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Dlx1&2 and *Mash1* Transcription Factors Control MGE and CGE Patterning and Differentiation through Parallel and Overlapping Pathways

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Here we define the expression of ~100 transcription factors (TFs) in progenitors and neurons of the developing mouse medial and caudal ganglionic eminences, anlage of the basal ganglia and pallial interneurons. We have begun to elucidate the transcriptional hierarchy of these genes with respect to the *Dlx* homeodomain genes, which are essential for differentiation of most γ-aminobutyric acidergic projection neurons of the basal ganglia. This analysis identified *Dlx*-dependent and *Dlx*-independent pathways. The *Dlx*-independent pathway depends in part on the function of the *Mash1* basic helix-loop-helix (b-HLH) TF. These analyses define core transcriptional components that differentially specify the identity and differentiation of the globus pallidus, basal telencephalon, and pallial interneurons.

Keywords: CGE, *Dlx*, *Mash*, MGE, transcription factor

Introduction

The combination of transcription factors (TFs) that are expressed in a cell are a fundamental signature of its identity. This information is essential for understanding the transcriptional networks that are operating to control the state of the cell, whether during development or in maturity. Furthermore, understanding the transcriptional hierarchy provides useful information for engineering stem and progenitor cells to become cells of specific phenotypes. Toward elucidating the TF codes expressed in stem/progenitor and their derivatives in the developing basal ganglia and their derivatives, including cortical interneurons, we have systematically identified and characterized the expression of TFs in the prenatal mouse subpallium, defining those TFs that are expressed in stem/progenitors and those expressed in postmitotic cells. Previously, we had identified 2 major transcriptional pathways in the developing subpallium, regulated by the *Dlx1&2* and *Mash1* genes (Anderson, Eisenstat, et al. 1997; Anderson, Qiu, et al. 1997; Casarosa et al. 1999; Yun et al. 2002; Castro et al. 2006; Long et al. 2007, 2009). Here we evaluated the effects of null mutations of *Dlx1&2*, *Mash1*, or *Dlx1&2* and *Mash1* on the expression of many of these subpallial TFs. In previous publications, we focused on the TF codes and effect of the *Dlx1&2* and *Mash1* mutations on the developing septum, lateral ganglionic eminence (LGE), and olfactory bulb (Long et al. 2007, 2009).

Here we investigated these parameters in the medial ganglionic eminence (MGE) and caudal ganglionic eminence (CGE). The MGE is the anlage for the pallidum (globus pallidus [GP] are related pallidal cell groups), interneurons that tangentially migrate to the pallium (cortex and hippocampus), striatum (Sussel et al. 1999; Marín and Rubenstein 2001; Colombo et al. 2007; Wonders and Anderson 2006; Xu et al. 2008), and oligodendrocytes (Kessaris et al. 2006; Petryniak et al. 2007). The CGE is the anlage for distinct subtypes of pallial interneurons (Xu et al. 2004; Butt et al. 2005; Wonders and Anderson 2006; Miyoshi et al. 2007); it is currently unknown whether the CGE also produces neurons that remain in the subpallium.

Our analysis, based on gene expression array data, followed by in situ hybridization, provides a nearly comprehensive description of the TFs expressed in stem/progenitor cells and their derivatives of the embryonic day (E) 15.5 MGE and CGE in mice with different dosages of *Dlx1&2* and *Mash1*.

Materials and Methods

RNA Preparation and Gene Expression Array Analysis

RNA was isolated from E15.5 mouse embryos using the dissected cortex, the combined LGE and MGE and their mantle, or the MGE from control (mixture of wild type and $Dlx1/2^{+/-}$; ratio not known) or $Dlx1/2^{-/-}$ brains (Cobos et al. 2007; Long et al. 2009). RNA was purified and shipped to the National Institute of Health Neuroscience Consortium (TGEN, Phoenix, AZ; http://arrayconsortium.tgen.org/) where biotin-labeled cRNA hybridization probes were generated using the Affymetrix's GeneChip IVT Labeling Kit (Santa Clara, CA), which simultaneously performs in vitro transcription (a linear ~20- to 60-fold amplification) and biotin labeling. The samples were hybridized to the Affymetrix Mouse Genome 430 2.0 array. TGEN uses GeneChip Operating Software (GCOS) to scan the arrays and to perform a statistical algorithm that determines the signal intensity of each gene (for details, see Cobos et al. 2007; Long et al. 2009); the hybridization results of these arrays are available at http://arrayconsortium.tgen.org and are entitled as ruben-affy-mouse-313340 (MGE project) and 2R01MH049428-11 (LGE/MGE and cortex project).

Animals

Mice were maintained in standard conditions with food and water ad libitum. All experimental procedures were approved by the Committee on Animal Health and Care at the University of California, San Francisco (UCSF). Mouse colonies were maintained at UCSF in accordance with National Institutes of Health and UCSF guidelines. Mouse strains with a null allele of *Dlx1&2* and *Mash1* were used in this study (Anderson, Qiu, et al. 1997; Casarosa et al. 1999). These strains were maintained by backcrossing to C57BL/6J mice. For staging of embryos, midday of the "vaginal" plug was calculated as E0.5. Polymerase chain reaction genotyping was performed as described (Anderson, Qiu, et al. 1997; Casarosa et al. 1999). Because no obvious differences in the phenotypes of $Dlx1\&2^{+/+}$ and $Dlx1\&2^{+/-}$ and $Mash1^{+/+}$ and $Mash1^{+/-}$ brains have been detected, they were both used as controls.

Tissue Preparation and In Situ Hybridization

Preparation of sectioned embryos and in situ hybridization were performed using digoxigenin riboprobes on 20-μm frozen sections cut on a cryostat using methods described in Long et al. (2007).

Results

Identification of TFs Expressed in Cells of the Embryonic Mouse MGE and CGE

To understand mechanisms that regulate patterning and differentiation of the mouse embryonic basal ganglia and its derivatives, such as the striatum, GP, and telencephalic interneurons, we have attempted to identify most of the TFs that have key roles in regulating its development at E15.5. We used gene expression array analysis of RNA prepared from E15.5 mouse basal ganglia (LGE, MGE, and CGE combined), MGE, and neocortex, followed by informatic methods to identify known TFs. We then compared the expression of the basal ganglia versus the cortex, control versus *Dlx1&2*$^{-/-}$ basal ganglia, and control versus *Dlx1&2*$^{-/-}$ MGE; this is an extension of the analysis of the LGE and septum that we recently reported (Long et al. 2009).

Table 1 lists TFs that are expressed in the E15.5 basal ganglia, providing expression levels in the basal ganglia, MGE, and cortex. We have eliminated TFs whose expression was below the level of 30 units (we usually cannot detect expression by in situ hybridization of most genes below this level) and general transcriptional components. In this paper, we have focused on TFs expressed in the MGE and CGE. We do not have array results for the CGE; however, as it is composed of both LGE and MGE parts (Flames et al. 2007), we believe that the basal ganglia sample approximates the CGE, although it is probably biased toward the dorsal CGE (dCGE) (also see Supplementary Table 1 for LGE/dCGE differences). Flames et al. (2007) proposed subdivisions of the LGE, MGE, CGE, and preoptic area (POA)—these were based on analysis at E11.5 and E13.5; those subdivisions are more difficult to discern at E15.5, so herein we have not used this nomenclature.

To evaluate the array results, we used in situ hybridization on coronal sections of E15.5 forebrain as indicated by asterisks in Table 1 (Figs 1 and 2; Supplementary Figs 1, 2, and 3). These results largely confirm the TF expression in the subpallium and allowed us to identify TFs that are expressed in progenitor zone (ventricular zone [VZ] and subventricular zone [SVZ]) and mantle zone (MZ) of the LGE, MGE, and CGE. For example, this analysis identifies several TFs that are expressed in the GP (*Arx*, *Dlx1*, *ER81 (Etv1)*, *Gbx1*, *Lhx6*, *Lhx7(8)*, *Oct6 (POU3F1)*, *ROR-beta*, *TCF4*, *Tshz2*, and *Zfp521 (Evi30)*) (Figs 1 and 2). It also enabled us to evaluate differential expression between the LGE, MGE, and CGE.

TFs that are specifically expressed in progenitor cells of MGE are *Lhx6*, *Lhx7(8)*, *Nkx2.1*; those preferentially expressed in the MGE (compared with the LGE) include *ER81 (Etv1)*, *Sox4*, and *Sox11* (also see Flames et al. 2007). TFs that are expressed in progenitor cells of the LGE and not detected in the MGE by this assay include *ESRG*, *FoxP1*, *FoxP2*, *FoxP4*, *Sp8*; TFs preferentially expressed in the LGE (compared with the MGE) include *ATBF1 (Zfbx3)*, *COUP-TF1 (NR2F1)*, *CTIP2 (Bcl11b)*, *Ebf1*, *Islet1*, *Meis1*, *Meis2*, *Oct6 (POU3F1)*, *Pbx1*, *Pbx3*, *Six3*, and *TCF4*. Of note, the MGE expression of many of these genes is within a narrow corridor between SVZ and mantle and may correspond to the ventral migration of LGE cells (López-Bendito et al. 2006).

The CGE contains at least 2 subdivisions; the ventral part is a caudal extension of the MGE and the dorsal part is a caudal extension of the LGE (Flames et al. 2007). However, whereas the LGE is largely dedicated to generating projection neurons of the striatum, accumbens, olfactory tubercle, and interneurons of the olfactory bulb (Long et al. 2007, 2009), the dCGE is known to generate interneurons of the neocortex and hippocampus (Xu et al. 2004; Butt et al. 2005; Miyoshi et al. 2007). Thus, there must be molecular differences in the progenitors of the LGE and CGE. Therefore, we qualitatively compared TF expression in the VZ/SVZ of these regions using in situ hybridization (Figs 1 and 2). Supplementary Table 1 summarizes our nonquantitative conclusions. We found TFs that are preferentially expressed in the LGE (green color, i.e., *ATBF1* [*Zfbx3*] and *Islet1*), equally expressed in the LGE and dCGE (yellow color, i.e., *Dlx* genes), and preferentially expressed in the dCGE (red color, i.e., *Arx*, *COUP-TFI (NR2F1)*, *Mash1*, *Prox1*, *Sall3*, *Sox1*, *Sox4*, and *Sp9*).

Below we describe how loss of either *Dlx1&2* or *Mash1* function affects the expression of many of these TFs and selected non-TFs. The analysis was performed using in situ hybridization on E15.5 coronal sections; in general, the gene expression array results (Table 1) were in accord with the histological analysis. Analysis at E12.5 was also performed in the *Dlx1&2*$^{-/-}$ mutants for selected genes (column 1 in Table 1) and showed similar results (data not shown) (for the LGE and septum, see Long et al. 2009).

Dlx1&2 Functions in the MGE

Dlx1&2 are required, to varying degrees, to promote expression of several TFs in MGE progenitors (VZ and SVZ), including *Arx*, *bMaf*, *Brn4*, *Cux2*, *Dlx1*, *Dlx2*, *Dlx5*, *Dlx6*, *ER81 (Etv1)*, *Gli1*, *Lhx6*, *Lhx7*, *Pbx1*, *Peg3*, *Sox4*, *Sox11*, and *Vax1* (Fig. 1), and non-TFs, including *CXCR4*, *CXCR7 (RDC1)*, *CyclinD2*, *GAD67*, *Gucy1a3*, *Shb*, *Tiam2*, and *Thbs* (Supplementary Fig. 1).

Dlx1&2 repress the expression of a set of TFs, including *antisense-Dlx6*, *COUP-TF1*, *Ctip2*, *Gbx1*, *Gsh1*, *Gsh2*, *Id2*, *Id4*, *Ikaros*, *Islet1*, *Lhx2*, *Mash1*, *Nkx2.1*, *Olig2*, *Otp*, *Prox1*, *Sall3*, *Six3*, and *Sox1* (Fig. 2), and non-TFs, including *Dact1* and *PKR1* (Supplementary Fig. 1). Some TFs do not show a discernable expression change, such as *Sp9* (Fig. 1*HHH–III′*). The MGE produces several types of cells including projection neurons of the GP and interneurons that migrate to the cortex and hippocampus. *Dlx1&2*$^{-/-}$ mutants produce a small GP but with reduced numbers of neurons expressing *ER81 (Etv1)*, *Gbx1*, *Gbx2*, *Lhx6*, *Lhx7/8*, *Lmo3*, *Meis1*, *Oct6 (POU3F1)*, *Pbx3*, *RORβ*, *Tcf4*, *Sema3a*, *Tshz2*, and *Zpf521* (Fig. 1) and non-TFs *Cad8*, *Gad67 (Gad1)*, *Robo2*, and *Sema3a* (Supplementary Fig. 1). On the other hand, some TFs show increased expression in the MGE mantle zone including *ATBF1*, *Ebf1*, *ESRG*, *Fez*, *FoxP2*, *Islet1*, and *Pbx3* (Fig. 2); this may be due to ectopic accumulation of cells from striatal and/or POA migrations (López-Bendito et al. 2006) or ectopic expression of these TFs in the pallidal MZ.

Most interneuron precursors fail to migrate into the cortex in *Dlx1&2*$^{-/-}$ mutants (Anderson, Eisenstat, et al. 1997; Pleasure et al. 2000; Cobos et al. 2005) and appear to remain as ectopia in the basal ganglia, some of which express *neuropilin 2* (*NP2*) (Marín et al. 2001). Here we show that these ectopia to form in a caudal position within the CGE and continue to express many TF and non-TF markers characteristic of immature interneurons, including *bMaf*, *ErbB4*, *Lhx6*, *NP2*, and *neurexophilin-1* (*Nphx*) (Supplementary Fig. 2). Surprisingly, there are also large ectopia that express GP markers: *ER81 (Etv1)* and *Nkx2.1 (Ttf1)* (Supplementary Fig. 2). Finally, there are ectopia

Table 1

List of TFs expressed in the E15.5 basal ganglia (BG) and identified gene expression array analysis (not including general TFs)

E12 ISH	E15 ISH	TF name	BG/cortex	Ctx expression	BG expression	BG expression−/−	MGE expression	MGE expression−/−
		TFs						
	*	*Arx*	4.64	487	2262	618	3648	948
		Asb2	3.18	11	35	32	11	14
		Asb4	4.81	16	77	125	419	992
	*	*ATBF1*	30.44	18	548	503	124	246
	*	*BF1 (FoxG1)*	0.75	5121	3856	3116	4142	4781
		Bhlhb5	0.32	3822	1219	1053	28	189
	*	*Brn2 (POU3F2)*	0.56	149	83	93	89	86
	*	*Brn4 (POU3F4)*	7.18	28	201	114	501	209
	*	*Brn5 (POU6F1)*	0.16	19	3	10	1	6
	*	*CoupTF1 (NR2F1)*	1.31	2414	3154	3561	1479	3857
		CoupTFII (NR2F2)	3.38	102	345	339	73	110
	*	*Ctip1 (Bcl11a, Evi9)*	1.06	1522	1616	1052	891	692
	*	*Ctip2 (Bcl11b, Rit-1b)*	1.43	1106	1579	792	1441	1748
	*	*Cux2*	*					
		Dach2	2.79	33	92	171	153	277
	*	*Dbx1*	1.83	18	33	27	17	28
	*	*Dlx1*	9.84	120	1181	7	3278	2
*	*	*Dlx2*	7.06	50	353	15	1069	15
	*	*Dlx5*	7.76	96	745	80	751	49
	*	*Dlx6*	9.80	15	147	21	172	5
	*	*Dlx6 antisense (Evf1, Evf2)*	4.00	11	44	4	22	2
	*	*Dlxin*	*					
	*	*Ebf1*	21.33	27	576	132	144	96
		Ebf2	1.59	17	27	42	17	29
*	*	*Ebf3*	0.66	74	49	198	12	50
	*	*Egr3*	2.05	21	43	18	45	25
	*	*Emx1*	0.31	323	100	75	33	4
	*	*Emx2*	0.43	356	152	177	105	94
*	*	*ER81 (Etv1)*	3.23	66	213	137	820	283
	*	*ESRG (ESRRG, NR3B3)*	1.57	21	33	26	20	25
	*	*Evi3 (Zfp521, EHZF)*	*					
		Fah	3.55	22	78	42	59	36
	*	*Fez (FezF1)*	*					
	*	*Fez-l (FezF2)*	*					
	*	*FoxO3a*	0.75	4	3	8	17	19
	*	*FoxO4*	0.46	13	6	36	17	23
	*	*FoxP1*	2.60	149	388	122	96	81
	*	*FoxP2*	2.53	34	86	90	31	14
	*	*FoxP4 (mFKHLA)*	1.30	43	56	43	30	38
	*	*FXR1alpha1 (NR1H4)*	2.10	10	21	14	30	21
*	*	*Gbx1*	*					
	*	*Gbx2*	5.33	9	48	63	15	124
	*	*Gli1 (Zfp5)*	*					
*	*	*Gsh1*	7.57	7	53	156	112	484
	*	*Gsh2*	14.77	13	192	205	185	308
	*	*Hes1*	0.64	171	110	88	131	93
*	*	*Hes5*	1.36	167	227	290	702	689
	*	*HesR1 (Hey1)*	1.10	106	117	144	137	107
		Hlx1	3.00	11	33	27	2	11
*	*	*Id2*	0.12	1870	230	686	122	729
	*	*Id4*	0.98	197	194	196	214	294
	*	*Ikaros (ZNFN1A1)*	2.27	11	25	25	36	31
	*	*Islet1*	254.00	6	1524	1318	1339	2264
		Klf4	1.33	36	48	45	54	88
		Klf5	7.14	21	150	92	62	298
	*	*Lhx1 (Lim1)*	0.36	14	5	22	8	15
	*	*Lhx2*	0.31	2120	650	774	428	853
*	*	*Lhx6*	2.67	46	123	98	130	167
	*	*Lhx7*	252.50	2	505	224	1513	166
		Lhx9	0.68	200	135	200	4	123
	*	*Lmo1*	0.62	569	351	362	1764	1048
	*	*Lmo3 (Rbtn3)*	*					
	*	*Lmo4*	2.19	360	789	581	697	761
	*	*Maf A*	*					
	*	*Maf B*	1.07	122	131	167	53	72
	*	*Maf C*	1.04	28	29	20	18	3
	*	*Mash1 (Ascl1)*	4.51	63	284	330	536	941
		Med6	6.03	64	386	229	344	275
	*	*Mef2c*	0.44	398	174	151	48	26
	*	*Meis1*	4.67	49	229	154	115	142
	*	*Meis2 (MRG1B)*	1.23	1489	1827	1275	1067	1078
		Msc (MyoR)	3.21	14	45	49	2	4
		Myt1l	0.74	1018	757	933	276	566
		Neurog2	0.23	1508	341	211	9	66
	*	*Nex1 (NeuroD6)*	0.12	5163	620	881	1	78
	*	*NHLH2 (Hen2, NSCL2)*	*					
	*	*Nkx2.1 (Ttf1)*	0.46	65	30	47	31	24

Table 1
Continued

E12 ISH	E15 ISH	TF name	BG/cortex	Ctx expression	BG expression	BG expression−/−	MGE expression	MGE expression−/−
	*	*Nkx2.2*	7.00	3	21	30	1	20
	*	*Nkx5.1 (Hmx1)*	1.67	3	5	3	4	2
		Nkx5.2	*					
	*	*Nkx6.1*	15.00	1	15	8	14	16
	*	*Nkx6.2*	1.24	100	124	112	76	74
	*	*Nolz1 (Zfp503)*	*					
	*	*Npas1*	0.50	12	6	2	16	5
		Nr4a2	0.72	132	95	114	37	50
	*	*Nur77 (NR4A1)*	1.18	65	77	51	36	26
*	*	*Oct6 (POU3F1)*	1.30	74	96	25	76	62
		Olig1	5.73	79	453	638	318	896
	*	*Olig2*	5.66	29	164	281	654	859
*	*	*Otp*	0.88	8	7	16	9	15
	*	*Otx1*	0.31	129	40	53	2	22
*	*	*Otx2*	0.46	76	35	71	151	359
	*	*Pak3*	1.33	179	238	526	230	395
	*	*Pax6*	0.42	200	83	103	27	16
*	*	*Pbx1*	1.03	180	186	126	191	137
	*	*Pbx3*	7.28	76	553	671	209	700
	*	*Peg3 (End4, Gcap4, Pw1, Zfp102)*	1.22	381	463	818	512	568
	*	*Phox2a (Arix, Pmx2)*	0.73	22	16	22	4	10
	*	*Prox1*	3.07	14	43	56	34	94
	*	*RALDH3 (ALDH6)*	*					
	*	*RARß*	14.26	19	271	40	56	28
	*	*RORb*	0.70	60	42	83	31	39
	*	*RXRg (NR2B3)*	21.29	14	298	48	57	26
*	*	*Sall3 (msal1, spalt)*	0.84	68	57	145	90	171
	*	*Sim1*	1.19	37	44	44	35	38
	*	*Six3*	9.70	33	320	153	188	253
		Solt	1.09	238	260	200	759	340
	*	*Sox1*	3.60	15	54	35	105	140
	*	*Sox11*	0.77	6142	4716	3765	5005	3203
	*	*Sox4*	0.73	827	602	865	507	1046
		Sox5	0.17	1023	172	223	243	347
		Sox6	1.14	258	294	406	711	836
		Sox8	4.24	29	123	85	63	96
*	*	*Sp8 (Btd)*	2.41	63	152	81	44	6
	*	*Sp9*	*					
	*	*Tbr1*	0.33	646	215	270	29	5
	*	*Tbr2*	0.16	1030	169	111	2	1
	*	*TCF4*	0.30	2771	818	1245	2066	2272
	*	*Tle4*	*					
	*	*Tlx*	1.44	16	23	18	9	4
		Tox	0.86	182	156	257	215	532
		Trp53	0.82	124	102	80	109	120
	*	*Trp53bp1*	0.66	212	140	153	127	91
	*	*Tshz1*	*					
	*	*Tshz2*	*					
*	*	*Vax1*	2.69	13	35	22	31	17
		Zbtb20	0.76	507	385	746	139	359
		Zfhx1b	0.31	1037	321	388	310	140
	*	*Zfp618*	*					
	*	*Zic1*	1.45	593	859	1592	613	604
		Non-TFs						
	*	*Adamts5*	1.39	31	43	28	133	31
		Adrenergic receptor, alpha 2a	7.38	13	96	102	41	50
		Ankyrin repeat and SOCS box-containing protein 4	7.13	15	107	152	784	1425
		B3galt5	12.00	5	60	29	94	72
		Bcl11b	1.43	1106	1579	792	1441	1748
		Bdkrb1	1.69	16	27	10	17	21
		Cad7, type 2	0.44	55	24	159	29	38
	*	*Cad8*	*					
		Calb1	9.41	34	320	177	236	174
		Calcr	3.20	5	16	91	42	88
		Camk2a	10.26	23	236	122	50	136
		Cap1	1.13	460	518	309	222	369
		Carnitine deficiency-associated gene expressed in ventricle 3	0.14	63	9	60	3	15
	*	*Ccr4*	0.23	22	5	23	3	3
		Cd69	1.63	8	13	4	1	5
		Cfh	2.00	10	20	11	13	5
		Clca1	1.60	5	8	39	24	35
		Clca2	1.50	10	15	31	4	18
		Coatomer protein complex, subunit gamma 2, antisense 2	4.23	242	1024	1680	74	363
		Cobl	9.40	10	94	87	24	25
	*	*Crabp1*	6.88	68	468	116	113	12
	*	*Crym*	0.35	329	114	41	18	33
	*	*CXCR4*	0.88	307	271	81	415	211
	*	*CXCR7*	3.96	408	1614	708	4602	1651

Table 1
Continued

E12 ISH	E15 ISH	TF name	BG/cortex	Ctx expression	BG expression	BG expression−/−	MGE expression	MGE expression−/−
	*	*CyclinD2*	1.18	1802	2126	1631	3051	1700
	*	*Dact1*	*					
		Dlc1	1.79	78	140	73	45	40
	*	*Drd1a*	1.32	31	41	17	5	4
		Egfl6	0.29	21	6	21	13	15
		Erbb2ip	0.42	95	40	150	73	47
	*	*ErbB4*	4.67	3	14	7	12	2
		Gabra1	9.57	7	67	94	18	40
	*	*GAD1 (GAD67)*	5.46	321	1753	1358	583	520
		GABA-A receptor, subunit gamma 1	6.11	9	55	78	18	12
		Gcnt2	1.90	50	95	42	70	58
		Gng4	5.00	15	75	77	14	39
		Gpr88	110.50	2	221	50	23	18
		Granulin	1.35	287	388	194	360	402
		Gucy1a3	2.62	188	492	179	655	174
		H2-K	2.77	13	36	12	2	13
		H2-Q1	1.96	26	51	17	32	18
		Hist1h1c	1.99	139	277	128	473	288
		Htr3a	2.07	92	190	62	223	113
		Ivd	0.87	229	199	96	144	183
		Kcnj9	10.67	3	32	24	9	1
		Kruppel-like factor 5	7.14	21	150	92	62	298
		Lck	2.80	10	28	15	10	19
		Lgals1	1.96	266	522	274	1212	589
		Lor	13.50	2	27	35	10	16
		Mbp	2.10	70	147	105	115	119
		Moxd1	6.60	5	33	25	7	16
		Myh6	13.08	13	170	89	59	95
		Ncdn	1.89	323	610	309	269	390
	*	*NP2*	1.18	150	177	248	17	57
	*	*Nphx*	*					
		Npy2r	7.75	4	31	30	36	26
		Olfm3	32.00	4	128	159	78	63
		Omg	12.25	4	49	65	37	91
		Ostb	9.33	3	28	18	23	5
	*	*Penk1*	13.31	13	173	79	39	34
		Phka1	0.44	36	16	38	32	17
	*	*PK2*	*					
	*	*PKR1*	*					
		Pla2g4b	0.93	126	117	52	121	124
		Plaa	0.48	33	16	78	21	25
		Pre B-cell leukemia TF 3	15.65	122	1909	2013	696	2110
		Presenilin 1	0.50	10	5	11	9	7
		Prok2	11.80	5	59	63	27	108
		Protease, cysteine, 2 (NEDD8 specific)	0.33	69	23	86	118	82
		Purg	1.61	57	92	48	52	58
		Pyruvate carboxylase	1.34	41	55	17	10	17
		Rbp1	9.70	213	2066	889	2917	1452
		Resp18	129.50	2	259	245	33	61
		Rnasep1	1.61	62	100	43	107	106
	*	*Robo2*	*					
		Rpl22	1.70	2909	4952	2182	7650	5885
		Rrbp1	1.08	37	40	7	18	21
		Rrp4	2.14	14	30	15	13	11
		S100 calcium-binding protein A10 (calpactin)	2.04	103	210	103	520	189
		Scmh1	0.35	98	34	212	43	33
	*	*Sema3a*	0.93	195	181	185	59	76
		Sema6d	0.61	33	20	84	38	26
	*	*Shb*	1.31	274	358	178	410	213
		Slco1a1	2.36	11	26	5	6	7
		Snx6	1.46	174	254	129	258	110
		Spp1	4.84	31	150	57	32	17
		Syndecan 1	1.75	193	338	160	470	301
		Syt6	1.73	48	83	41	54	21
		Tac1	86.55	11	952	457	310	174
	*	*Thbs1*	0.87	204	178	69	166	86
	*	*Tiam2*	0.27	895	239	55	438	42
		Top2b	13.95	19	265	44	56	19
		Trhr	0.40	45	18	28	4	5
		Uty	0.77	135	104	7	45	80
		V1ra5	0.50	2	1	8	2	9
	*	*Viaat*	10.76	42	452	38	617	24
		Vsnl1	7.82	28	219	255	54	133
		Wnt5a	0.83	82	68	38	110	87
		Wnt7a	2.01	160	321	158	513	441
		Zfp145; PLZF	2.10	31	65	29	2	2

Note: Asterisks in columns 1 and 2 indicate if the results were verified by in situ hybridization. Columns to the right indicate the raw hybridization scores for the individual genes for hybridization using RNA isolated from control E15.5 cortex, combined LGE, MGE, and CGE (BG), MGE, or from *Dlx1&2*$^{-/-}$ BG (for details, see Long et al. 2009) or *Dlx1&2*$^{-/-}$ MGE (for details, see Cobos et al. 2007). ISH, in situ hybridization.

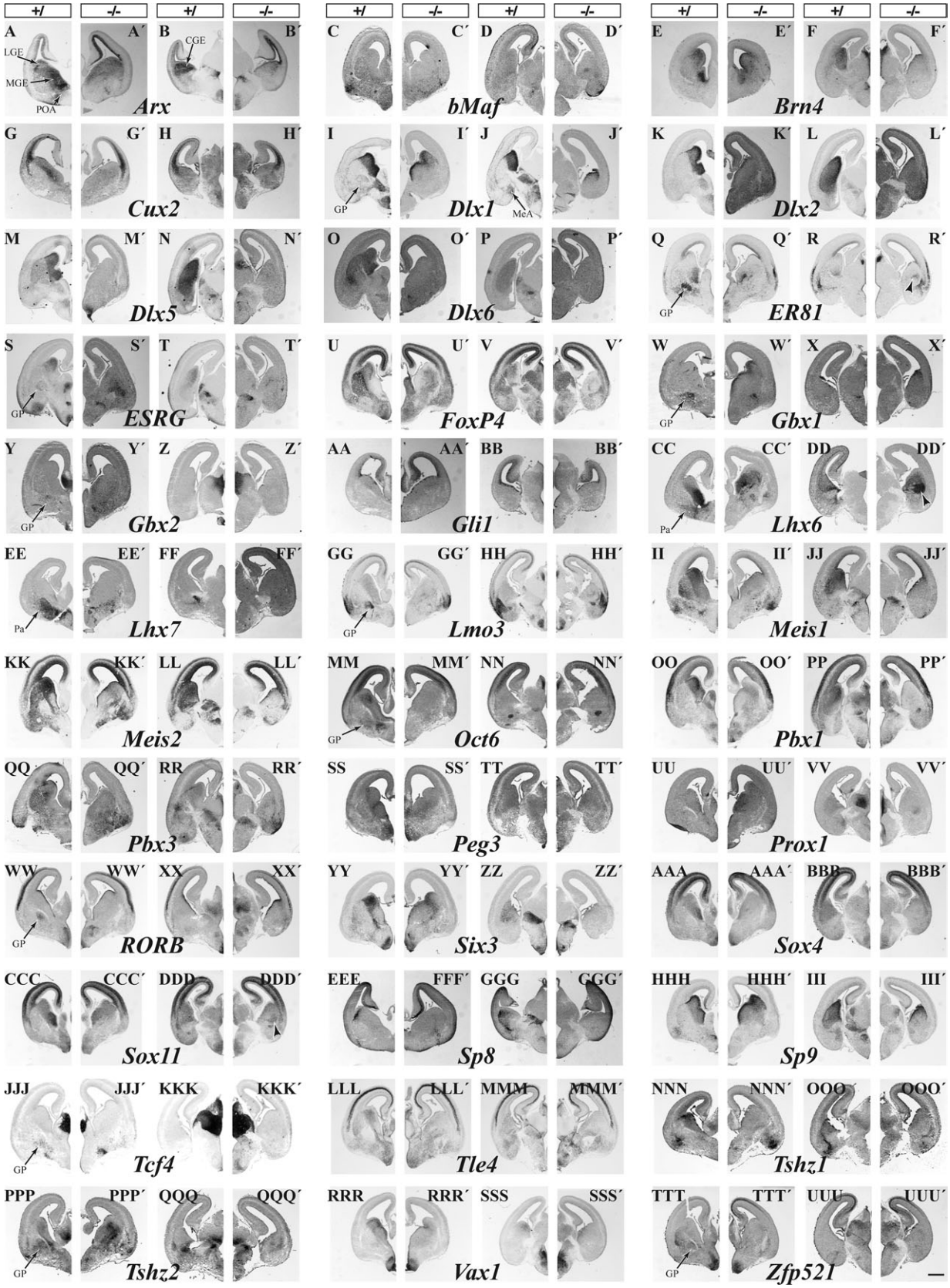

Figure 1. TFs whose expression is reduced in either the LGE/MGE (left pair) or CGE (right pair) in the *Dlx1&2*$^{-/-}$ mutants as shown by in situ hybridization on coronal hemisections from E15.5 forebrains. Control: left section; *Dlx1&2*$^{-/-}$: right section. Magnification bar: 500 μm.

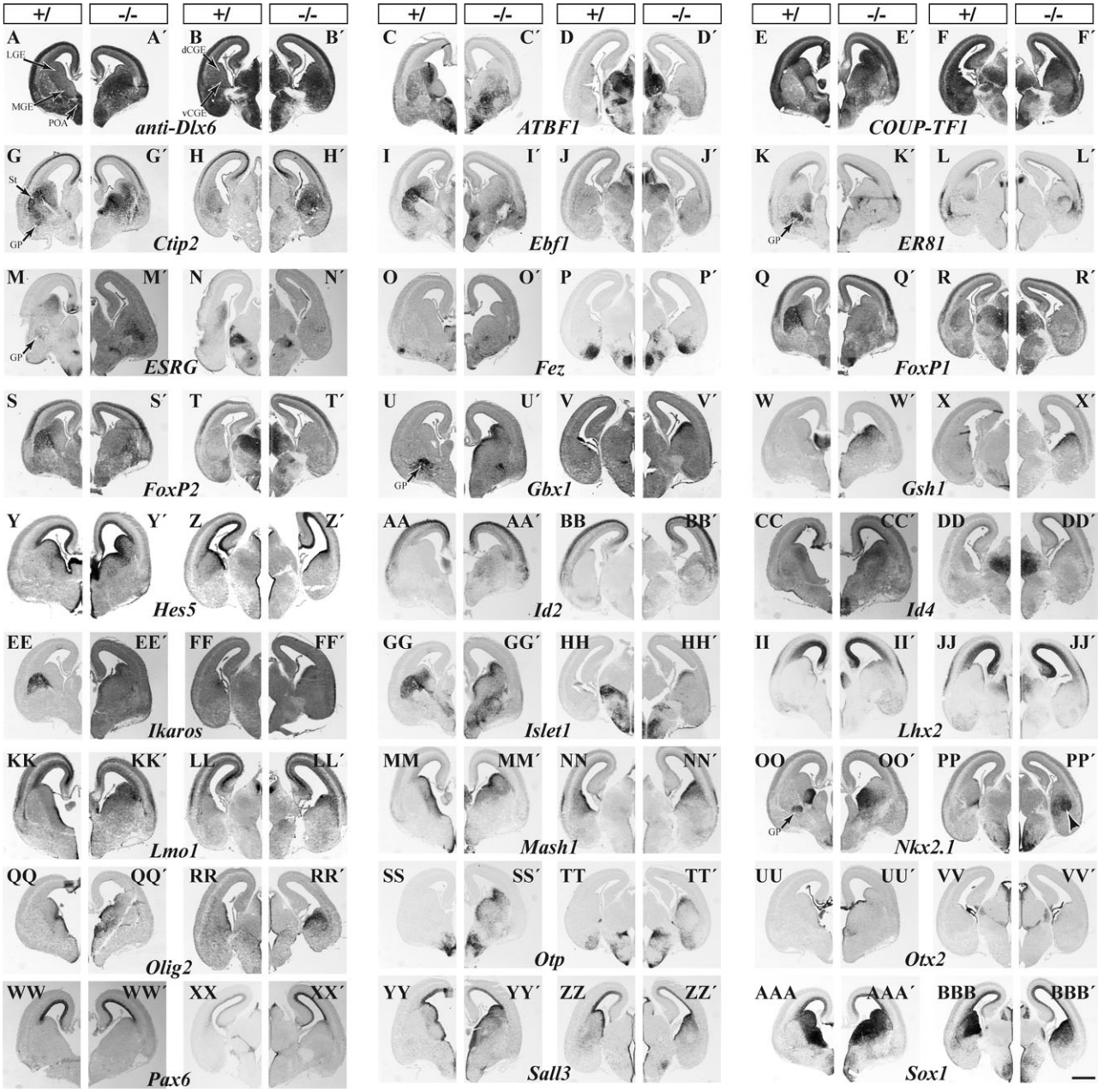

Figure 2. TFs whose expression is increased in either the LGE/MGE (left pair) or CGE (right pair) in the *Dlx1&2*$^{-/-}$ mutants as shown by in situ hybridization on coronal hemisections from E15.5 forebrains. Control: left section; *Dlx1&2*$^{-/-}$: right section. Magnification bar: 500 μm.

expressing other genes (*Pbx1*, *Prox1*, and *Sox11*) that currently are not known to mark specific cell types (Supplementary Fig. 2).

Dlx1&2 Functions in the CGE

Dlx1&2 are required to promote expression of several TFs in the CGE including *Arx*, *Brn4*, *Dlx1,2,5,6*, *ESRG*, *FoxP1*, *FoxP4*, *Meis1*, *Meis2*, *Oct6 (POU3F1)*, *Pbx1*, *Pbx3*, *Prox1*, *Six3*, *Sox4*, *Sox11*, *Sp8*, *Tle4*, *Tshz1*, and *Vax1* and non-TFs including *CXCR4*, *CXCR7 (RDC1)*, *ErbB4*, *Gad67 (Gad1)*, *Gucy1a3*, *Robo2*, *Shh*, *Tiam2*, and *Thbs*. The reduction of some genes probably corresponds to the block of MGE-derived interneuron tangential migration (i.e., *bMaf*, *Cux2*, *Lhx6*; Fig. 1*C–D′*, *G–H′*,*CC–DD′*).

Dlx1&2 repress the expression of several TFs including *antisense-Dlx6*, *COUP-TFI (NR2F1)*, *Ctip2 (Bcl11b)*, *Gbx1*, *Gsh1*, *Gsh2*, *Id2*, *Ikaros*, *Islet1*, *Lmo1*, *Mash1*, *Olig2*, *Otp*, and *Sall3* and several non-TFs including *Dact1* and *PKR1* (Fig. 2). Several genes show little change in expression including *FoxP2*, *Hes5*, *Id4*, *Lhx2*, *Otx2*, *Pax6*, *Sox1*, and *Sp9* (Figs 1 and 2); this is unlike the LGE, which has increased *Hes5*, *Lhx2*, and *Sp9* expression (Figs 1 and 2; Supplementary Table 2; Anderson, Qiu, et al. 1997; Yun et al. 2002; Long et al. 2007, 2009).

Finally, as noted above, there are ectopic accumulations of cells in the CGE expressing several markers characteristic of the GP or cortical interneurons (i.e., *bMaf*, *ER81 (Etv1)*, *ErbB4*, *Lhx6*, *Nkx2.1 (Ttf1)*, *NP2*, *Nphx*, *Pbx1*, *Prox1*, and *Sox11*; Supplementary Fig. 2).

Dlx1&2$^{-/-}$;Mash1$^{-/-}$ Compound Mutants Define Genes Epistatic to Dlx1&2, Mash1, or Both Dlx1&2 and Mash1

Whereas many aspects of MGE and CGE differentiation are lost in the *Dlx1&2$^{-/-}$* mutants, many aspects are maintained (Figs 1 and 2; Supplementary Fig. 1 and Supplementary Table 2). The maintained characteristics may be regulated by TFs whose expression persists in mutant LGE progenitors (Fig. 2) (Long et al. 2009). A good candidate of this type of TF is *Mash1*, due to its overexpression in the *Dlx1&2$^{-/-}$* mutants (Fig. 2) (Yun et al. 2002; Long et al. 2009). As MASH1 and DLX2 proteins are coexpressed in progenitors of the dorsal LGE (dLGE) (Porteus et al. 1994; Yun et al. 2002), they have the potential to regulate the developmental programs of these cells in parallel and/or in series. Here, we explored the hypothesis that *Mash1* has a critical role in maintaining certain aspects of MGE and CGE differentiation in the *Dlx1&2$^{-/-}$* mutants, as it does in the LGE (Long et al. 2009).

We studied the expression of TFs and selected other genes in the MGE and CGE in *Dlx1&2$^{-/-}$*, *Mash1$^{-/-}$*, and *Dlx1&2$^{-/-}$;Mash1$^{-/-}$* mutants at E15.5, concentrating on genes whose expression persists in *Dlx1&2$^{-/-}$* mutants (Supplementary Fig. 3). Expression of these genes fell into 4 general classes (Supplementary Table 3):

Class I genes appear to be epistatic only to *Dlx1&2$^{-/-}$*. Expression of Class Ia genes (*ER81*, *Gli1*, *Gsh1*, and *Sp8*) is reduced or lost in the *Dlx1&2$^{-/-}$* mutants and is not overtly affected in the *Mash1$^{-/-}$* mutants, and the triple mutant phenocopies the *Dlx1&2$^{-/-}$* mutant. Class Ib genes are ectopically expressed in the *Dlx1&2$^{-/-}$* mutants and are not overtly modified by loss of *Mash1$^{-/-}$*.

Class II genes appear to be epistatic only to *Mash1$^{-/-}$* (i.e., *Hes5^{CGE}*: i.e., in only the CGE).

Class III genes appear to be altered in both the *Dlx1&2$^{-/-}$* and *Mash1$^{-/-}$* mutants, and in most cases, these phenotypes are exacerbated in the triple mutants. There are 5 subtypes of Class III genes based on their differentiation responses in the *Dlx1&2$^{-/-}$* and *Mash1$^{-/-}$* mutants:

1. IIIa (Arx^{MGE}, Dlx1, Dlx5, and Gad67^{CGE}): decreased in Dlx1&2$^{-/-}$ and increased in Mash1$^{-/-}$;
2. IIIb (Arx^{CGE}, Meis2, Six3, Sox1, Sp9, and Vax1): decreased in both Dlx1&2$^{-/-}$ and Mash1$^{-/-}$;
3. IIIc (Dact1, Hes5^{MGE}, Olig2, and Sox1): increased in Dlx1&2$^{-/-}$ and decreased in Mash1$^{-/-}$;
4. IIId (*Islet 1*): increased in the VZ of the CGE (and LGE; but reduced in the SVZ and MZ of the LGE) and not greatly modified by *Mash1* dosage; and
5. IIIe (*Tshz2*): increased in *Dlx1&2$^{-/-}$* and ectopic in *Mash1$^{-/-}$*.

Class IV (ER81 in the MGE) genes show a modest decrease in the number of labeled GP neurons in *Dlx1&2$^{-/-}$*, *Mash1$^{-/-}$*, and *Dlx1&2$^{-/-}$;Mash1$^{-/-}$* mutants.

Of note, several TFs continue to be expressed in the *Dlx1&2$^{-/-}$;Mash1$^{-/-}$* mutants, albeit generally at lower levels, in the CGE (*Gsh1*, *Islet1*, *Olig2*, and *Sp9*) and MGE (*ER81*, *Islet1*, *Olig2*, and *Sp9*) (Supplementary Fig. 3), demonstrating that some fundamental aspects of CGE and MGE specification such as *GAD67* expression (Supplementary Fig. 1) are not fully dependent on *Dlx* and *Mash1*.

Discussion

We report a comprehensive analysis of the TFs, excluding general TFs, that are expressed in the developing (E15.5) mouse MGE and CGE. Although we cannot rule out that there are additional important TFs, we expect that they will be present in our gene expression array data sets (http://arrayconsortium.tgen.org) and are entitled as ruben-affy-mouse-313340 (MGE project) and 2R01MH049428-11 (LGE/MGE and cortex project). This paper complements our TF analysis in developing septum and LGE (Long et al. 2009) and the work of Flames et al. (2007), which used a subset of these TFs to define E13.5 subpallial progenitor zones. We then defined the response of many of these genes in mice lacking expression of *Dlx1&2*, *Mash1*, or *Dlx1&2* and *Mash1*. We expect that this analysis will provide an important foundation for establishing the transcriptional circuitry that controls cell fate and differentiation of MGE and CGE derivatives. This information will be extremely useful in understanding normal pathways that control the development and evolution of the basal ganglia and pallial interneurons and will help predict the effect of mutations, whether they are in experimental animals or in humans. Furthermore, understanding the transcriptional hierarchies will be essential in engineering stem cells.

TF Profile in the VZ and SVZ of the dCGE

Flames et al. (2007) demonstrated that the CGE is a composite of the caudal LGE (the dCGE) and MGE (the ventral part). Here we show that although the dCGE does share many properties with the LGE, there are several important differences (Supplementary Table 1). We find TFs that are preferentially expressed in the LGE (green color, i.e., *ATBF1 (Zfbx3)* and *Islet1*), equally expressed in the LGE and dCGE (yellow color, i.e., Dlx genes), and preferentially expressed in the dCGE (red color, i.e., *Arx*, *COUP-TFI (NR2F1)*, *Mash1*, *Prox1*, *Sall3*, *Sox1*, *Sox4*, and *Sp9*). We propose that 1) the TFs preferentially expressed in the LGE are important in development of striatal projection neurons and olfactory bulb interneurons; 2) the TFs preferentially expressed in the dCGE are important in development of cortical interneurons (subsets of neuropeptide-Y-, calretinin-, and vasoactive intestinal peptide-expressing pallial interneurons; see Zhao et al. 2008); and 3) TFs that are equally expressed in the LGE and dCGE have general roles in regulating the development of telencephalic γ-aminobutyric acidergic (GABAergic) neurons.

Within the dCGE, there is a VZ and a SVZ, but a MZ is not clearly distinct; this feature is exemplified by the expression of the Dlx genes whose combinatorial expression defines these 3 differentiation zones in the LGE and MGE (Fig. 1). At this point, it is unclear whether or not the CGE produces subpallial nuclei, although a caudal nucleus, such as the central nucleus of the amygdala, is a possibility (Carney et al. 2006; García-López et al. 2008).

It is possible that the CGE primarily consists of a large SVZ where pallial interneurons are produced and partially mature. In addition, many MGE-derived interneurons (*Lhx6^{+}*) migrate through the CGE; it is possible that local CGE factors regulate their development; this idea is discussed below in the section of subpallial neuronal ectopia in the *Dlx1&2$^{-/-}$* mutants.

Role of Dlx1&2 in dCGE Development

Dlx1&2 have a profound role in promoting differentiation of the dCGE, as exemplified by the reduced expression of *Arx*, *Brn4*, *Dlx5*, *Dlx6*, *ESRG*, *FoxP4*, *Meis1*, *Meis2*, *Pbx1*, *Pbx3*, *Prox1*, *Six3*, *Sox4*, *Sox11*, *Sp8*, *Tle4*, *Tshz1*, and *Vax1* in the *Dlx1&2$^{-/-}$* mutants.

These findings are similar to the phenotype of dLGE and septum, but not ventral parts of these primordia (Long et al. 2009). Thus, the dLGE and dCGE share similar dependence on *Dlx1&2*.

In the dLGE and dCGE, *Dlx1&2* may have their strongest functions in the SVZ, as exemplified by reduced *Sp9* expression in the SVZ and not in the VZ. Furthermore, *Dlx1&2* are required to repress the expression of *COUP-TFI*, *Ctip2*, *Mash1*, and *Sall3*, supporting the model that *Dlx1&2* promote the maturation of SVZ progenitors (see Yun et al. 2002; Long et al. 2007, 2009). Below we discuss the role of *Mash1* in CGE development and the effect of removing *Mash1* function in *Dlx1&2*$^{-/-}$ mutants. Elevated levels of *antisense-Dlx6* transcripts are intriguing (Fig. 2), suggesting that the *Dlx1&2* are required to repress this potential inhibitor of *Dlx6* function (Faedo et al. 2004; Feng et al. 2006).

Several genes are ectopically expressed in the mutant dCGE, including markers of the MGE (*Gsh1* and *Gbx1*), the ventral pallium (*Id2* and *Lmo1*), dLGE (*Ikaros* and *Islet1*), and diencephalon (*Otp*) (Fig. 2). These results show that *Dlx1&2* are required to specify the identity of dCGE progenitors, a finding that is also apparent in the dLGE (Long et al. 2009).

MGE TFs and the Function of Dlx1&2

Several TFs appear to preferentially, or exclusively, mark MGE progenitors and their derivatives: *Cux2*, *ER81*, *Gbx1*, *Gbx2*, *Gsh1*, *Lhx6*, *Lhx7*, *Nkx2.1 (TTF-1)*, *Nkx6.2*, *Prox1*, *ROR-beta*, and *TCF4*. The MGE also shares molecular features with the LGE/dCGE, such as expression of *Arx*, *Brn4 (POU3f4)*, *Dlx1&2/5/6*, *Mash1*, *Sp9*, and *Vax1* (Figs 1 and 2; Flames et al. 2007).

The preoptic (POA) progenitor and mantle zones are rostroventral to the MGE (Flames et al. 2007) and express many of the same genes as the MGE but also have their distinct molecular features, including expression of *COUP-TFI*, *Dbx1*, *Lhx2*, *Nkx5.1*, *Nkx5.2* (*Hmx2* and *Hmx3*), and *Nkx6.2* (Wang et al. 2004; Flames et al. 2007). Here we did not explicitly investigate the effect of the *Dlx1/2* and *Mash1* mutations on POA development.

Although regional identity of *Dlx1&2*$^{-/-}$ MGE does not appear to be greatly disturbed, its differentiation of the GP and pallial interneurons is impeded, perhaps secondary to increased notch signaling, as reflected by increased *Mash1* and Hes5 expression (Fig. 2; see Yun et al. 2002). Increased expression of the *Pak3* kinase has also been implicated in cytoskeletal dysregulation leading to premature neurite extension and inhibition of migration (Cobos et al. 2007).

Regional identity of the mutant MGE is probably preserved by the continued expression of *Nkx2.1* (which may be increased; Fig. 2), a TF required from MGE specification (Sussel et al. 1999). However, despite preserved *Nkx2.1* expression, expression of *Cux2* and *Lhx7(8)* are clearly reduced (Fig. 1). *Cux2* is expressed in tangentially migrating interneurons (Cobos et al. 2006), and its function is linked to the development of reelin-expressing interneurons (Cubelos et al. 2008). *Lhx7(8)* is expressed in the SVZ of the ventral MGE (Flames et al. 2007) and its derivatives in the pallidum and striatal interneurons where it is required for the cholinergic phenotype (Zhao et al. 2003; Mori et al. 2004; Fragkouli et al. 2005).

Lhx6 is expressed in MGE progenitors and in pallidal neurons, striatal interneurons, and pallial interneurons, and it promotes tangential migration, integration into the cortical plate, and differentiation of somatotstatin^{+} and parvalbumin^{+} interneurons (Sussel et al. 1999; Marin et al. 2000; Alifragis et al. 2004; Liodis et al. 2007; Zhao et al. 2008). Its expression is reduced in the MGE of the *Dlx1&2*$^{-/-}$ mutant (Fig. 1; Petryniak et al. 2007) and is reduced in the cortex, secondary to reduced interneuron migration (Cobos et al. 2006).

Here we report a large *Lhx6*$^{+}$ ectopia in a superficial part of the dCGE (Fig. 1; Supplementary Fig. 2), suggesting that these cells correspond to interneurons that have failed to migrate to the cortex. However, this ectopia is also *Nkx2.1*$^{+}$ (Fig. 2; Supplementary Fig. 2). As *Nkx2.1* is not expressed in pallial interneurons (Sussel et al. 1999), this suggests several interesting possibilities, including the following: 1) *Dlx1&2* are required to repress *Nkx2.1* in interneurons—perhaps persistent *Nkx2.1* expression contributes to the defect in tangential migration and 2) this ectopia could be a misplaced GP. We think that this hypothesis is less likely because the ectopia also expresses *Nphx*, a marker of tangentially migrating interneurons and not of the GP, and because the ectopia does not express the following GP markers: *Arx*, *Dlx1*, *Gbx1*, *Lhx6*, *Lhx7(8)*, *Oct6 (POU3F1)*, *ROR-beta*, *TCF4*, *Tshz2*, and *Zfp521 (Evi30)* (Fig. 1). There are other ectopia in the CGE and MGE that are located outside of the *Lhx6/Nkx2.1/Nphx* ectopia and that express *ER81*, *ErbB4*, *NP2*, *Prox1*, and *Sox11* (Supplementary Fig. 2); these may correspond to distinct subtypes of neurons that failed to disperse (for *NP2*, see Marín et al. 2001).

Transcriptional and Neurogenic Pathways Downstream of Dlx1&2 and Mash1

We studied the expression of TFs and selected other genes in the MGE and CGE in *Dlx1&2*$^{-/-}$, *Mash1*$^{-/-}$, and *Dlx1&2*$^{-/-}$; *Mash1*$^{-/-}$ mutants at E15.5, concentrating on genes whose expression persists in *Dlx1&2*$^{-/-}$ mutants (Supplementary Fig. 3). Expression of these genes fell into 4 general classes (Supplementary Table 3). Ongoing studies are aimed to establish whether these phenotypes are the result of direct transcriptional control. Several TFs continue to be expressed in the *Dlx1&2*$^{-/-}$;*Mash1*$^{-/-}$ mutants, albeit generally at lower levels, in the CGE (*Gsh1*, *Islet1*, *Olig2*, and *Sp9*) and MGE (*ER81*, *Islet1*, *Olig2*, and *Sp9*) (Supplementary Fig. 3); in addition, *GAD67* expression is weakly maintained. Thus, *ER81*, *Gsh1*, *Islet1*, *Olig2*, *Sp9*, or other TFs (i.e., *Gsh2* or *Nkx2.1*) may be maintaining some of the fundamental features of the embryonic basal ganglia in the triple mutant. Furthermore, whereas some telencephalic cell types are reduced in *Dlx1&2*$^{-/-}$, *Mash1*$^{-/-}$, and *Dlx1&2*$^{-/-}$;*Mash1*$^{-/-}$ mutants (striatal, pallidal, and pallial GABAergic, dopaminergic, and cholinergic neurons) (Marin et al. 2000; Long et al. 2007, 2009), oligodendrocyte generation is promoted (Petryniak et al. 2007). Finally, early stages of neurogenesis are dependent on *Mash1* (i.e., *Sox1*), and not on *Dlx1&2* (Supplementary Fig. 3). Thus, this analysis is beginning to dissect the complementary roles of *Dlx1&2* and *Mash1* in promoting the differentiation of the subpallium.

Non-TF Gene Dysregulation in Dlx1&2$^{-/-}$ *Mutants*

The subpallial progenitor zones produce GABAergic, cholinergic, and dopaminergic neurons, oligodendrocytes, and astrocytes. The *Dlx* genes are essential for the differentiation of many of these neurons (Marin et al. 2000; Yun et al. 2002; Long et al. 2007) and repress glial differentiation (Yun et al. 2002;

Petryniak et al. 2007). We have begun to identify some of the key effector genes whose expression is downstream (directly/indirectly) of *Dlx1&2*. Supplementary Figure 2 shows some salient examples; others are described in Cobos et al. (2007) and Long et al. (2009).

Dlx1&2 promote GABAergic differentiation through promoting expression of the enzymes that synthesize GABA: *GAD67 (Gad1)* and *GAD65 (Gad2)* and the pump that concentrates GABA in synaptic vesicles (*vGAT*) (Supplementary Fig. 1; Anderson et al. 1999; Stühmer et al. 2002; Long et al. 2007, 2009; Eisenstat D, Cobos I and Rubenstein JLR, unpublished data).

Alterations in migration may be contributed by reduced expression of cytokine receptors (*CXCR4* and *CXCR7*) and the neuregulin receptor, *ErbB4*. Migration defect may also be contributed by alterations in *Gucy1a3*, *NP2*, *Robo2*, *Sbb*, *Thbs*, and *Tiam2* expression (Supplementary Fig. 1). Defective differentiation of striatal and pallidal neurons is indicated by reduced expression of *Cad8*, *Robo2*, and *Sema3a* and several other genes (see Long et al. 2009).

Dlx1&2 repress several non-TFs in progenitor cells including *Dact1* and *PK2* (Supplementary Fig. 1; for additional genes, see Cobos et al. 2007 and Long et al. 2009); some of these are complementary to changes in the *Mash1*$^{-/-}$ mutant (Supplementary Fig. 3). These changes in progenitor properties are associated with persistent *CyclinD2* expression in the LGE/dCGE SVZ (Supplementary Fig. 1) and elevated proliferation in the SVZ of the LGE/dCGE (Anderson, Qiu, et al. 1997). On the other hand, there is reduced *CyclinD2* expression in the MGE SVZ, which is consistent with the reduce proliferation of this region (Anderson, Qiu, et al. 1997; Petryniak et al. 2007). We do not know why proliferation in the LGE/dCGE and MGE respond differently in the *Dlx1&2*$^{-/-}$ mutant.

Supplementary Material

Supplementary materials can be found at http://www.cercor.oxfordjournals.org/.

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Notes

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