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A Time and a Place for Nkx2-1 in Interneuron Specification and Migration

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Abstract

The homeobox transcription factor, *Nkx2-1*, plays multiple roles during forebrain development. Using restricted genetic ablation of *Nkx2-1*, in this issue of *Neuron*, Butt et al. show that *Nkx2-1* in telencephalic progenitors regulates interneuron subtype specification, while Nóbrega-Pereira et al. demonstrate that postmitotic *Nkx2-1* regulates migration and sorting of interneurons to the striatum or cortex by controlling the expression of the guidance receptor, Neuropilin-2.

Nkx2-1 Is a Multifunctional Transcription Factor

A single transcription factor can participate in multiple developmental events as cells progress down a particular neuronal lineage. For example, a specific transcription factor may specify neuronal fate in a progenitor cell and subsequently regulate processes such as migration or differentiation in a postmitotic neuron. Such distinct developmental roles have now been described for the homeobox transcription factor, *Nkx2-1*. *Nkx2-1* regulates the identity of neuronal progenitor cells, mediates neuronal subtype specification, and directs neuronal migration. *Nkx2-1* is expressed in the basal telencephalon as early as the 11 somite stage and maintains its expression in defined structural regions of the developing basal telencephalon including the septum, anterior entopeduncular area, and preoptic area as well as the medial ganglionic eminence (MGE), a subregion of the ventral embryonic germinal zones known as the ganglionic eminences (Sussel et al., 1999). Interestingly, unlike the *Dlx* homeobox transcription factors, which are expressed in the lateral ganglionic eminence (LGE), caudal ganglionic eminence (CGE), and MGE (Flames et al., 2007), *Nkx2-1* is absent from the LGE and CGE (Sussel et al., 1999), suggesting a specific role in MGE neurogenesis.

The observation that *Nkx2-1* is expressed in the MGE ventricular and sub-ventricular progenitor zones as well as in postmitotic cells provided an early clue that *Nkx2-1* could play multiple roles in MGE neurogenesis (Sussel et al., 1999). In this issue of *Neuron*, Butt et al. (2008) and Nóbrega-Pereira et al. (2008) build upon our previous understanding of *Nkx2-1* by describing the critical role that *Nxk2-1* plays during distinct temporal windows in the regional specification of the ventral telencephalon, fate determination of MGE progenitors, and sorting and migration of MGE-derived cells.

Nkx2-1 Helps Determine Interneuron Subtype Identity

GABAergic interneurons are remarkably diverse and are subdivided by morphology, connectivity, electrophysiology, and the expression of molecular markers (Markram et al., 2004). The majority of cortical interneurons can be classified by largely nonoverlapping

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expression of parvalbumin (PV), calretinin (CR), and sosmatostatin (SST). Most cortical interneurons are generated in the MGE and CGE, and their fates are determined by the place and time of their specification. PV- and SST-expressing interneurons are generated first and arise primarily from the MGE, while CR- and VIP-expressing interneurons are born later and arise in the CGE (Butt et al., 2005; Fogarty et al., 2007). In this issue of *Neuron*, Butt et al. (2008) demonstrate that *Nkx2-1* controls the regional identity of MGE progenitors and influences the cell-fate specification of MGE-derived interneurons in a temporally defined manner.

Butt et al. (2008) use a conditional loss-of-function approach to determine the role of *Nkx2-1* in the specification of interneuron subtypes. Using a tamoxifen-inducible Cre recombinase under the control of the *Olig2* locus in combination with a Cre-responsive fluorescent reporter, the authors obtain temporal control over the removal of *Nkx2-1* and the ability to genetically mark the recombined cells. This approach expands previous work by allowing the authors to examine the long-term fate consequences of *Nkx2-1* loss of function in vivo rather than embryonically or in culture systems as necessitated by the perinatal lethality of *Nkx2-1* knockouts (Sussel et al., 1999; Xu et al., 2004).

Previous studies using *Nkx2-1* null mice have shown that constitutive loss of *Nkx2-1* leads to a shrinkage of the MGE (MGE*) as well as a ventral-to-dorsal transformation of the MGE* with the MGE acquiring LGE markers and producing LGE derivatives, such as the caudate putamen, at the expense of MGE derivatives such as the globus pallidus, cortical interneurons, and cholinergic striatal neurons (Sussel et al., 1999). Similarly, Butt and colleagues (2008) observe that early loss of *Nkx2-1* function favors the production of typically LGE-derived striatal medium spiny neurons at the expense of MGE-derived inhibitory cortical interneurons. These animals develop spontaneous seizures and abnormal behavior, presumably due to the reduction in inhibitory cortical activity. Interestingly, loss of *Nkx2-1* at E9.5, E10.5, or E12.5 did not lead to the reduction in size of the MGE seen with the traditional knockout suggesting a very early role for *Nkx2-1* in the initial formation of the MGE (before E9.5) and then slightly later (E9.5–E12.5) in the specification of MGE identity.

In order to interrogate the postnatal subtype specification of cortical interneurons, Butt et al. (2008) utilize their conditional knockout approach combined with immunohistochemistry and electrophysiological recordings. Previous studies have used in vitro and in vivo transplantation of MGE cells from *Nkx2-1* knockout mice to establish the necessity of this transcription factor for specifying both the PV and SST interneuronal classes (Du et al., 2008; Liodis et al., 2007; Xu et al., 2004). Consistent with these studies, both early and later (E9.5–E12.5) loss of *Nkx2-1* causes a significant cell-autonomous decrease in MGE-derived subtypes (fast spiking and nonfast spiking PV^+ and SST^+ interneurons). Interestingly, Butt and colleagues (2008) extend our previous understanding by finding a concomitant increase in CGE-derived subtypes (adapting and late spiking CR^+ and VIP^+ interneurons). Furthermore, loss of *Nkx2-1* at E12.5 does not reduce the number of GABAergic cortical neurons or produce abnormal behavior as do the earlier manipulations.

Taken together, this new study suggests that *Nkx2-1* plays a central role in the temporal specification of ventral telencephalic progenitors and acts as a molecular determinant for neuronal subtypes (Figure 1). Early on, *Nkx2-1* is involved in the structural formation of the MGE (Sussel et al., 1999). From E9.5–E10.5 *Nkx2-1* is critical for specifying the molecular identity of the MGE as a source of cortical interneurons and not LGE derivatives, such as striatal medium spiny neurons, and also acts to repress a CGE identity and the production of CGE derivatives such as VIP-and CR-expressing interneurons. However, the decision to make GABAergic cortical interneurons seems be independent of *Nkx2-1* by E12.5.

Thus the activation and inhibition of multiple *Nkx2-1* targets seems to induce MGE identity while repressing the neighboring fates of the LGE and CGE. The dual actions of *Nkx2-1* might be reflected by its ability to act as both a transcriptional repressor and activator. The TN domain near the N terminus of *NKX2-1* might recruit transcriptional repressors, while the C-terminal NK2-SD domain could function in transcriptional activation. Thus, *Nkx2-1* might direct "MGE-like" neurogenesis by activating MGE-specific genetic programs while repressing LGE/CGE programs. In support of this notion, *Nkx2-1* maintains the expression of MGE molecules such as *Lhx6*, *Lhx7*, and *Shh* and represses LGE markers such as SCRIP and GOLF1 and CGE markers such as CoupTFII. *Lhx6* acts as a downstream effector of *Nkx2-1* in the specification of PV- and SST-expressing interneurons, since *Nkx2-1* directly regulates *Lhx6* expression. *Lhx6* null mice have defects in PV and SST interneuron formation, and ectopic expression of *Lhx6* in *Nkx2-1* null mice can rescue PV and SST interneuron generation (Du et al., 2008; Liodis et al., 2007).

Ventral LGE and dorsal MGE markers such as *Nkx6–2* and *Gli1* are expanded throughout the MGE of early conditional *Nkx2.1* knockouts (Butt et al., 2008). Recent molecular marker analysis of MGE, LGE, and CGE progenitor domains suggests that the CGE may not contain progenitor pools that are intrinsically different from those found in the LGE (Flames et al., 2007). This suggests that CR-producing progenitors within the CGE could be defined as extensions of the caudal pole of the LGE. Together with the fact that *Nkx6–2* progenitors give rise to cortical CR^+ interneurons (Fogarty et al., 2007), an alternative interpretation of Butt et al. (2008)'s data could be that expansion of *Nkx6–2* expression throughout the MGE causes a transformation from ventral MGE-derived neurons to dorsal MGE-derived and/or ventral LGE-derived neurons. Thus, rather than an MGE to CGE transformation, conditional loss of *Nkx2-1* function may cause an MGE to LGE transformation along the entire anterior-to-posterior axis. The identification of CGE-specific markers and additional experiments that define the function of *Nkx6–2* in interneuron specification are needed to address these alternatives.

Nkx2-1 Helps Direct Interneuron Migration

Not only has *Nkx2-1* been linked to regional specification and cell-fate determination, but a role in cellular migration is also coming to light. In their studies of cell-fate determination, Du et al. (2008) suggest that *Nkx2-1* is a direct activator of the LIM-homeodomain-containing transcription factor *Lhx6*, previously shown to be absent in *Nkx2-1* knockout mice (Sussel et al., 1999). Interestingly, loss of *Lhx6* causes a delay in tangential interneuron migration and disrupts the distribution of interneurons within the cortex (Alifragis et al., 2004; Liodis et al., 2007; Zhao et al., 2008). Mechanistically, *Lhx6* may mediate these effects by regulating the expression of receptors including BrbB4, CXCR4, and CXCR7 (Zhao et al., 2008). In conjunction with the respecification of MGE precursors into LGE derivatives, the reduction in GABA-positive neurons in the cortex of *Nkx2-1* null mice may be explained by a migratory defect due to the loss of *Lhx6* expression in these mice (Du et al., 2008; Sussel et al., 1999). However, Nóbrega-Pereira et al. (2008) uncover a function for *Nkx2-1* in the migration and targeting of both cortical and striatal interneurons through the direct regulation of chemorepulsive signaling molecules.

Nóbrega-Pereira et al. (2008) expand our understanding of *Nkx2-1* by addressing its postmitotic role in sorting striatal and cortical interneurons (Figure 1). *Nkx2-1*-expressing progenitors generate two types of interneurons: those that downregulate Nkx2-1 and migrate to the cortex and those that maintain *Nkx2-1* expression and travel to the striatum (Nóbrega-Pereira et al., 2008; Sussel et al., 1999). The authors manipulate this sorting decision by overexpressing NKX2-1 in the MGE of organotypic slice cultures. NKX2-1 overexpression leads to an accumulation of cells in the basal ganglia and a reduction in cells that reach the neocortex.

Interestingly, they show, using structure-function analysis, that the homeodomain is required for this phenotype while the N-terminal repressor domain and the C-terminal activator domains are not necessary.

To extend these findings to a loss-of-function paradigm, Nóbrega-Pereira et al. (2008) created conditional *Nkx2-1* knockout mouse using a Cre-recombinase under the control of the *Lhx6* promoter, which turns on in postmitotic MGE-derived cells. Surprisingly, the loss of *Nkx2-1* postmitotically has a profound effect on the number of PV^+ and cholinergic $ChAT^+$ neurons that migrate to the striatum. There is no detectable increase in the number of cortical MGE-derived cells or in cell death, and thus the final distribution of these cells still needs to be determined. Interestingly, in contrast to *Nkx2-1*'s function in MGE-derived progenitors as a fate determinant of PV, SST, and cholinergic neurons, the number of SST neurons is unaffected following postmitotic loss of *Nkx2-1* (Butt et al., 2008; Du et al., 2008; Sussel et al., 1999; Xu et al., 2004).

Nóbrega-Pereira et al. (2008) provide a mechanism for the sorting phenotype by showing that postmitotic loss of *Nkx2-1* inhibits migration to the striatum by disinhibiting the expression of the Semaphorin receptor Neuropilin-2 (*Nrp2*). Semaphorin-3A and Semaphorin-3F (*Sema3A/F*) are expressed in the striatum and exert a repulsive force on *Nrp1*- and *Nrp2*-expressing cells destined for the neocortex (Marin et al., 2001). When overexpressed in MGE-derived cells, NKX2-1 prevents *Sema3A/F*-mediated repulsion and downregulates *Nrp2* directly by binding the promoter in a homeodomain-dependent manner. In support of this finding, Butt et al. (2008) also report a substantial loss of striatal interneurons in their conditional *Nkx2-1* loss-of-function manipulations that target progenitors rather than postmitotic neurons.

Homeobox Transcription Factors in Neuronal Specification, Migration, and Connectivity

Together, Butt et al. (2008) and Nóbrega-Pereira et al. (2008) paint a picture in which *Nkx2-1* plays a primary role in directing the formation and identity of the developing MGE as well as the postmitotic fate and migration of MGE-derived cells. Is there a precedent for other homeobox proteins to factor in such a wide range of cellular processes? The *Dlx1* and *Dlx2* homeobox transcription factors are also expressed by progenitors within the ventral telencephalon, regulate the fate and migration of ventral precursors, and promote GABAergic interneuron differentiation. Similar to NKX2-1, DLX1, and DLX2 bind to the *Nrp2* promoter and inhibit transcription of *Nrp2* (Le et al., 2007). However, in contrast to *Nkx2-1*, Dlx1&2-mediated inhibition of *Nrp2* is predicted to promote interneuron migration toward the cortex (Le et al., 2007). Together, these studies suggest cortically directed interneuron migration requires the controlled timing and expression of guidance molecules. Whether *Dlx1&2* and *Nkx2-1* functionally interact within interneurons to regulate migration remains to be determined.

Furthermore, *Dlx1&2* also play a critical role in directing MGE progenitors toward the GABAergic interneuron fate as their expression is necessary to repress precocious oligodendrocyte precursor formation (Petryniak et al., 2007). After specification, DLX proteins promote GABAergic interneuron differentiation likely through the transcriptional activation of interneuron-specific genes such as glutamic acid decarboxylase, the key enzyme in GABA synthesis. In this role, DLX homeobox proteins may establish general interneuronal genetic programs while *Nkx2-1* provides subtype specification. Further work to understand the interaction of these and other transcription factors in interneuron specification, migration guidance, and connectivity will provide interesting avenues of future research.

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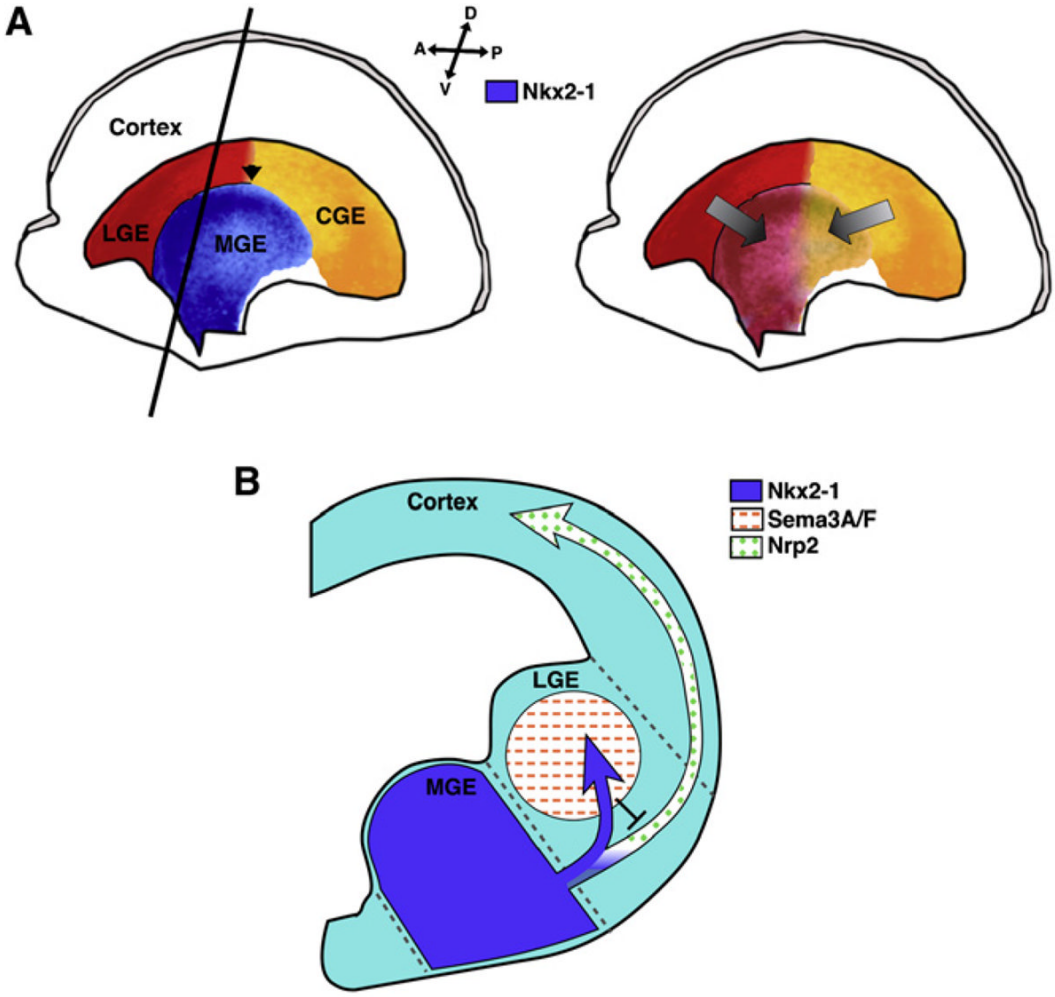

Figure 1.

(A) (Left) Schematic of an E12.5 mouse brain highlighting the three major proliferative zones of the ventral telencephalon. *Nkx2-1* expression defines the medial ganglionic eminence (MGE, blue). Dorsal to the MGE and separated by an anatomical sulcus is the lateral ganglionic eminence (LGE, red). Posterior to the end of the sulcus (arrowhead) where the MGE and LGE fuse is the caudal ganglionic eminence (CGE, orange). *Nkx2-1* is also expressed in the septum and preoptic area (not shown). The straight line delineates the coronal slice shown in (B). (Right) Early and late removal of *Nkx2-1* expression in MGE progenitors changes the fate of MGE-derived precursors in a temporally distinct manner such that they acquire LGE and CGE characteristics. See Butt et al. (2008) for details.

(B) Schematic of a coronal section of a developing mouse brain highlighting the routes of migration for MGE-derived *Nkx2-1*-expressing interneurons. One stream of interneurons maintains expression of Nkx2-1 (blue) and migrates to the striatum while the other downregulates *Nkx2-1* expression and migrates to the cortex. Nobrega-Pereira et al. (2008) show that *Nkx2-1* expression underlies the sorting mechanism for cortical and striatal interneurons by repressing Neuropilin-2 receptor expression (*Nrp2*, green dots). *Nkx2-1*$^{-}$/*Npn2*$^{+}$ cells are repelled by the expression of Semaphorin-3A and -3F (*Sema3A/F*, red bars) in the striatum and migrate to the cortex.