one of the most well-known instances being mutations in the ZRS enhancer of *Shh* that lead to limb malformations (Lettice et al., 2003). There are a few examples of enhancers whose deletion was shown to cause clear neurodevelopmental phenotypes. For instance, deletion of an enhancer regulating *Fezf2* resulted in loss of *Fezf2* expression and anatomical changes in the brain characterized by loss of specification of corticospinal neurons (Shim et al., 2012). On the other hand, a study that targeted four ultraconserved enhancers, which included enhancers predicted to regulate

brain-expressed genes that produced severe phenotypes when knocked out, found that mice homozygous for the enhancer deletion allele for each of the four enhancers did not have gross neurodevelopmental or neurological phenotypes (Ahituv et al., 2007), indicating that even the evolutionarily most conserved enhancers may not necessarily have functions at the organism level that are required for viability.

Two related explanations may account for the mixed consequences of enhancer loss of function in these mouse models. The first is that there is a high level of functional redundancy in enhancers that regulate a specific locus. In this model, loss of function of a single enhancer is masked by the activity of another enhancer that drive a similar expression pattern, as has been observed for “shadow” enhancers in *Drosophila* (Lagha et al., 2012). In a similar model, enhancers may not be functionally redundant, but they act in a combinatorial manner where each enhancer acts to fine tune the expression of a gene. In this model, careful phenotyping would be necessary to identify the changes caused by loss of enhancer function. Outside the brain, this general paradigm was recently demonstrated in a study where deletions of craniofacial enhancers produced subtle morphological changes (Attanasio et al., 2013). While there remain a small number of regulatory loss-of-function models in mouse, results from human genetic studies, especially GWAS, suggest a significant role for regulatory variation across phenotypes, including in neurodevelopmental disorders (Ripke et al., 2013). It is quite possible that each regulatory circuit will be different, with strong effects possible for loss of function at some enhancers and weak or no observable phenotypes seen when other enhancers are deleted or when sequence variation changes enhancer activity.

Conclusions and Major Outstanding Questions

The combination of detailed functional dissection of the roles of individual TFs with genome-wide approaches to mapping gene expression, enhancer activity, and chromatin dynamics reveal an emerging picture of the role of transcriptional regulation in forebrain development. These studies link cell-specific expression of TFs during processes such as proliferation, differentiation, migration, and synapse development to target genes via binding at TF binding motifs found in promoter-proximal sequences and distal enhancers. These regulatory circuits are emerging as tightly linked to the evolution of the human brain and to human neurodevelopmental disorders, with initial indications of specific developmental cortical subregions and neuronal classes that may be affected by mutations that contribute to disorders such as autism and schizophrenia. Figure 5 summarizes findings from recent studies highlighting the role of gene regulation in evolution and neurodevelopmental disorders.

With the availability of new technologies for epigenomic and functional profiling of regulatory sequences, availability of patient-specific models such as induced pluripotent stem cells and precision-engineered animal models, where is the field headed? We now discuss major challenges for three areas: basic biological mechanisms underlying gene regulation, the role of regulatory elements and chromatin structure in brain development, and linking findings from human genetic studies to biological mechanisms focusing on gene regulation.

Challenge 1: Basic Mechanisms

General models of gene regulation via TF binding and activation or repression through cofactor recruitment, changes in chromatin accessibility, and DNA looping are becoming clearer, yet many of the basic mechanisms required for this process are still poorly defined. TF binding sites can be predicted based on various experimental methods and can be validated using direct measures of interaction; however, it appears that a large proportion of the validated binding sites of many TFs may not be functionally relevant (White et al., 2013), and that combinatorial binding of TFs may govern function (Teng et al., 2014). Consistent with these observations, levels of individual TF expression do not have extensive global effects as measured by TF knock-down and expression profiling (Cusanovich et al., 2014). There are additionally unresolved questions regarding regulatory function and the balance of conservation versus binding site turnover within enhancers across evolution (Villar et al., 2014), the order and spacing of binding sites within enhancers (Smith et al., 2013), the requirement for specific TF binding interactions within enhancers (Teng et al., 2014), and the relationship between structural contact through looping and transcriptional activation (DeMare et al., 2013; Kieffer-Kwon et al., 2013).

A second major mechanistic question is how enhancers drive expression of a specific gene or set of target genes given the observation that many enhancers appear to skip nearby transcription start sites to interact with more distal targets (Zhang et al., 2013). Although recent advances mapping chromatin interactions enable mapping of enhancer-promoter interactions, the mechanism for establishing specificity has been studied only for a very small number of loci. It is clear that enhancers vastly outnumber their transcribed targets. The model of specific enhancers driving cell-type or tissue-specific expression patterns is attractive and is true at a genomic scale, yet it appears that many genes are controlled by complex landscapes of potentially redundant regulatory elements and that loss of function of even highly conserved enhancers near critical genes may not produce significant phenotypic effects (Ahituv et al., 2007). Recent studies suggest that enhancer redundancy is required to maintain robust expression patterns (Lagha et al., 2012) and that arrays of regulatory elements with correlated activity may enable tighter quantitative or qualitative regulation of expression (Whyte et al., 2013); however, this remains a relatively unexplored area especially in mammalian systems.

At this time, no single known epigenomic signature is sufficient to identify all enhancers and distinguish enhancers that are active with perfect specificity (Cotney et al., 2012), and an active area of research is honing prediction algorithms that are based on regulatory sequence composition, epigenomic signatures, and genomic structure, and in developing methods to enable function-based screening of regulatory element activity as described above. A central assumption to the current models is that the census of TFs present combined with the relevant regulatory sequence substrate represents the information necessary for interpreting endogenous enhancer function, yet the field remains a long way from determining the rules that govern the processes involved in the establishment and function of mammalian regulatory circuitry and in predicting the effects of perturbations to these systems.

Challenge 2: Gene Regulation in Forebrain Development

The framework and major actors in many TF signaling networks active in control of differentiation across a variety of neuronal subtypes are becoming clearer, with emerging *Dlx1/2* and *Nkx2-1* networks in the LGE/CGE/MGE detailed above. However, these existing pictures are likely to represent a gross simplification of the intrinsic and external factors directing developmental processes in the brain. Moving from single genes to networks will require systems level analysis of brain development. Efforts are already underway to map the transcriptome (Kang et al., 2011; Miller et al., 2014) and connectome (Oh et al., 2014) of developing brains, and significant resources will be developed toward developing new technologies. Genomic analysis has led the way, with major accomplishments using RNA-seq to understand transcriptional differences underlying normal and pathogenic brain development (Kang et al., 2008; Miller et al., 2014; Voineagu et al., 2011). In the future, the direct and indirect regulatory interactions that drive brain development will be revealed at a systems level with the level of rigor and detail that is currently only possible via in depth examination of individual circuits.

In contrast to the substantial effort dedicated to mapping TF activity in the developing brain, other aspects of the regulatory circuitry orchestrating normal and pathogenic neurodevelopmental processes remain underexplored. While genome-wide studies have established that the dynamic activity of tens of thousands of enhancers orchestrates spatiotemporal gene expression in forebrain development, mapping and epigenomic and functional characterization of these elements in the human genome remains a priority. Furthermore, technology exists to establish enhancer-promoter interactions (Fullwood and Ruan, 2009; Lieberman-Aiden et al., 2009), which will be critical to understanding the control of loci involved in specific processes or implicated in specific disorders. Of the tens of thousands of enhancers predicted in functional genomics studies of brain development, only a fraction of the target genes have been predicted with experimental confirmation of required regulatory activity available for only a small subset of these predictions (e.g., Shim et al., 2012). It is unlikely that current centralized efforts will have the bandwidth to profile all of the multidimensional axes of brain subregions, temporal stages across differentiation and development, and the different lineages represented in the brain. Nonetheless, the technology required to map and characterize regulatory elements is accessible and individual research groups can now profile specific systems of interest. Through these combined efforts, a more complete picture of the location, function, and targets of neural regulatory elements should emerge.

Complementary to characterizing the activity of individual regulatory elements genome-wide across the multidimensional space of brain development is the less studied role of chromatin remodeling and epigenetic marking in brain development. Early studies in this area suggest a central role for chromatin remodeling factors in forebrain development, substantial transcriptional control via chromatin restriction as lineage specification proceeds in the brain, and widespread changes in DNA methylation patterns across brain development (Lister et al., 2013; Ronan et al., 2013; Zhu et al., 2013). Further studies are necessary to

understand the mechanism and requirement for these processes in neurodevelopment. Finally, the availability of genomic data sets, functional genomic assays, and efficient genome modification technology will enable deeper exploration of the regulatory circuits and activities of specific TFs in brain development. With new technologies for precision genome engineering emerging (Bedell et al., 2012; Christian et al., 2010; Jinek et al., 2012; Mali et al., 2013), we foresee rapid expansion in the understanding of the interplay of specific genes that control neural lineage specification, regionalization, and function. Through the results of these studies, it will be possible to ask more detailed questions regarding the role of regulatory sequences and chromatin remodeling processes in the development and function of the brain and potentially in the role of noncoding sequence in the evolution of the human brain and cognitive capacity.

Challenge 3: Gene Regulation in Neurodevelopmental Disorders

As described above, neurodevelopmental disorders appear to be driven in part by changes in gene expression levels during development, highlighted by the dosage sensitivity observed in genes identified by CNV and exome screening. The compelling evidence generated by recent attempts to intersect neurodevelopmental transcriptome data with genes linked to autism and schizophrenia is, as of now, more suggestive than conclusive regarding the pathophysiology of these disorders (Parikshak et al., 2013; Voineagu et al., 2011; Willsey et al., 2013). Further work is required to map the affected cell types and document the molecular changes and cell biology associated with changes in expression of susceptibility genes. Additionally, there are two major unexplored potential driver mechanisms behind neurodevelopmental disorders. First is the effect of noncoding sequence variation. As regulatory elements are predicted to direct robust expression patterns in the developing brain, it is likely that sequence variation that changes the function of these elements has the potential to drive changes in gene expression and downstream dosage-sensitivity-driven effects. Although many GWAS have identified noncoding regions as associated with phenotypes such as autism and schizophrenia, the causal variants have yet to be characterized. In a recent example of a large effect noncoding variant with severe phenotypic consequences in the brain, a short deletion in regulatory sequence was identified in subjects exhibiting gyral abnormalities (Bae et al., 2014). The deletion variant disrupted the regulatory function, leading to a change of expression of isoforms of *GPR56* and resulting in changes in the cortical expression pattern of this gene, which resulted in restricted polymicrogyria surrounding the Sylvian fissure that is linked to intellectual and language difficulty and seizures. Another suggestive finding is the duplication of the region containing *VIPR2* in schizophrenia, where a minimal duplication of noncoding sequence was observed in cases (Vacic et al., 2011).

The current list of characterized causal variants found in noncoding regions appears exceptionally small when viewed in light of the current prominence and success of screening coding sequence (e.g., exome sequencing). In the future, whole genome analysis will enable unbiased examination of noncoding sequences as well, but there will be major challenges connected to the expected large number of variants and the difficulty of predicting functionality of affected noncoding sequence (Jiang

et al., 2013). The expansion of regulatory element mapping and characterization described above will enable screening of noncoding regions. In the meantime, targeted examination of noncoding regions may be a viable intermediate approach, where predicted functional or conserved noncoding elements around susceptibility genes could be screened at a volume sufficient to start to dissect differences between case and control population variation at these loci.

An area poised for large-scale growth in the near future is the characterization of epigenomic or epigenetic changes in the brain (or in induced pluripotent stem cell-derived differentiated neurons) of individuals afflicted with neurological and neurodevelopmental disorders. These studies are confounded by the difficulty of assigning causal relationships for observed differences in driving original pathophysiological processes or in contributing to later manifestations of the traits. Nonetheless, the emergence of consistent patterns associated with specific phenotypes has the potential to provide evidence for a role of stable epigenetic changes that will ultimately lead to better understanding of the forces contributing to neurodevelopmental disorders and could provide a link between environmental factors and long-term changes in gene regulation.

The identification of specific regulatory elements or of widespread epigenetic changes will additionally generate novel targets for therapeutic intervention. Already, major changes are underway in the molecular diagnosis of neurodevelopmental disorders that enable better clinical care (Krystal and State, 2014), as well as the development of promising targeted therapeutic approaches (Gross et al., 2012). The ability to target the CRISPR/Cas9 or TALE systems to specific sequences has been harnessed to build synthetic regulatory proteins that can act to change gene expression or epigenomic markings at specific regulatory elements (Mendenhall et al., 2013). In the not-too-distant future, it may be possible to use synthetic regulators to modify gene dosage of target genes involved in key processes in specific neuronal cell populations implicated via studies of transcriptional regulation in normal and pathogenic brain development. With the combination of new technologies and the rapidly growing understanding of the role of transcriptional regulation in neurodevelopment, this is an exciting and rapidly changing field that has the potential to transform our understanding of the evolution, development, and function of the human brain.

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REFERENCES

Ahituv, N., Zhu, Y., Visel, A., Holt, A., Afzal, V., Pennacchio, L.A., and Rubin, E.M. (2007). Deletion of ultraconserved elements yields viable mice. *PLoS Biol.* 5, e234.

- Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* **23**, 185–188.
- Anderson, S.A., Eisenstat, D.D., Shi, L., and Rubenstein, J.L. (1997a). Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. *Science* **278**, 474–476.
- Anderson, S.A., Qiu, M., Bulfone, A., Eisenstat, D.D., Meneses, J., Pedersen, R., and Rubenstein, J.L. (1997b). Mutations of the homeobox genes Dlx-1 and Dlx-2 disrupt the striatal subventricular zone and differentiation of late born striatal neurons. *Neuron* **19**, 27–37.
- Andersson, R., Gebhard, C., Miguel-Escalada, I., Hoof, I., Bornholdt, J., Boyd, M., Chen, Y., Zhao, X., Schmid, C., Suzuki, T., et al.; FANTOM Consortium (2014). An atlas of active enhancers across human cell types and tissues. *Nature* **507**, 455–461.
- Ariza-Cosano, A., Visel, A., Pennacchio, L.A., Fraser, H.B., Gómez-Skarmeta, J.L., Irimia, M., and Bessa, J. (2012). Differences in enhancer activity in mouse and zebrafish reporter assays are often associated with changes in gene expression. *BMC Genomics* **13**, 713–531.
- Arnold, C.D., Gerlach, D., Stelzer, C., Boryń, Ł.M., Rath, M., and Stark, A. (2013). Genome-wide quantitative enhancer activity maps identified by STARR-seq. *Science* **339**, 1074–1077.
- Atanasio, C., Nord, A.S., Zhu, Y., Blow, M.J., Li, Z., Liberton, D.K., Morrison, H., Plajzer-Frick, I., Holt, A., Hosseini, R., et al. (2013). Fine tuning of craniofacial morphology by distant-acting enhancers. *Science* **342**, 1241006–1241006.
- Azim, E., Jabaudon, D., Fame, R.M., and Macklis, J.D. (2009). SOX6 controls dorsal progenitor identity and interneuron diversity during neocortical development. *Nat. Neurosci.* **12**, 1238–1247.
- Bae, B.-I., Tietjen, I., Atabay, K.D., Evrony, G.D., Johnson, M.B., Asare, E., Wang, P.P., Murayama, A.Y., Im, K., Lisgo, S.N., et al. (2014). Evolutionarily dynamic alternative splicing of GPR56 regulates regional cerebral cortical patterning. *Science* **343**, 764–768.
- Ballas, N., Grunseich, C., Lu, D.D., Speh, J.C., and Mandel, G. (2005). REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis. *Cell* **121**, 645–657.
- Batista-Brito, R., and Fishell, G. (2013). The generation of cortical interneurons. In *Patterning and Cell Type Specification in the Developing CNS and PNS*, J.L.R. Rubenstein and P. Rakic, eds. (Elsevier), pp. 503–518.
- Batista-Brito, R., Rossignol, E., Hjerling-Leffler, J., Denaxa, M., Wegner, M., Lefebvre, V., Pachnis, V., and Fishell, G. (2009). The cell-intrinsic requirement of Sox6 for cortical interneuron development. *Neuron* **63**, 466–481.
- Bedell, V.M., Wang, Y., Campbell, J.M., Poshusta, T.L., Starker, C.G., Krug, R.G., 2nd, Tan, W., Penheiter, S.G., Ma, A.C., Leung, A.Y.H., et al. (2012). In vivo genome editing using a high-efficiency TALEN system. *Nature* **491**, 114–118.
- Bhatia, S., Bengani, H., Fish, M., Brown, A., Divizia, M.T., de Marco, R., Damante, G., Grainger, R., van Heyningen, V., and Kleinjan, D.A. (2013). Disruption of autoregulatory feedback by a mutation in a remote, ultraconserved PAX6 enhancer causes aniridia. *Am. J. Hum. Genet.* **93**, 1126–1134.
- Birney, E., Stamatoyannopoulos, J.A., Dutta, A., Guigó, R., Gingeras, T.R., Margulies, E.H., Weng, Z., Snyder, M., Dermitzakis, E.T., Thurman, R.E., et al.; ENCODE Project Consortium; NISC Comparative Sequencing Program; Baylor College of Medicine Human Genome Sequencing Center; Washington University Genome Sequencing Center; Broad Institute; Children’s Hospital Oakland Research Institute (2007). Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* **447**, 799–816.
- Blow, M.J., McCulley, D.J., Li, Z., Zhang, T., Akiyama, J.A., Holt, A., Plajzer-Frick, I., Shoukry, M., Wright, C., Chen, F., et al. (2010). ChIP-Seq identification of weakly conserved heart enhancers. *Nat. Genet.* **42**, 806–810.
- Bogdanović, O., Fernandez-Miñán, A., Tena, J.J., de la Calle-Mustienes, E., Hidalgo, C., van Kruysbergen, I., van Heeringen, S.J., Veenstra, G.J., and Gómez-Skarmeta, J.L. (2012). Dynamics of enhancer chromatin signatures mark the transition from pluripotency to cell specification during embryogenesis. *Genome Res.* **22**, 2043–2053.
- Butt, S.J.B., Sousa, V.H., Fuccillo, M.V., Hjerling-Leffler, J., Miyoshi, G., Kimura, S., and Fishell, G. (2008). The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes. *Neuron* **59**, 722–732.
- Cajal, S.R.Y. (1899). *Textura del Sistema Nervioso del Hombre y de los Vertebrados*. (Imprenta N. Moya).
- Casici, T. (2011). Development: hourglass theory gets molecular approval. *Nat. Rev. Genet.* **12**, 76–76.
- Castro, D.S., Martynoga, B., Parras, C., Ramesh, V., Pacary, E., Johnston, C., Drechsel, D., Lebel-Potter, M., Garcia, L.G., Hunt, C., et al. (2011). A novel function of the proneural factor Ascl1 in progenitor proliferation identified by genome-wide characterization of its targets. *Genes Dev.* **25**, 930–945.
- Chapuy, B., McKeown, M.R., Lin, C.Y., Monti, S., Roemer, M.G.M., Qi, J., Rahl, P.B., Sun, H.H., Yeda, K.T., Doench, J.G., et al. (2013). Discovery and characterization of super-enhancer-associated dependencies in diffuse large B cell lymphoma. *Cancer Cell* **24**, 777–790.
- Chen, Y.-J.J., Vogt, D., Wang, Y., Visel, A., Silberberg, S.N., Nicholas, C.R., Danjo, T., Pollack, J.L., Pennacchio, L.A., Anderson, S., et al. (2013). Use of “MGE enhancers” for labeling and selection of embryonic stem cell-derived medial ganglionic eminence (MGE) progenitors and neurons. *PLoS ONE* **8**, e61956.
- Christian, M., Cermak, T., Doyle, E.L., Schmidt, C., Zhang, F., Hummel, A., Bogdanove, A.J., and Voytas, D.F. (2010). Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics* **186**, 757–761.
- Clancy, B., Finlay, B.L., Darlington, R.B., and Anand, K.J.S. (2007). Extrapolating brain development from experimental species to humans. *Neurotoxicology* **28**, 931–937.
- Close, J., Xu, H., De Marco Garcia, N., Batista-Brito, R., Rossignol, E., Rudy, B., and Fishell, G. (2012). Satb1 is an activity-modulated transcription factor required for the terminal differentiation and connectivity of medial ganglionic eminence-derived cortical interneurons. *J. Neurosci.* **32**, 17690–17705.
- Cobos, I., Calcagnotto, M.E., Vilaythong, A.J., Thwin, M.T., Noebels, J.L., Baraban, S.C., and Rubenstein, J.L.R. (2005). Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. *Nat. Neurosci.* **8**, 1059–1068.
- Cobos, I., Borello, U., and Rubenstein, J.L.R. (2007). Dlx transcription factors promote migration through repression of axon and dendrite growth. *Neuron* **54**, 873–888.
- Colasante, G., Collombat, P., Raimondi, V., Bonanomi, D., Ferrai, C., Maira, M., Yoshikawa, K., Mansouri, A., Valtorta, F., Rubenstein, J.L.R., and Broccoli, V. (2008). Arx is a direct target of Dlx2 and thereby contributes to the tangential migration of GABAergic interneurons. *J. Neurosci.* **28**, 10674–10686.
- Colombo, E., Collombat, P., Colasante, G., Bianchi, M., Long, J., Mansouri, A., Rubenstein, J.L.R., and Broccoli, V. (2007). Inactivation of Arx, the murine ortholog of the X-linked lissencephaly with ambiguous genitalia gene, leads to severe disorganization of the ventral telencephalon with impaired neuronal migration and differentiation. *J. Neurosci.* **27**, 4786–4798.
- Cooper, G.M., Stone, E.A., Asimenos, G., Green, E.D., Batzoglou, S., and Sidow, A.; NISC Comparative Sequencing Program (2005). Distribution and intensity of constraint in mammalian genomic sequence. *Genome Res.* **15**, 901–913.
- Cotney, J., Leng, J., Oh, S., DeMare, L.E., Reilly, S.K., Gerstein, M.B., and Noonan, J.P. (2012). Chromatin state signatures associated with tissue-specific gene expression and enhancer activity in the embryonic limb. *Genome Res.* **22**, 1069–1080.
- Cotney, J., Leng, J., Yin, J., Reilly, S.K., DeMare, L.E., Emera, D., Ayoub, A.E., Rakic, P., and Noonan, J.P. (2013). The evolution of lineage-specific regulatory activities in the human embryonic limb. *Cell* **154**, 185–196.
- Creyghton, M.P., Cheng, A.W., Welstead, G.G., Kooistra, T., Carey, B.W., Steine, E.J., Hanna, J., Lodato, M.A., Frampton, G.M., Sharp, P.A., et al. (2010). Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc. Natl. Acad. Sci. USA* **107**, 21931–21936.
- Cuddapah, S., Jothi, R., Schones, D.E., Roh, T.-Y., Cui, K., and Zhao, K. (2009). Global analysis of the insulator binding protein CTCF in chromatin

- barrier regions reveals demarcation of active and repressive domains. *Genome Res.* 19, 24–32.
- Cusanovich, D.A., Pavlovic, B., Pritchard, J.K., and Gilad, Y. (2014). The functional consequences of variation in transcription factor binding. *PLoS Genet.* 10, e1004226.
- DeMare, L.E., Leng, J., Cotney, J., Reilly, S.K., Yin, J., Sarro, R., and Noonan, J.P. (2013). The genomic landscape of cohesin-associated chromatin interactions. *Genome Res.* 23, 1224–1234.
- Denaxa, M., Kalaitzidou, M., Garefalaki, A., Achimastou, A., Lasrado, R., Maes, T., and Pachnis, V. (2012). Maturation-promoting activity of SATB1 in MGE-derived cortical interneurons. *Cell Reports* 2, 1351–1362.
- Dickel, D.E., Zhu, Y., Nord, A.S., Wylie, J.N., Akiyama, J.A., Afzal, V., Plajzer-Frick, I., Kirkpatrick, A., Göttgens, B., Bruneau, B.G., et al. (2014). Function-based identification of mammalian enhancers using site-specific integration. *Nat. Methods* 11, 566–571.
- Domazet-Lošo, T., and Tautz, D. (2010). A phylogenetically based transcriptome age index mirrors ontogenetic divergence patterns. *Nature* 468, 815–818.
- Du, T., Xu, Q., Ocbina, P.J., and Anderson, S.A. (2008). NKX2.1 specifies cortical interneuron fate by activating Lhx6. *Development* 135, 1559–1567.
- ENCODE Project Consortium (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature* 489, 57–74.
- Flandin, P., Kimura, S., and Rubenstein, J.L.R. (2010). The progenitor zone of the ventral medial ganglionic eminence requires Nkx2-1 to generate most of the globus pallidus but few neocortical interneurons. *J. Neurosci.* 30, 2812–2823.
- Fragkouli, A., Hearn, C., Errington, M., Cooke, S., Grigoriou, M., Bliss, T., Stylianopoulou, F., and Pachnis, V. (2005). Loss of forebrain cholinergic neurons and impairment in spatial learning and memory in LHX7-deficient mice. *Eur. J. Neurosci.* 21, 2923–2938.
- Fragkouli, A., van Wijk, N.V., Lopes, R., Kessar, N., and Pachnis, V. (2009). LIM homeodomain transcription factor-dependent specification of bipotential MGE progenitors into cholinergic and GABAergic striatal interneurons. *Development* 136, 3841–3851.
- Frazer, K.A., Pachter, L., Poliakov, A., Rubin, E.M., and Dubchak, I. (2004). VISTA: computational tools for comparative genomics. *Nucleic Acids Res.* 32, W273–9.
- Friocourt, G., and Parnavelas, J.G. (2010). Mutations in ARX Result in Several Defects Involving GABAergic Neurons. *Front. Cell. Neurosci.* 4, 4.
- Fullwood, M.J., and Ruan, Y. (2009). ChIP-based methods for the identification of long-range chromatin interactions. *J. Cell. Biochem.* 107, 30–39.
- Fullwood, M.J., Liu, M.H., Pan, Y.F., Liu, J., Xu, H., Mohamed, Y.B., Orlov, Y.L., Velkov, S., Ho, A., Mei, P.H., et al. (2009). An oestrogen-receptor-alpha-bound human chromatin interactome. *Nature* 462, 58–64.
- Ghanem, N., Yu, M., Long, J., Hatch, G., Rubenstein, J.L.R., and Ekker, M. (2007). Distinct cis-regulatory elements from the Dlx1/Dlx2 locus mark different progenitor cell populations in the ganglionic eminences and different subtypes of adult cortical interneurons. *J. Neurosci.* 27, 5012–5022.
- Gong, S., Zheng, C., Doughty, M.L., Losos, K., Didkovsky, N., Schambra, U.B., Nowak, N.J., Joyner, A., Leblanc, G., Hatten, M.E., and Heintz, N. (2003). A gene expression atlas of the central nervous system based on bacterial artificial chromosomes. *Nature* 425, 917–925.
- Green, R.E., Krause, J., Briggs, A.W., Maricic, T., Stenzel, U., Kircher, M., Patterson, N., Li, H., Zhai, W., Fritz, M.H.-Y., et al. (2010). A draft sequence of the Neandertal genome. *Science* 328, 710–722.
- Gross, C., Berry-Kravis, E.M., and Bassell, G.J. (2012). Therapeutic strategies in fragile X syndrome: dysregulated mGluR signaling and beyond. *Neuropsychopharmacology* 37, 178–195.
- Gulsuner, S., Walsh, T., Watts, A.C., Lee, M.K., Thornton, A.M., Casadei, S., Rippey, C., Shahin, H., Nimgaonkar, V.L., Go, R.C., et al.; Consortium on the Genetics of Schizophrenia (COGS); PAARTNERS Study Group (2013). Spatial and temporal mapping of de novo mutations in schizophrenia to a fetal prefrontal cortical network. *Cell* 154, 518–529.
- Hébert, J.M., and Fishell, G. (2008). The genetics of early telencephalon patterning: some assembly required. *Nat. Rev. Neurosci.* 9, 678–685.
- Hehr, U., Pineda-Alvarez, D.E., Uyanik, G., Hu, P., Zhou, N., Hehr, A., Schell-Apacik, C., Altus, C., Daumer-Haas, C., Meiner, A., et al. (2010). Heterozygous mutations in SIX3 and SHH are associated with schizencephaly and further expand the clinical spectrum of holoprosencephaly. *Hum. Genet.* 127, 555–561.
- Hill, D.P., and Wurst, W. (1993). Gene and enhancer trapping: mutagenic strategies for developmental studies. *Curr. Top. Dev. Biol.* 28, 181–206.
- Hnisz, D., Abraham, B.J., Lee, T.I., Lau, A., Saint-André, V., Sigova, A.A., Hoke, H.A., and Young, R.A. (2013). Super-enhancers in the control of cell identity and disease. *Cell* 155, 934–947.
- Howard, M.A., Rubenstein, J.L.R., and Baraban, S.C. (2014). Bidirectional homeostatic plasticity induced by interneuron cell death and transplantation in vivo. *Proc. Natl. Acad. Sci. USA* 111, 492–497.
- Jafari, S., Alkhori, L., Schleiffer, A., Brochtrup, A., Hummel, T., and Alenius, M. (2012). Combinatorial activation and repression by seven transcription factors specify *Drosophila* odorant receptor expression. *PLoS Biol.* 10, e1001280.
- Jiang, Y.-H., Yuen, R.K.C., Jin, X., Wang, M., Chen, N., Wu, X., Ju, J., Mei, J., Shi, Y., He, M., et al. (2013). Detection of clinically relevant genetic variants in autism spectrum disorder by whole-genome sequencing. *Am. J. Hum. Genet.* 93, 249–263.
- Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., and Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337, 816–821.
- Kagey, M.H., Newman, J.J., Bilodeau, S., Zhan, Y., Orlando, D.A., van Berkum, N.L., Ebmeier, C.C., Goossens, J., Rahl, P.B., Levine, S.S., et al. (2010). Mediator and cohesin connect gene expression and chromatin architecture. *Nature* 467, 430–435.
- Kalinka, A.T., Varga, K.M., Gerrard, D.T., Preibisch, S., Corcoran, D.L., Jarrells, J., Ohler, U., Bergman, C.M., and Tomancak, P. (2010). Gene expression divergence recapitulates the developmental hourglass model. *Nature* 468, 811–814.
- Kammandel, B., Chowdhury, K., Stoykova, A., Aparicio, S., Brenner, S., and Gruss, P. (1999). Distinct cis-essential modules direct the time-space pattern of the Pax6 gene activity. *Dev. Biol.* 205, 79–97.
- Kang, J.H., Ahn, K.S., Heo, S.Y., Won, J.Y., and Shim, H. (2008). Gene targeting in mouse embryos mediated by RecA and modified single-stranded oligonucleotides. *In Vitro Cell. Dev. Biol. Anim.* 44, 57–62.
- Kang, H.J., Kawasawa, Y.I., Cheng, F., Zhu, Y., Xu, X., Li, M., Sousa, A.M.M., Pletikos, M., Meyer, K.A., Sedmak, G., et al. (2011). Spatio-temporal transcriptome of the human brain. *Nature* 478, 483–489.
- Khaitovich, P., Muetzel, B., She, X., Lachmann, M., Hellmann, I., Dietzsch, J., Steigele, S., Do, H.-H., Weiss, G., Enard, W., et al. (2004). Regional patterns of gene expression in human and chimpanzee brains. *Genome Res.* 14, 1462–1473.
- Kheradpour, P., Ernst, J., Melnikov, A., Rogov, P., Wang, L., Zhang, X., Alston, J., Mikkelsen, T.S., and Kellis, M. (2013). Systematic dissection of regulatory motifs in 2000 predicted human enhancers using a massively parallel reporter assay. *Genome Res.* 23, 800–811.
- Kieffer-Kwon, K.-R., Tang, Z., Mathe, E., Qian, J., Sung, M.-H., Li, G., Resch, W., Baek, S., Pruett, N., Grøntved, L., et al. (2013). Interactome maps of mouse gene regulatory domains reveal basic principles of transcriptional regulation. *Cell* 155, 1507–1520.
- King, M.C., and Wilson, A.C. (1975). Evolution at two levels in humans and chimpanzees. *Science* 188, 107–116.
- Kitamura, K., Yanazawa, M., Sugiyama, N., Miura, H., Iizuka-Kogo, A., Kusaka, M., Omichi, K., Suzuki, R., Kato-Fukui, Y., Kamiirisa, K., et al. (2002). Mutation of ARX causes abnormal development of forebrain and testes in mice and X-linked lissencephaly with abnormal genitalia in humans. *Nat. Genet.* 32, 359–369.

- Kitamura, K., Itou, Y., Yanazawa, M., Ohsawa, M., Suzuki-Migishima, R., Umeki, Y., Hohjoh, H., Yanagawa, Y., Shinba, T., Itoh, M., et al. (2009). Three human ARX mutations cause the lissencephaly-like and mental retardation with epilepsy-like pleiotropic phenotypes in mice. *Hum. Mol. Genet.* **18**, 3708–3724.
- Kohwi, M., and Doe, C.Q. (2013). Temporal fate specification and neural progenitor competence during development. *Nat. Rev. Neurosci.* **14**, 823–838.
- Konopka, G., Bomar, J.M., Winden, K., Coppola, G., Jonsson, Z.O., Gao, F., Peng, S., Preuss, T.M., Wohlschlegel, J.A., and Geschwind, D.H. (2009). Human-specific transcriptional regulation of CNS development genes by FOXP2. *Nature* **462**, 213–217.
- Kothary, R., Clapoff, S., Darling, S., Perry, M.D., Moran, L.A., and Rossant, J. (1989). Inducible expression of an hsp68-lacZ hybrid gene in transgenic mice. *Development* **105**, 707–714.
- Krystal, J.H., and State, M.W. (2014). Psychiatric disorders: diagnosis to therapy. *Cell* **157**, 201–214.
- Kulaeva, O.I., Nizovtseva, E.V., Polikanov, Y.S., Ulianov, S.V., and Studitsky, V.M. (2012). Distant activation of transcription: mechanisms of enhancer action. *Mol. Cell. Biol.* **32**, 4892–4897.
- Kurokawa, D., Kiyonari, H., Nakayama, R., Kimura-Yoshida, C., Matsuo, I., and Aizawa, S. (2004). Regulation of Otx2 expression and its functions in mouse forebrain and midbrain. *Development* **131**, 3319–3331.
- Kurokawa, D., Ohmura, T., Sakurai, Y., Inoue, K., Suda, Y., and Aizawa, S. (2014). Otx2 expression in anterior neuroectoderm and forebrain/midbrain is directed by more than six enhancers. *Dev. Biol.* **387**, 203–213.
- Lagha, M., Bothma, J.P., and Levine, M. (2012). Mechanisms of transcriptional precision in animal development. *Trends Genet.* **28**, 409–416.
- Lai, H.C., Meredith, D.M., and Johnson, J.E. (2013). bHLH factors in neurogenesis and neuronal subtype specification. In *Patterning and Cell Type Specification in the Developing CNS and PNS*, J.L.R. Rubenstein and P. Rakic, eds. (Elsevier), pp. 333–354.
- Lee, A.T., Gee, S.M., Vogt, D., Patel, T., Rubenstein, J.L., and Sohal, V.S. (2014). Pyramidal neurons in prefrontal cortex receive subtype-specific forms of excitation and inhibition. *Neuron* **81**, 61–68.
- Lenhard, B., Sandelin, A., and Carninci, P. (2012). Metazoan promoters: emerging characteristics and insights into transcriptional regulation. *Nat. Rev. Genet.* **13**, 233–245.
- Lettice, L.A., Heaney, S.J.H., Purdie, L.A., Li, L., de Beer, P., Oostra, B.A., Goode, D., Elgar, G., Hill, R.E., and de Graaff, E. (2003). A long-range Shh enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly. *Hum. Mol. Genet.* **12**, 1725–1735.
- Lettice, L.A., Daniels, S., Sweeney, E., Venkataraman, S., Devenney, P.S., Gautier, P., Morrison, H., Fantes, J., Hill, R.E., and FitzPatrick, D.R. (2011). Enhancer-adoption as a mechanism of human developmental disease. *Hum. Mutat.* **32**, 1492–1499.
- Lieberman-Aiden, E., van Berkum, N.L., Williams, L., Imakaev, M., Ragoczy, T., Telling, A., Amit, I., Lajoie, B.R., Sabo, P.J., Dorschner, M.O., et al. (2009). Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**, 289–293.
- Liodis, P., Denaxa, M., Grigoriou, M., Akufo-Addo, C., Yanagawa, Y., and Pachnis, V. (2007). Lhx6 activity is required for the normal migration and specification of cortical interneuron subtypes. *J. Neurosci.* **27**, 3078–3089.
- Lister, R., Mukamel, E.A., Nery, J.R., Urich, M., Puddifoot, C.A., Johnson, N.D., Lucero, J., Huang, Y., Dwork, A.J., Schultz, M.D., et al. (2013). Global epigenomic reconfiguration during mammalian brain development. *Science* **341**, 1237905–1237905.
- Long, J.E., Garel, S., Depew, M.J., Tobet, S., and Rubenstein, J.L.R. (2003). DLX5 regulates development of peripheral and central components of the olfactory system. *J. Neurosci.* **23**, 568–578.
- Long, J.E., Garel, S., Alvarez-Dolado, M., Yoshikawa, K., Osumi, N., Alvarez-Buylla, A., and Rubenstein, J.L.R. (2007). Dlx-dependent and -independent regulation of olfactory bulb interneuron differentiation. *J. Neurosci.* **27**, 3230–3243.
- Long, J.E., Cobos, I., Potter, G.B., and Rubenstein, J.L.R. (2009a). Dlx1&2 and Mash1 transcription factors control MGE and CGE patterning and differentiation through parallel and overlapping pathways. *Cereb. Cortex* **19** (Suppl 1), i96–i106.
- Long, J.E., Swan, C., Liang, W.S., Cobos, I., Potter, G.B., and Rubenstein, J.L.R. (2009b). Dlx1&2 and Mash1 transcription factors control striatal patterning and differentiation through parallel and overlapping pathways. *J. Comp. Neurol.* **512**, 556–572.
- MacDonald, J.L., Fame, R.M., Azim, E., Shnyder, S.J., Molyneaux, B.J., Arlotta, P., and Macklis, J.D. (2013). Specification of cortical projection neurons. In *Patterning and Cell Type Specification in the Developing CNS and PNS*, J.L.R. Rubenstein and P. Rakic, eds. (Elsevier), pp. 475–502.
- Machon, O., van den Bout, C.J., Backman, M., Røskov, Ø., Caubit, X., Fromm, S.H., Geronimo, B., and Krauss, S. (2002). Forebrain-specific promoter/enhancer D6 derived from the mouse Dach1 gene controls expression in neural stem cells. *Neuroscience* **112**, 951–966.
- Mali, P., Yang, L., Esvelt, K.M., Aach, J., Guell, M., DiCarlo, J.E., Norville, J.E., and Church, G.M. (2013). RNA-guided human genome engineering via Cas9. *Science* **339**, 823–826.
- Mao, R., Page, D.T., Merzlyak, I., Kim, C., Tecott, L.H., Janak, P.H., Rubenstein, J.L.R., and Sur, M. (2009). Reduced conditioned fear response in mice that lack Dlx1 and show subtype-specific loss of interneurons. *J. Neurodev. Disord.* **1**, 224–236.
- Mariani, J., Favaro, R., Lancini, C., Vaccari, G., Ferri, A.L., Bertolini, J., Tonoli, D., Latorre, E., Caccia, R., Ronchi, A., et al. (2012). Emx2 is a dose-dependent negative regulator of Sox2 telencephalic enhancers. *Nucleic Acids Res.* **40**, 6461–6476.
- Marsh, E., Fulp, C., Gomez, E., Nasrallah, I., Minarcik, J., Sudi, J., Christian, S.L., Mancini, G., Labosky, P., Dobyns, W., et al. (2009). Targeted loss of Arx results in a developmental epilepsy mouse model and recapitulates the human phenotype in heterozygous females. *Brain* **132**, 1563–1576.
- McCarroll, S.A., and Hyman, S.E. (2013). Progress in the genetics of polygenic brain disorders: significant new challenges for neurobiology. *Neuron* **80**, 578–587.
- McClellan, J., and King, M.-C. (2010). Genetic heterogeneity in human disease. *Cell* **141**, 210–217.
- McKinsey, G.L., Lindtner, S., Trzcinski, B., Visel, A., Pennacchio, L.A., Huylebroeck, D., Higashi, Y., and Rubenstein, J.L.R. (2013). Dlx1&2-dependent expression of Zfhx1b (Sip1, Zeb2) regulates the fate switch between cortical and striatal interneurons. *Neuron* **77**, 83–98.
- McLean, C.Y., Reno, P.L., Pollen, A.A., Bassan, A.I., Capellini, T.D., Guenther, C., Indjeian, V.B., Lim, X., Menke, D.B., Schaar, B.T., et al. (2011). Human-specific loss of regulatory DNA and the evolution of human-specific traits. *Nature* **471**, 216–219.
- Mendenhall, E.M., Williamson, K.E., Reyon, D., Zou, J.Y., Ram, O., Jung, J.K., and Bernstein, B.E. (2013). Locus-specific editing of histone modifications at endogenous enhancers. *Nat. Biotechnol.* **31**, 1133–1136.
- Miller, J.A., Ding, S.-L., Sunkin, S.M., Smith, K.A., Ng, L., Szafer, A., Ebbert, A., Riley, Z.L., Royall, J.J., Aiona, K., et al. (2014). Transcriptional landscape of the prenatal human brain. *Nature* **508**, 199–206.
- Molyneaux, B.J., Arlotta, P., Menezes, J.R.L., and Macklis, J.D. (2007). Neuronal subtype specification in the cerebral cortex. *Nat. Rev. Neurosci.* **8**, 427–437.
- Montavon, T., Soshnikova, N., Mascrez, B., Joye, E., Thevenet, L., Splinter, E., de Laat, W., Spitz, F., and Duboule, D. (2011). A regulatory archipelago controls Hox genes transcription in digits. *Cell* **147**, 1132–1145.
- Murtha, M., Tokcaer-Keskin, Z., Tang, Z., Strino, F., Chen, X., Wang, Y., Xi, X., Basilio, C., Brown, S., Bonneau, R., et al. (2014). FIREWACH: high-throughput functional detection of transcriptional regulatory modules in mammalian cells. *Nat. Methods* **11**, 559–565.
- Neph, S., Stergachis, A.B., Reynolds, A., Sandstrom, R., Borenstein, E., and Stamatoyanopoulos, J.A. (2012). Circuitry and dynamics of human transcription factor regulatory networks. *Cell* **150**, 1274–1286.

- Nobrega, M.A., Ovcharenko, I., Afzal, V., and Rubin, E.M. (2003). Scanning human gene deserts for long-range enhancers. *Science* *302*, 413–413.
- Nóbrega-Pereira, S., Kessar, N., Du, T., Kimura, S., Anderson, S.A., and Marin, O. (2008). Postmitotic Nkx2-1 controls the migration of telencephalic interneurons by direct repression of guidance receptors. *Neuron* *59*, 733–745.
- Nord, A.S., Blow, M.J., Attanasio, C., Akiyama, J.A., Holt, A., Hosseini, R., Phouanenavong, S., Plajzer-Frick, I., Shoukry, M., Afzal, V., et al. (2013). Rapid and pervasive changes in genome-wide enhancer usage during mammalian development. *Cell* *155*, 1521–1531.
- Nowick, K., Gernat, T., Almaas, E., and Stubbs, L. (2009). Differences in human and chimpanzee gene expression patterns define an evolving network of transcription factors in brain. *Proc. Natl. Acad. Sci. USA* *106*, 22358–22363.
- O’Leary, D.D.M., Stocker, A.M., and Zembrzycki, A. (2013). Area patterning of the mammalian cortex. In *Patterning and Cell Type Specification in the Developing CNS and PNS*, J.L.R. Rubenstein and P. Rakic, eds. (Elsevier), pp. 61–85.
- O’Roak, B.J., Deriziotis, P., Lee, C., Vives, L., Schwartz, J.J., Girirajan, S., Karakoc, E., Mackenzie, A.P., Ng, S.B., Baker, C., et al. (2011). Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nat. Genet.* *43*, 585–589.
- O’Roak, B.J., Vives, L., Fu, W., Egerton, J.D., Stanaway, I.B., Phelps, I.G., Carvill, G., Kumar, A., Lee, C., Ankenman, K., et al. (2012a). Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science* *338*, 1619–1622.
- O’Roak, B.J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B.P., Levy, R., Ko, A., Lee, C., Smith, J.D., et al. (2012b). Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature* *485*, 246–250.
- Odom, D.T., Dowell, R.D., Jacobsen, E.S., Gordon, W., Danford, T.W., MacIsaac, K.D., Rolfe, P.A., Conboy, C.M., Gifford, D.K., and Fraenkel, E. (2007). Tissue-specific transcriptional regulation has diverged significantly between human and mouse. *Nat. Genet.* *39*, 730–732.
- Oh, S.W., Harris, J.A., Ng, L., Winslow, B., Cain, N., Mihalas, S., Wang, Q., Lau, C., Kuan, L., Henry, A.M., et al. (2014). A mesoscale connectome of the mouse brain. *Nature* *508*, 207–214.
- Oksenberg, N., Stevison, L., Wall, J.D., and Ahituv, N. (2013). Function and regulation of AUTS2, a gene implicated in autism and human evolution. *PLoS Genet.* *9*, e1003221.
- Olivetti, P.R., and Noebels, J.L. (2012). Interneuron, interrupted: molecular pathogenesis of ARX mutations and X-linked infantile spasms. *Curr. Opin. Neurobiol.* *22*, 859–865.
- Ovcharenko, I., Nobrega, M.A., Loots, G.G., and Stubbs, L. (2004). ECR Browser: a tool for visualizing and accessing data from comparisons of multiple vertebrate genomes. *Nucleic Acids Res.* *32*, W280–6.
- Parikhshak, N.N., Luo, R., Zhang, A., Won, H., Lowe, J.K., Chandran, V., Horvath, S., and Geschwind, D.H. (2013). Integrative functional genomic analyses implicate specific molecular pathways and circuits in autism. *Cell* *155*, 1008–1021.
- Parker, S.C.J., Stitzel, M.L., Taylor, D.L., Orozco, J.M., Erdos, M.R., Akiyama, J.A., van Bueren, K.L., Chines, P.S., Narisu, N., Black, B.L., et al.; NISC Comparative Sequencing Program; National Institutes of Health Intramural Sequencing Center Comparative Sequencing Program Authors; NISC Comparative Sequencing Program Authors (2013). Chromatin stretch enhancer states drive cell-specific gene regulation and harbor human disease risk variants. *Proc. Natl. Acad. Sci. USA* *110*, 17921–17926.
- Pattabiraman, K., Golonzka, O., Lindtner, S., Nord, A.S., Taher, L., Hoch, R., Silberberg, S.N., Zhang, D., Chen, B., Zeng, H., et al. (2014). Transcriptional regulation of enhancers active in protodomains of the developing cerebral cortex. *Neuron* *82*, 989–1003.
- Patwardhan, R.P., Hiatt, J.B., Witten, D.M., Kim, M.J., Smith, R.P., May, D., Lee, C., Andrie, J.M., Lee, S.-I., Cooper, G.M., et al. (2012). Massively parallel functional dissection of mammalian enhancers in vivo. *Nat. Biotechnol.* *30*, 265–270.
- Pennacchio, L.A., Ahituv, N., Moses, A.M., Prabhakar, S., Nobrega, M.A., Shoukry, M., Minovitsky, S., Dubchak, I., Holt, A., Lewis, K.D., et al. (2006). In vivo enhancer analysis of human conserved non-coding sequences. *Nature* *444*, 499–502.
- Petryniak, M.A., Potter, G.B., Rowitch, D.H., and Rubenstein, J.L.R. (2007). Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. *Neuron* *55*, 417–433.
- Pletikos, M., Sousa, A.M.M., Sedmak, G., Meyer, K.A., Zhu, Y., Cheng, F., Li, M., Kawasawa, Y.I., and Sestan, N. (2014). Temporal specification and bilaterality of human neocortical topographic gene expression. *Neuron* *81*, 321–332.
- Poitras, L., Yu, M., Lesage-Pelletier, C., Macdonald, R.B., Gagné, J.-P., Hatch, G., Kelly, I., Hamilton, S.P., Rubenstein, J.L.R., Poirier, G.G., and Ekker, M. (2010). An SNP in an ultraconserved regulatory element affects Dlx5/Dlx6 regulation in the forebrain. *Development* *137*, 3089–3097.
- Pollard, K.S., Salama, S.R., King, B., Kern, A.D., Dreszer, T., Katzman, S., Siepel, A., Pedersen, J.S., Bejerano, G., Baertsch, R., et al. (2006). Forces shaping the fastest evolving regions in the human genome. *PLoS Genet.* *2*, e168.
- Potter, G.B., Petryniak, M.A., Shevchenko, E., McKinsey, G.L., Ekker, M., and Rubenstein, J.L.R. (2009). Generation of Cre-transgenic mice using Dlx1/Dlx2 enhancers and their characterization in GABAergic interneurons. *Mol. Cell. Neurosci.* *40*, 167–186.
- Prabhakar, S., Noonan, J.P., Pääbo, S., and Rubin, E.M. (2006a). Accelerated evolution of conserved noncoding sequences in humans. *Science* *314*, 786–786.
- Prabhakar, S., Poulin, F., Shoukry, M., Afzal, V., Rubin, E.M., Couronne, O., and Pennacchio, L.A. (2006b). Close sequence comparisons are sufficient to identify human *cis*-regulatory elements. *Genome Res.* *16*, 855–863.
- Prabhakar, S., Visel, A., Akiyama, J.A., Shoukry, M., Lewis, K.D., Holt, A., Plajzer-Frick, I., Morrison, H., Fitzpatrick, D.R., Afzal, V., et al. (2008). Human-specific gain of function in a developmental enhancer. *Science* *321*, 1346–1350.
- Qiu, M., Bulfone, A., Martinez, S., Meneses, J.J., Shimamura, K., Pedersen, R.A., and Rubenstein, J.L. (1995). Null mutation of Dlx-2 results in abnormal morphogenesis of proximal first and second branchial arch derivatives and abnormal differentiation in the forebrain. *Genes Dev.* *9*, 2523–2538.
- Quint, M., Drost, H.-G., Gabel, A., Ullrich, K.K., Bönn, M., and Grosse, I. (2012). A transcriptomic hourglass in plant embryogenesis. *Nature* *490*, 98–101.
- Rada-Iglesias, A., Bajpai, R., Swigut, T., Brugmann, S.A., Flynn, R.A., and Wysocka, J. (2011). A unique chromatin signature uncovers early developmental enhancers in humans. *Nature* *470*, 279–283.
- Rakic, P. (1988). Specification of cerebral cortical areas. *Science* *241*, 170–176.
- Ravasi, T., Suzuki, H., Cannistraci, C.V., Katayama, S., Bajic, V.B., Tan, K., Akalin, A., Schmeier, S., Kanamori-Katayama, M., Bertin, N., et al. (2010). An atlas of combinatorial transcriptional regulation in mouse and man. *Cell* *140*, 744–752.
- Ripke, S., O’Dushlaine, C., Chambert, K., Moran, J.L., Kähler, A.K., Akterin, S., Bergen, S.E., Collins, A.L., Crowley, J.J., Fromer, M., et al.; Multicenter Genetic Studies of Schizophrenia Consortium; Psychosis Endophenotypes International Consortium; Wellcome Trust Case Control Consortium 2 (2013). Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nat. Genet.* *45*, 1150–1159.
- Ronan, J.L., Wu, W., and Crabtree, G.R. (2013). From neural development to cognition: unexpected roles for chromatin. *Nat. Rev. Genet.* *14*, 347–359.
- Royo, J.L., Bessa, J., Hidalgo, C., Fernández-Miñán, A., Tena, J.J., Roncero, Y., Gómez-Skarmeta, J.L., and Casares, F. (2012). Identification and analysis of conserved *cis*-regulatory regions of the MEIS1 gene. *PLoS ONE* *7*, e33617.
- Rubenstein, J.L.R., and Campbell, K. (2013). Neurogenesis in the Basal Ganglia. (Elsevier).
- Rubenstein, J.L.R., and Rakic, P. (2013). Patterning and Cell Type Specification in the Developing CNS and PNS. (Elsevier).

- Schmidt, D., Wilson, M.D., Ballester, B., Schwale, P.C., Brown, G.D., Marshall, A., Kutter, C., Watt, S., Martinez-Jimenez, C.P., Mackay, S., et al. (2010). Five-vertebrate ChIP-seq reveals the evolutionary dynamics of transcription factor binding. *Science* **328**, 1036–1040.
- Schwartz, R.J., and Olson, E.N. (1999). Building the heart piece by piece: modularity of *cis*-elements regulating *Nkx2-5* transcription. *Development* **126**, 4187–4192.
- Schwartz, S., Zhang, Z., Frazer, K.A., Smit, A., Riemer, C., Bouck, J., Gibbs, R., Hardison, R., and Miller, W. (2000). PipMaker—a web server for aligning two genomic DNA sequences. *Genome Res.* **10**, 577–586.
- Seybold, B.A., Stanco, A., Cho, K.K.A., Potter, G.B., Kim, C., Sohal, V.S., Rubenstein, J.L.R., and Schreiner, C.E. (2012). Chronic reduction in inhibition reduces receptive field size in mouse auditory cortex. *Proc. Natl. Acad. Sci. USA* **109**, 13829–13834.
- Shen, Y., Yue, F., McCleary, D.F., Ye, Z., Edsall, L., Kuan, S., Wagner, U., Dixon, J., Lee, L., Lobanenkov, V.V., and Ren, B. (2012). A map of the *cis*-regulatory sequences in the mouse genome. *Nature* **488**, 116–120.
- Shim, S., Kwan, K.Y., Li, M., Lefebvre, V., and Sestan, N. (2012). *cis*-regulatory control of corticospinal system development and evolution. *Nature* **486**, 74–79.
- Shlyueva, D., Stampfel, G., and Stark, A. (2014). Transcriptional enhancers: from properties to genome-wide predictions. *Nat. Rev. Genet.* **15**, 272–286.
- Siepel, A., Bejerano, G., Pedersen, J.S., Hinrichs, A.S., Hou, M., Rosenbloom, K., Clawson, H., Spieth, J., Hillier, L.W., Richards, S., et al. (2005). Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res.* **15**, 1034–1050.
- Silbereis, J.C., Nobuta, H., Tsai, H.-H., Heine, V.M., McKinsey, G.L., Meijer, D.H., Howard, M.A., Petryniak, M.A., Potter, G.B., Alberta, J.A., et al. (2014). *Olig1* function is required to repress *dlx1/2* and interneuron production in Mammalian brain. *Neuron* **81**, 574–587.
- Smith, R.P., Taher, L., Patwardhan, R.P., Kim, M.J., Inoue, F., Shendure, J., Ovcharenko, I., and Ahituv, N. (2013). Massively parallel decoding of mammalian regulatory sequences supports a flexible organizational model. *Nat. Genet.* **45**, 1021–1028.
- Stamatoyannopoulos, G. (2005). Control of globin gene expression during development and erythroid differentiation. *Exp. Hematol.* **33**, 259–271.
- Stamatoyannopoulos, J.A., Snyder, M., Hardison, R., Ren, B., Gingeras, T., Gilbert, D.M., Groudine, M., Bender, M., Kaul, R., Canfield, T., et al.; Mouse ENCODE Consortium (2012). An encyclopedia of mouse DNA elements (Mouse ENCODE). *Genome Biol.* **13**, 418.
- Stanco, A., Pla, R., Vogt, D., Chen, Y., Mandal, S., Walker, J., Hunt, R.F., Lindtner, S., Erdman, C., Pieper, A.A., et al. (2014). NPAS1 represses the generation of specific subtypes of cortical interneurons. *Neuron* **84**, 940–943.
- Stefflova, K., Thybert, D., Wilson, M.D., Streeter, I., Aleksic, J., Karagianni, P., Brazma, A., Adams, D.J., Talianidis, I., Marioni, J.C., et al. (2013). Cooperativity and rapid evolution of cobound transcription factors in closely related mammals. *Cell* **154**, 530–540.
- Stergachis, A.B., Neph, S., Reynolds, A., Humbert, R., Miller, B., Paige, S.L., Vernot, B., Cheng, J.B., Thurman, R.E., Sandstrom, R., et al. (2013). Developmental fate and cellular maturity encoded in human regulatory DNA landscapes. *Cell* **154**, 888–903.
- Suda, Y., Kokura, K., Kimura, J., Kajikawa, E., Inoue, F., and Aizawa, S. (2010). The same enhancer regulates the earliest *Emx2* expression in caudal forebrain primordium, subsequent expression in dorsal telencephalon and later expression in the cortical ventricular zone. *Development* **137**, 2939–2949.
- Sussel, L., Marin, O., Kimura, S., and Rubenstein, J.L. (1999). Loss of *Nkx2.1* homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: evidence for a transformation of the pallidum into the striatum. *Development* **126**, 3359–3370.
- Teng, L., He, B., Gao, P., Gao, L., and Tan, K. (2014). Discover context-specific combinatorial transcription factor interactions by integrating diverse ChIP-Seq data sets. *Nucleic Acids Res.* **42**, e24–e24.
- Theil, T., Aydin, S., Koch, S., Grotewold, L., and Rütger, U. (2002). Wnt and Bmp signalling cooperatively regulate graded *Emx2* expression in the dorsal telencephalon. *Development* **129**, 3045–3054.
- Thompson, C.L., Ng, L., Menon, V., Martinez, S., Lee, C.-K., Glattfelder, K., Sunkin, S.M., Henry, A., Lau, C., Dang, C., et al. (2014). A high-resolution spatiotemporal atlas of gene expression of the developing mouse brain. *Neuron* **83**, 309–323.
- Vacic, V., McCarthy, S., Malhotra, D., Murray, F., Chou, H.-H., Peoples, A., Makarov, V., Yoon, S., Bhandari, A., Corominas, R., et al. (2011). Duplications of the neuropeptide receptor gene *VIPR2* confer significant risk for schizophrenia. *Nature* **471**, 499–503.
- van den Berghe, V., Stappers, E., Vandesande, B., Dimidschstein, J., Kroes, R., Francis, A., Conidi, A., Lesage, F., Dries, R., Cazzola, S., et al. (2013). Directed migration of cortical interneurons depends on the cell-autonomous action of *Sip1*. *Neuron* **77**, 70–82.
- van Heyningen, V., and Williamson, K.A. (2002). *PAX6* in sensory development. *Hum. Mol. Genet.* **11**, 1161–1167.
- Verkerk, A.J., Pieretti, M., Sutcliffe, J.S., Fu, Y.H., Kuhl, D.P., Pizzuti, A., Reiner, O., Richards, S., Victoria, M.F., Zhang, F.P., et al. (1991). Identification of a gene (*FMR-1*) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* **65**, 905–914.
- Villar, D., Flicek, P., and Odom, D.T. (2014). Evolution of transcription factor binding in metazoans - mechanisms and functional implications. *Nat. Rev. Genet.* **15**, 221–233.
- Visel, A., Minovitsky, S., Dubchak, I., and Pennacchio, L.A. (2007). VISTA Enhancer Browser—a database of tissue-specific human enhancers. *Nucleic Acids Res.* **35**, D88–D92.
- Visel, A., Akiyama, J.A., Shoukry, M., Afzal, V., Rubin, E.M., and Pennacchio, L.A. (2009a). Functional autonomy of distant-acting human enhancers. *Genomics* **93**, 509–513.
- Visel, A., Blow, M.J., Li, Z., Zhang, T., Akiyama, J.A., Holt, A., Plajzer-Frick, I., Shoukry, M., Wright, C., Chen, F., et al. (2009b). ChIP-seq accurately predicts tissue-specific activity of enhancers. *Nature* **457**, 854–858.
- Visel, A., Taher, L., Girgis, H., May, D., Golonzka, O., Hoch, R.V., McKinsey, G.L., Pattabiraman, K., Silberberg, S.N., Blow, M.J., et al. (2013). A high-resolution enhancer atlas of the developing telencephalon. *Cell* **152**, 895–908.
- Vogt, D., Hunt, R.F., Mandal, S., Sandberg, M., Silberberg, S.N., Nagasawa, T., Yang, Z., Baraban, S.C., and Rubenstein, J.L.R. (2014). *Lhx6* directly regulates *Arx* and *CXCR7* to determine cortical interneuron fate and laminar position. *Neuron* **82**, 350–364.
- Voineagu, I., Wang, X., Johnston, P., Lowe, J.K., Tian, Y., Horvath, S., Mill, J., Cantor, R.M., Blencowe, B.J., and Geschwind, D.H. (2011). Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* **474**, 380–384.
- Waclaw, R.R., Allen, Z.J., 2nd, Bell, S.M., Erdélyi, F., Szabó, G., Potter, S.S., and Campbell, K. (2006). The zinc finger transcription factor *Sp8* regulates the generation and diversity of olfactory bulb interneurons. *Neuron* **49**, 503–516.
- Waclaw, R.R., Ehrman, L.A., Pierani, A., and Campbell, K. (2010). Developmental origin of the neuronal subtypes that comprise the amygdalar fear circuit in the mouse. *J. Neurosci.* **30**, 6944–6953.
- Wang, Y., Dye, C.A., Sohal, V., Long, J.E., Estrada, R.C., Roztocil, T., Lufkin, T., Deisseroth, K., Baraban, S.C., and Rubenstein, J.L.R. (2010). *Dlx5* and *Dlx6* regulate the development of parvalbumin-expressing cortical interneurons. *J. Neurosci.* **30**, 5334–5345.
- Wang, B., Lufkin, T., and Rubenstein, J.L.R. (2011). *Dlx6* regulates molecular properties of the striatum and central nucleus of the amygdala. *J. Comp. Neurol.* **519**, 2320–2334.
- Wang, B., Long, J.E., Flandin, P., Pla, R., Waclaw, R.R., Campbell, K., and Rubenstein, J.L.R. (2013). Loss of *Gsx1* and *Gsx2* function rescues distinct phenotypes in *Dlx1/2* mutants. *J. Comp. Neurol.* **521**, 1561–1584.
- White, M.A., Myers, C.A., Corbo, J.C., and Cohen, B.A. (2013). Massively parallel *in vivo* enhancer assay reveals that highly local features determine

the *cis*-regulatory function of ChIP-seq peaks. *Proc. Natl. Acad. Sci. USA* **110**, 11952–11957.

Whyte, W.A., Orlando, D.A., Hnisz, D., Abraham, B.J., Lin, C.Y., Kagey, M.H., Rahl, P.B., Lee, T.I., and Young, R.A. (2013). Master transcription factors and mediator establish super-enhancers at key cell identity genes. *Cell* **153**, 307–319.

Willsey, A.J., Sanders, S.J., Li, M., Dong, S., Tebbenkamp, A.T., Muhle, R.A., Reilly, S.K., Lin, L., Fertuzinhos, S., Miller, J.A., et al. (2013). Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism. *Cell* **155**, 997–1007.

Yun, K., Fischman, S., Johnson, J., Hrabě de Angelis, M., Weinmaster, G., and Rubenstein, J.L.R. (2002). Modulation of the notch signaling by *Mash1* and *Dlx1/2* regulates sequential specification and differentiation of progenitor cell types in the subcortical telencephalon. *Development* **129**, 5029–5040.

Zerucha, T., Stühmer, T., Hatch, G., Park, B.K., Long, Q., Yu, G., Gambarotta, A., Schultz, J.R., Rubenstein, J.L., and Ekker, M. (2000). A highly conserved enhancer in the *Dlx5/Dlx6* intergenic region is the site of cross-regulatory interactions between *Dlx* genes in the embryonic forebrain. *J. Neurosci.* **20**, 709–721.

Zhang, Y., Wong, C.-H., Birnbaum, R.Y., Li, G., Favaro, R., Ngan, C.Y., Lim, J., Tai, E., Poh, H.M., Wong, E., et al. (2013). Chromatin connectivity maps reveal dynamic promoter-enhancer long-range associations. *Nature* **504**, 306–310.

Zhao, Y., Marín, O., Hermes, E., Powell, A., Flames, N., Palkovits, M., Rubenstein, J.L.R., and Westphal, H. (2003). The LIM-homeobox gene *Lhx8* is required for the development of many cholinergic neurons in the mouse forebrain. *Proc. Natl. Acad. Sci. USA* **100**, 9005–9010.

Zhao, Y., Flandin, P., Long, J.E., Cuesta, M.D., Westphal, H., and Rubenstein, J.L.R. (2008). Distinct molecular pathways for development of telencephalic interneuron subtypes revealed through analysis of *Lhx6* mutants. *J. Comp. Neurol.* **510**, 79–99.

Zhao, Y., Flandin, P., Vogt, D., Blood, A., Hermes, E., Westphal, H., and Rubenstein, J.L.R. (2014). *Ldb1* is essential for development of *Nkx2.1* lineage derived GABAergic and cholinergic neurons in the telencephalon. *Dev. Biol.* **385**, 94–106.

Zhou, Q.-P., Le, T.N., Qiu, X., Spencer, V., de Melo, J., Du, G., Plews, M., Fonseca, M., Sun, J.M., Davie, J.R., and Eisenstat, D.D. (2004). Identification of a direct *Dlx* homeodomain target in the developing mouse forebrain and retina by optimization of chromatin immunoprecipitation. *Nucleic Acids Res.* **32**, 884–892.

Zhu, J., Adli, M., Zou, J.Y., Verstappen, G., Coyne, M., Zhang, X., Durham, T., Miri, M., Deshpande, V., De Jager, P.L., et al. (2013). Genome-wide chromatin state transitions associated with developmental and environmental cues. *Cell* **152**, 642–654.