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Transcriptional Regulation of Enhancers Active in Protodomains of the Developing Cerebral Cortex

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SUMMARY

Elucidating the genetic control of cerebral cortical (pallial) development is essential for understanding function, evolution, and disorders of the brain. Transcription factors (TFs) that embryonically regulate pallial regionalization are expressed in gradients, raising the question of how discrete domains are generated. We provide evidence that small enhancer elements active in protodomains integrate broad transcriptional information. CreER^{T2} and GFP expression from 14 different enhancer elements in stable transgenic mice allowed us to define a comprehensive regional fate map of the pallium. We explored transcriptional mechanisms that control the activity of the enhancers using informatics, in vivo occupancy by TFs that regulate cortical patterning (*CoupTFI, Pax6*, and *Pbx1*), and analysis of enhancer activity in *Pax6* mutants. Overall, the results provide insights into how broadly expressed patterning TFs regulate the activity of small enhancer elements that drive gene expression in pallial protodomains that fate map to distinct cortical regions.

INTRODUCTION

At the core of cortical development lie transcriptional programs that orchestrate a sequence of processes beginning with specification of the cortical anlage and its regional subdivisions, or the protomap (Rakic, 2009; O'Leary et al., 2013). Ongoing work has identified a set of transcription factors (TFs) that control the size and areal identities of pallial subdivisions. These include *CoupTFI*, *Dmrta2 (Dmrt5)*, *Emx2*, *Lef1*, *Lhx2*, *Pax6*, and *Sp8* (Bishop et al., 2000; Galceran et al., 2000; Yun et al., 2001; Mallamaci and Stoykova, 2006; Armentano et al., 2007; Sahara et al., 2007; Faedo et al., 2008; Mangale et al., 2008; Chou et al., 2009; Konno et al., 2012; Borello et al., 2013; Saulnier et al., 2013). Each of these TFs is expressed in distinct gradients in progenitor cells of the pallial ventricular zone (VZ). For instance, *Pax6* is expressed in rostrocaudal and ventrodorsal gradients; *Pax6* loss of function in mice results in a respecification of cortical regions along both its rostrocaudal and ventrodorsal axes (Bishop et al., 2000; Yun et al., 2001). Despite the subdivision of the pallium into discrete structural/molecular units (e.g., the medial, dorsal, lateral, and ventral pallium [MP, DP, LP, and VP]; Puelles et al., 2000), to date the TFs that are known to control regional fate are expressed in gradients across these subdivisions, raising the intriguing question of how these gradients are interpreted in an integrative fashion to generate sharply delineated pallial subdivisions and later adult cortical regions.

One mechanism that could solve this conundrum would be that enhancer elements integrate TF expression to generate gene activation in distinct pallial subdivisions, much in the way that regional fate is generated in the cellular blastoderm of *Drosophila* embryos (Lagha et al., 2012). While this general paradigm had previously been supported through anecdotal reports of individual pallial enhancers identified in gene-centric studies (Kammandel et al., 1999; Theil et al., 2002; van den Bout et al., 2002; Ahituv et al., 2007; Colasante et al., 2008), a recent more comprehensive screen for forebrain enhancers that includes spatial activity data for ~145 human enhancers that are active in the embryonic day (E) 11.5 mouse telencephalon enables a rigorous and systematic search for enhancers involved in

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prepatterning of the pallium (Visel et al., 2013). Here we present evidence that enhancers integrate information from TF gradients in the embryonic day E11.5 mouse pallium to generate distinct expression domains. Using a panel of 14 human enhancers carefully selected based on their in vivo activity patterns, we generated a set of stable mouse transgenic lines that express $CreER^{T2}$ and GFP in distinct domains within the developing pallium. Leveraging this unique set of reporter mice, we derived fate maps that elucidate the embryonic origin of pallial subdivisions. Furthermore, we used a combination of bioinformatics, chromatin immunoprecipitation sequencing (ChIP-seq), and in vivo studies to elucidate the regulation of these enhancers by major pallial transcription factors including COUPTFI, PAX6, and PBX1. Overall, we propose that the enhancers defined through this study identify protodomains of the pallial neuroepithelium, which may be fundamental units of cortical development and evolution.

RESULTS

Pallial Protodomains Identified by Enhancer Activity Using Transient Transgenic Assay

To define enhancers potentially marking neuroepithelial subdivisions in the E11.5 pallium, we mined a previously described large collection of enhancers active in the developing telencephalon, assayed using transient transgenic mouse *LacZ* expression (Visel et al., 2013). We identified more than 40 enhancers that showed regional pallial expression, many of which showed intrapallial boundaries (Figures 1A–1C and Figure S1 available online). For instance, in the MP, several enhancer lines showed nested patterns of expression, varying between a small dorsocaudal domain (643), a domain in the ventral caudomedial telencephalon (653), a larger domain that includes the entire caudomedial telencephalon (192), and the entire dorsomedial and caudomedial region including the primordial septum (348) (Figure 1C). Regional patterns of activity were also observed for enhancers expressed in the DP, LP, and VP (Figures 1A and 1B). We mapped these expression limits onto a model schema of the E11.5 pallial neuroepithelium, from which we hypothesize the existence of a set of sharply delimited pallial progenitor domains or protodomains (A-I) (Figure 1D; Table S1).

Enhancer Activity of Pallial Enhancer $CreER^{T2}$-IRES-GFP Alleles

To test the idea that these human enhancers are active in protodomains that generate distinct pallial subdivisions, we produced stable transgenic mouse lines to characterize the properties of 14 enhancers that reproducibly exhibited boundaries in the E11.5 pallium (Figures 1A–1C and Figure S1; asterisks label the enhancers used to make stable lines).

We generated stable transgenic mouse lines that express $CreER^{T2}$-IRES-GFP and downstream of each one of the 14 selected “pallial” enhancers and a minimal Hsp68 promoter. We generated two to three founders for 10/14 of the lines; their expression domains were reproducible (Table S2). We further analyzed the properties of one founder for each enhancer.

To characterize the activity of each enhancer, we defined the GFP expression at E11.5 and compared the enhancer activity in the stable and transient transgenic assays. The stable lines showed enhancer activity patterns that closely resembled the transient transgenic assay (Table S2). We annotated the E11.5 expression domains on a flattened topologic representation of the embryonic pallium (right hemisphere), where stippled gray color indicates GFP expression (Figures 2I and 2I′ and Figures S2A–S2N). For instance, for enhancer 643, we observed progenitor GFP expression in the MP at E11.5 (Figures 2A–2H). On the other hand, enhancer 1,050 showed progenitor GFP expression in the DP and MP at E11.5 but was absent in the ventrolateral pallium (VLP) (Figures 2A′–2H′).

Next, we examined prenatal GFP expression at E12.5, E14.5, and E17.5 for all of the lines (Figures S2A–S2N and Table S2). In most cases, enhancer activity was strongest at E11.5 and was largely unchanged at E12.5 (Table S2). However, activity patterns of some of the enhancers were more dynamic. For instance, 636 was selectively active in the VLP at E10.5, but by E11.5, its activity was greatly reduced (Figure S2E). Activity of 12/14 enhancers decreased and/or became restricted to a smaller domain by E14.5 and E17.5 (Table S2). For instance, 218, 281, 653, and 1,318 activity was no longer detected in the pallium by E14.5. Three of the enhancers with MP expression (348, 643, and 1,006) were no longer active in the hippocampus but maintained activity in the hippocampal fissure, choroid plexus, and fimbrial area. The activity of 636, 840, and 1,172 became restricted to small populations of cells in the pallium at E17.5 (Figures S2E, S2I, and S2M). Enhancer 660, which was active in the caudoventral MP at E11.5, became active in the SVZ and superficial cortical layers of the DP at E17.5 (Figure S2H).

Fate Mapping Using Pallial Enhancer $CreER^{T2}$-IRES-GFP Alleles

To determine the identity of the cells whose progenitors have E11.5 enhancer activity, we performed fate map analyses by introducing the *Ai14 (tdTomato)* Cre reporter allele (Madisen et al., 2010) into the enhancer $CreER^{T2}$-IRES-GFP lines. We administered tamoxifen at E10.5 to induce $CreER^{T2}$ translocation to the nucleus, where it activated tdTomato expression and then performed neuroanatomical analyses at later stages. Because of the ~24–36 hr window of tamoxifen action (Hayashi and McMahon, 2002), we assessed enhancer activity at both E11.5 and E12.5 to better interpret the results of E10.5 tamoxifen treatment (Figure S2 and Table S2). Since prenatal tamoxifen treatment frequently led to fetal death around the time of delivery, we obtained fate-mapping data at E17.5 for all enhancer lines. However, we also obtained postnatal fate maps (P30) for a subset of the enhancers (192, 348, 636, 643, 653, and 660; Figure S2 and Table S3). We chose these enhancers because of their activity in the hippocampus; the hippocampus matures later than the neocortex; thus, P30 data helped analysis of the hippocampal fate map.

We annotated the fate map domains on a flattened topological representation of the maturing/mature pallium (Figures 2S and 2S′ and Figure S2). Here we indicated anatomical locations containing $tdTomato^{+}$ cells using a graded rating scale of 1–4: 1 (red) high density to 4 (green) almost no $tdTomato^{+}$ cells (Figures 2S and 2S′). For instance, 643, which showed E11.5 activity restricted to the MP, fate mapped to the rostrodorsal CA fields,

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