The origin and specification of cortical interneurons

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Abstract | GABA-containing interneurons are crucial to both the development and function of the cerebral cortex. Unlike cortical projection neurons, which have a relatively conserved set of characteristics, interneurons include multiple phenotypes that vary on morphological, physiological and neurochemical axes. This diversity, and the relatively late, context-dependent maturation of defining features, has challenged efforts to uncover the transcriptional control of cortical interneuron development. Here, we discuss recent data that are beginning to illuminate the origins and specification of distinct subgroups of cortical interneurons.

Fate-mapping approaches
Experiments that are designed to determine the relationship between the origin or genetic make-up of a cell and its differentiated fate.

Telencephalon
The anterior-most region of the neural tube, consisting of the cerebral cortex, basal ganglia, hippocampus, septal nuclei and olfactory bulb.

Subpallium
The regions of the telencephalon ventral to the cerebral cortex, including the basal ganglia.

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The proper functioning of the cerebral cortex requires neural networks produced by projection neurons and interneurons that primarily use the neurotransmitters glutamate and GABA (γ-aminobutyric acid), respectively. Interneurons, the ~25% of cortical neurons that have smooth or sparsely spiny dendrites and locally projecting axons, have a vital role in modulating cortical output and plasticity[1,2]. Cortical interneurons have also been implicated in developmental processes, including the regulation of neuronal proliferation and migration during corticogenesis and the development of cortical circuitry[3,4].

Despite their prominent roles in cortical function, progress in understanding interneuron fate determination has been relatively slow. Cortical interneurons accomplish specific functions through a remarkable diversity of subtypes, which are variably defined by their morphological, physiological and molecular characteristics[5] (BOX 1). This diversity, along with the attainment of subtype-defining characteristics only after weeks of postnatal maturation, has hindered efforts to connect the embryonic development of cortical interneurons to their differentiated fate. Consequently, little was known about the generation of cortical interneuron diversity until improved fate-mapping approaches and transgenic mice became available.

Here, we discuss recent developments in the study of the origins of cortical interneurons. The identification of distinct origins for interneuron subgroups has begun to drive studies of the transcriptional control of interneuron diversity. As such, this field is poised for a burst of discoveries that will have profound implications for our understanding of cortical function and dysfunction.

Origins of cortical interneurons

Whereas cortical projection neurons derive from the dorsal (pallial) telencephalon and migrate radially into the cortical mantle zone, immunolabelling for GABA late in rodent gestation has revealed streams of cells migrating tangentially across areal boundaries of the developing cerebral cortex[6]. Fluorescent dye labelling of cultured telencephali found a robust migration of cells from the subpallium into the overlying cortex[7], a result that is consistent with immunolabelling for the transcription factor distal-less homeobox 2 (DLX2) (REF. 8). Subsequent analyses of *Dlx1*/*Dlx2* mouse mutants, together with *in vivo* ablation experiments and co-labelling of migrating cells in slice cultures, suggested that this migration includes cortical interneurons[9–12]. Tangential migrations of putative interneurons have been identified in several mammalian species including mice[9,13], rats[7,14], ferrets[15] and humans[16] (for a recent review on interneuron migration, see REF. 17). In rodents and ferrets the subpallium appears to be the primary source of cortical interneurons, whereas one study reported that in human embryos most cortical interneurons undergo their terminal mitosis in the cortical subventricular zone[16]. Here, we address distinct regions within the telencephalon that have been implicated as origins of cortical interneurons, with a particular emphasis on the neurochemically defined interneuron subgroups that those regions generate (FIGS 1,2; TABLE 1).

Medial ganglionic eminence. Initial studies of subcortical to cortical migration showed labelled cells within the lateral ganglionic eminence (LGE)[7,9,10]. However, these studies did not distinguish between cells originating

Box 1 | Classification of cortical interneurons

Since the time of Cajal, who described the "cells with short axons" in cortical sections stained by the Golgi method[117], scientists investigating the cerebral cortex have studied neurons that are generally characterized by a locally projecting axon, aspiny or sparsely spiny dendrites, a cell soma that is smaller than most cortical pyramidal neurons of the same species, and that contain the neurotransmitter GABA (γ-aminobutyric acid). As new techniques have been developed, these cells, known as interneurons, have been characterized by morphology, connectivity, neurochemistry and the expression of ion physiology/channels[118]. Efforts to classify interneurons are underway, but are complicated by the variability found on all four axes listed above, to the point at which it has been suggested that in some respects interneurons exist as a continuous spectrum of characteristics rather than in distinct groups or types[119]. Both the extensive clustering of characteristics into distinct subgroups[24,27,118,120], and the differential origins and molecular specification of interneuron subgroups discussed here, suggest that there are indeed distinct subgroups and subtypes of interneurons. Here, subgroups are mainly distinguished by the expression of markers that are not necessarily relevant to their function. Although this approach has proved useful for initial efforts to connect interneuron origins and the effectors of their fate with a mature phenotype, the ultimate goal is to develop a transcriptional code for the generation of interneuron subgroups defined not by arbitrary marker proteins, but by their effects on postsynaptic neurons and neural networks.

within the LGE itself and those that had been generated elsewhere. Indeed, fluorescent dye labelling of the more ventrally located medial ganglionic eminence (MGE) in telencephalic slice cultures revealed a large stream of cells, many of which contain GABA, migrating to the neocortex[14]. Mice lacking the homeobox transcription factor *Nkx2.1*, in which normal MGE tissue fails to form, are missing this migration and have a ~50% reduction of GABA-positive cells in the neocortex just before birth compared with wild-type mice[18]. Comparisons of the migratory behaviour of LGE- and MGE-derived cells *in vitro* and *in vivo* have shown that MGE cells have a far greater propensity to migrate into the cortex[13,19,20].

As cortical interneurons in rodents mature over several weeks of postnatal development, slice culture experiments have proved inadequate to examine whether there are distinct origins of interneuron subtypes. Subsequent experiments involving transplants of genetically labelled MGE progenitors *in utero* into the embryonic MGE[19,21], the lateral ventricle[22], or *in vitro* onto a cortical feeder layer[23] showed that most MGE-derived interneurons contain either parvalbumin (PV) or somatostatin (SST). This expression defines two distinct neurochemical subgroups that also tend to have distinct physiological characteristics and connectivities, and that together comprise ~60% of the cortical interneurons in mice and rats[24,25] (FIG. 2). By contrast, these studies rarely found MGE-derived interneurons that contain calretinin (CR), a calcium binding protein that is largely non-overlapping with the SST or PV subgroups and is primarily labelled in cells with a vertically orientated, bipolar or bitufted morphology[26,27]. This suggests that most CR-positive interneurons originate from a spatially or temporally distinct source from those that contain PV or SST.

Caudal ganglionic eminence. In addition to the MGE, the caudal ganglionic eminence (CGE) is the other subcortical structure that is most strongly implicated in the generation of cortical interneurons[20,28,29]. Morphologically, the CGE exists as a fusion of the more rostral MGE and LGE, beginning at the coronal level of the mid- to caudal thalamus. There are two distinct molecular domains within the CGE that closely resemble caudal extensions of the MGE and LGE. The ventral-most CGE, like the MGE, expresses *Nkx2.1*. By contrast, the dorsal domain that protrudes into the lateral ventricle strongly expresses the transcription factor *Gsh2*, which is required for the proper patterning of the LGE[30], and *ER81*, a transcription factor that is expressed in olfactory bulb progenitors both in the LGE and in the adult subventricular zone[31].

Initial fate-mapping experiments with *in utero* isochronic, homotopic transplants of dissections that included both dorsal and ventral CGE at embryonic day (E) 13.5 showed that the CGE gives rise to deep-layer cortical interneurons, many of which contain PV or SST, but not CR[28]. This lack of CR-positive cells might be due to the timing of the experiment, as dissociation and/or transplantation of telencephalic progenitors appears to drive those cells out of the cell cycle, and in mice nearly all CR-positive interneurons undergo their final S-phase of the cell cycle after E14.5 (REF. 23). Indeed, selective dissection of the dorsal CGE (dCGE) at E14.5 gives rise to many CR-positive, bipolar cells after plating on a cortical feeder layer[23]. In addition, *in utero* isochronic, homotopic transplants of the dCGE at E15.5 generate CR-positive interneurons in the juvenile cortex that show distinct spiking characteristics indicative of that interneuron subgroup[21]. Finally, a recent analysis of a *Gad65*-GFP (green fluorescent protein) transgenic mouse line together with explant cultures also suggested that many cells migrating from the CGE become vertically orientated CR-containing interneurons[32].

Taken together, these data suggest that CR-positive interneurons are primarily generated in the NKX2.1-negative region of the dCGE. However, the ventral CGE might generate PV or SST-containing interneurons, although the caudal migration of MGE-born progenitors through the CGE en route to the cortex complicates this scenario[21,33]. Finally, evidence from studies on rats shows colocalization of SST and CR in a subset of deep-layer Martinotti cells[34]. Whether these cells derive from the MGE along with most SST interneurons or from the dCGE along with other CR interneurons remains to be seen. However, transplants of MGE progenitors directly into the neonatal cortex show rare but significant colocalization of SST and CR (C.P.W. and S.A.A., unpublished observations), suggesting at least a partial MGE contribution to this subgroup (FIG. 2).

Lateral ganglionic eminence. Although several studies have indicated that any LGE contribution to cortical interneurons is far smaller than that of the MGE[13,19,20], evidence in support of some LGE contribution deserves mention. First, *Nkx2.1* mutants lack normal MGE tissue, yet show only a ~50% reduction of cortical GABA-containing cells at E18.5 (REF. 18). Although this could be the result of an enhanced generation of CR-positive cells from dCGE-like neuroepithelium, in these mutants the LGE-like region also shows robust migration to the cortex at E15.5 (REFS 20,29). Second, slice culture

Parvalbumin
(PV). A calcium-binding protein that is localized to, and potentially acts as an endogenous buffer for, fast-spiking cortical interneurons.

Somatostatin
(SST). A neuropeptide that is localized to a subset of cortical interneurons.

Calretinin
(CR). A calcium-binding protein that is localized to a subset of cortical interneurons.

S-phase of the cell cycle
The phase of the cell cycle during which DNA replication takes place.

Bipolar cells
Small cells with narrow dendritic arborizations that extend vertically, often across the entire cortical thickness.

Martinotti cells
Cells containing axons that project towards cortical layer I, and that primarily target the distal-most dendrites of pyramidal neurons.

experiments in which progenitors were labelled with the S-phase marker bromodeoxyuridine (BrdU) suggested that a small number of LGE-derived cells, some of which co-label for GABA, do migrate from the LGE to the cortex[20]. Finally, explants taken from rat embryos in which the MGE has been removed continue to show robust migration from the LGE to the cortex, implying that the observed migration is not simply due to MGE cells migrating through the LGE[35]. One possible explanation for these mixed results is the relatively pleiotropic nature of the morphologically defined LGE, which consists of distinct progenitor domains along the dorsoventral axis that give rise to the lateral cortex, olfactory bulb interneurons and medium spiny striatal projection neurons[31]. In addition, migration from the LGE to the cortex has been shown to include oligodendrocytes after E14.5 (REF. 36). To summarise, the current data support at best a minor contribution from the LGE to the cortical interneuron population. The identities of these cells are not known, but do not seem to include many that contain SST, PV or CR[23].

Rostral migratory stream. In contrast to cells from the LGE, cells taken from the rostral migratory stream (RMS) at postnatal day (P) 0 can express *CR* when cultured on cortical feeder cells[23]. However, given that nearly all CR-positive cortical interneurons in the somatosensory cortex at P25 are born before E16.5 (REF. 23), it is difficult to assess the relevance of this finding. It might simply be a case of CR-positive interneurons of the olfactory bulb showing the capacity to survive and differentiate in a cortical environment. However, it is also possible that cells could leave the RMS prior to reaching the olfactory bulb and instead migrate to the cortex. In support of this model, immunohistochemical labelling for DLX1, which labels migrating interneuron precursors in the RMS, seems to label cells migrating from the RMS to the cortex[37]. Earlier migration from the rostral neuroepithelium of the lateral ventricle into layer I of the cortex has also been described for cells containing CR, calbindin and GABA[38–40]. Furthermore, immunohistochemical labelling for polysialic acid–neural cell adhesion molecule (PSA–NCAM) in adult rabbits reveals cells migrating from the RMS into the frontal cortical parenchyma[41]. Taken together, these results suggest the involvement of the RMS in the generation of cortical interneurons, with a particular emphasis on the subgroup that contains CR.

Septal region. Another subpallial region that might contribute interneurons to the cerebral cortex is the septal area. Initial speculation about the occurrence of migration from the septal region to the cortex was based on immunohistochemical labelling for DLX1 (REF. 37). More convincing evidence comes from the recent analysis of mouse mutants lacking the homeodomain-containing transcription factor *Vax1*, which is expressed in a pattern similar to that of *Dlx1* and *Dlx2* within the subcortical telencephalon[42]. At birth, *Vax1* mutants have a 30–44% reduction in GABA-containing cortical neurons compared with wild-type mice, with the greatest loss occurring in the rostral-most cortex. Whereas the MGE is reduced in size, the septal region is almost completely absent in these mutants. Experiments conducted using slice cultures show cells migrating from the ventrolateral septum into layer I of the rostral cortex; this migration is lost in the *Vax1* mutants. These data therefore provide evidence for a septal contribution to the cortical interneuron population, although further work is needed to definitively show this migration. These results might help to explain the large-scale migration of later-born interneurons from layer I into the cortical plate[40,43]. Whether this migration represents distinct subtypes of interneurons remains to be explored.

Cortex. Although several reports have shown that cultures of dorsal telencephalic progenitors have the capacity to generate GABA-containing cells[44–47], little evidence supports a cortical origin of cortical interneurons in rodents[23]. In ferrets, focal injections of the S-phase marker tritiated thymidine into the cortical proliferative zone at P1, when neurogenesis for the superficial cortical layers is ongoing, labelled many glutamatergic pyramidal neurons but essentially no neurons that contained GABA, PV, SST or CR when examined at P40 (REF. 15).

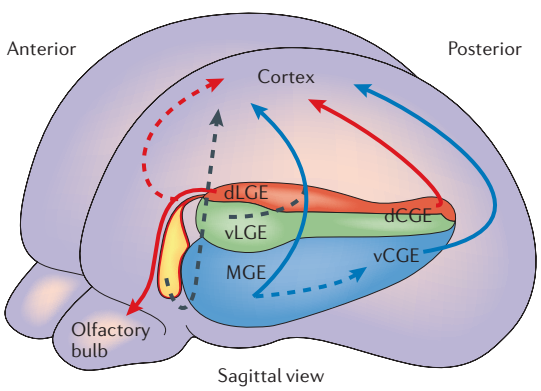

Figure 1 | **Migration pathways of cortical interneuron subgroups from the ventral telencephalon.** Schematic illustration of established (solid arrows) and proposed (dashed arrows) pathways of migration of cortical interneurons. Blue arrows represent somatostatin (SST)- or parvalbumin (PV)-containing interneuron progenitors. Red arrows represent calretinin (CR)-containing interneuron progenitors. Black arrows represent the potential migration of cortical interneuron progenitors, the subtype of which has yet to be determined. SST and PV interneurons primarily migrate from the *Nkx2.1*-expressing domain of the medial ganglionic eminence (MGE) and might also arise from the ventral caudal ganglionic eminence (vCGE), which also expresses *Nkx2.1* (blue shading). These interneurons have also been shown to migrate caudally from the MGE into the CGE[21,33]. CR interneurons primarily arise from the dorsal CGE (dCGE). The dorsal lateral ganglionic eminence (dLGE) expresses *ER81* (red shading) and also generates CR interneurons destined for the olfactory bulb, which might contribute to the cortical CR interneuron population. Progenitors from the ventral LGE (vLGE; green shading) also migrate to the cortex, and could represent an undetermined subgroup of cortical interneurons. Finally, *Vax1*-expressing progenitors of the septal region (yellow shading) might generate cortical interneurons of an unknown subgroup[42].

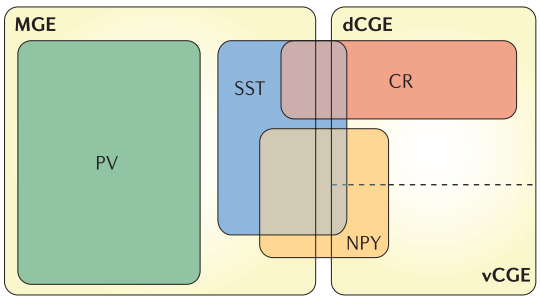

Figure 2 | **Relative contributions of the MGE and CGE to cortical interneuron neurochemical subgroups.** Parvalbumin (PV)-containing interneurons derive almost entirely from the medial ganglionic eminence (MGE). Somatostatin (SST)-containing interneurons are primarily generated in the MGE[23], but could also derive from the ventral caudal ganglionic eminence (vCGE) and the dorsal CGE (dCGE)[21]. Neuropeptide Y (NPY)-containing interneurons, which overlap ~50% with SST interneurons in the cortex, also derive from the MGE and vCGE, although the dCGE also generates a significant number of NPY cells that are not colocalized with SST[21]. Whether this represents a separate subgroup of NPY cells remains to be seen (FIG. 3). Calretinin (CR)-containing interneurons primarily originate in the dCGE. In certain interneurons (Martinotti cells) calretinin is colocalized with SST[34], which might represent a small contribution of the MGE to the overall calretinin interneuron population.

However, retroviral labelling of slice cultures of human embryonic forebrain suggested that most GABA-positive cells in the human cortex originate from mitoses in the cortical subventricular zone[16]. This intriguing finding awaits replication in humans and non-human primates, but is supported by the observation that *Nkx2.1*, a gene that is required for the specification of the MGE-derived interneuron subgroups in mice (see below), is strongly expressed in the cortical proliferative zone in humans but not in rodents[48].

Finally, it is noteworthy that an additional population of cortical interneurons could be born within the postnatal cerebral cortex. A recent study by Dayer *et al.*[49] found that a small percentage of postnatally born cells (measured using BrdU incorporation) in the adult rat cortex showed immunoreactivity for the neuron-specific marker NeuN (33/7624), and that a subset of those cells contained GABA and either CR or calbindin. These newly born interneurons were suggested to originate from NG2-positive progenitors within the cortex itself. Although the small number of cells makes the contribution of postnatal interneuronogenesis to the overall interneuron population difficult to assess, this finding might represent an additional source of cortical interneurons. Interestingly, NG2-positive cells isolated from the early postnatal mouse brain are multipotent and capable of generating GABA-containing neurons when grafted into the hippocampus[50,51].

Birthdating of cortical interneurons

Unlike interneuron progenitors of the olfactory bulb, which continue to proliferate as they migrate from the dorsal LGE through the RMS[52,53], cortical interneurons in mice that are generated in the MGE complete the S-phase of the cell cycle prior to beginning their migration to the cortex[54–56]. Birthdating of GABA-containing interneurons in rodent and ferret cortices reveals an 'inside-out' pattern similar to that established for projection neurons of the same layer. Deeper layer interneurons tend to leave the cell cycle prior to those destined for the superficial layers[57–59]. This tendency might apply mainly to the more plentiful MGE-derived subgroups, as birthdating of CR-positive cells shows a lack of laminar colocalization with non-GABA-positive cells (presumed to be mainly projection neurons) in the mouse visual cortex[60]. Furthermore, in the superficial rat cortex, SST-containing interneurons tend to be born earlier than those that contain vasoactive intestinal polypeptide (VIP)[61,62], a neuropeptide found mainly in vertically orientated cells that frequently colocalize with CR[63,64].

Specification of cortical interneurons

The findings that cortical interneurons appear to be postmitotic on the initiation of migration, and that subgroups show characteristic differences in terms of both the origins and the birthdating–laminar location relationship, have important implications with regard to interneuron specification. Clearly, the progenitors of the MGE- and CGE-derived interneuron subgroups have molecular differences at the time that they exit the cell cycle, but are they committed at this point to a particular fate or do signals encountered later establish their potential for differentiating into a given subgroup? A comparison of two MGE progenitor fate-mapping experiments suggests that key aspects of interneuron subgroup fate are likely to be specified as the progenitors leave the cell cycle, and not by factors encountered during migration. In one set of experiments, the neurochemical fates of MGE or CGE progenitors were assessed 2–4 weeks after the cells were dissociated and plated on high-density feeder layers made from neonatal cortex[23]. In a second study from a different group, both the neurochemical and physiological characteristics of MGE or CGE cells were tested after homotopic, isochronic transplantation of genetically labelled cells back into the MGE or CGE *in utero*[21]. Remarkably, a strong bias for CR-positive cells to derive from the dCGE, and SST- and PV-positive subgroups to come from the MGE, was found in both studies. Moreover, there was a similar ratio of PV- to SST-containing cells from the MGE in both studies. These results suggest that the fate of interneuron subgroups, as has also been shown for MGE-derived interneuron laminar fate[22], is specified on the basis of the signals that they encounter at their origins. Given the interest in using cortical interneuron transplantations to repress medically intractable seizures[65], or even as a drug delivery system[13], these results are highly encouraging in that subcortical to cortical migration does not appear to be required for at least some aspects of differentiated interneuron fate.

So, what signals are responsible for the specification of dCGE- versus MGE-derived cortical interneurons? The molecular basis for the neuronal fate differences between the dCGE and the LGE is unclear, as the

Table 1 | **Summary of interneuron neurochemical subgroup characteristics in mice**

Neurochemical marker	Relative % of GABA+ cells	Characteristic morphology	Axonal targeting on projection neurons*	Intrinsic physiology‡
Somatostatin	~30%	Small basket	Proximal dendrites/soma	RSNP
		Martinotti	Distal dendrites	BSNP
Parvalbumin	~50%	Large basket	Proximal dendrites/soma	FS
		Nest basket	Soma	FS/RSNP
		Chandelier	Axon initial segment	FS
Calretinin	~15%	Small bipolar	Proximal dendrites; also, other GABA+ cells	RSNP/BSNP

*Refers to characteristic axon targets, although other targets may exist; ‡refers to firing patterns elicited from intracellular injections of depolarizing current; +, -positive; BSNP, burst-spiking non-pyramidal; FS, fast spiking; RSNP, regular-spiking non-pyramidal. Table based on REFS 5,21,24,25,27,34,115,118.

proliferative zones of both structures express essentially the same genes[28]. Nevertheless, there must be crucial differences because, unlike dCGE cells, LGE cells placed onto cortical feeder layers give rise to few CR-positive neurons[23]. One such difference could be an effector of the transcription factors *Dlx1* and *Dlx2*, closely linked genes that are expressed throughout the subcortical telencephalon and that are required for most subcortical to cortical interneuron migration[9,20]. Cortical cultures from *Dlx1*/*Dlx2* nulls have reduced numbers of PV- and SST-containing interneurons, which suggests a role for these transcription factors in the maintenance of interneurons in a migration-permissive state. By contrast, bipolar CR-positive interneurons are essentially absent in these cultures, suggesting that *Dlx1* and *Dlx2* are involved in the specification of these cells[23].

Unlike the intriguing lack of identified molecular differences between the dCGE and the LGE, both overlapping and distinct gene expression patterns have been identified between the LGE/dCGE and the MGE[30]. Chief among these is *Nkx2.1*, a homeodomain-containing transcription factor that is expressed in the proliferative zone of the MGE and in the more ventrally located preoptic region[66]. *Nkx2.1* is downregulated in cortical interneurons prior to their entry into the cerebral cortex, but is maintained at least to juvenile ages in subsets of striatal interneurons[67]. In *Nkx2.1*-null mice, normal MGE tissue fails to form and there is a ventral expansion of LGE-like tissue[18]. SST and neuropeptide Y (NPY), detectable in a small number of cortical interneurons at E18.5, are absent from the cortex of *Nkx2.1* nulls[20]. To determine the requirement for *Nkx2.1* in specifying other interneuron subgroups in these perinatal lethal mutants, cortices from embryos at E18.5 were dissociated and maintained for 2–4 weeks *in vitro*[23]. Consistent with studies on the interneuron fate potentials of MGE progenitors, PV, SST and NPY were present in cultures of wild-type cortex but absent in those from *Nkx2.1* nulls. Transplantation of the MGE-like region of *Nkx2.1* nulls onto cortical feeder cultures from normal mice also failed to give rise to the PV-, SST- or NPY-containing phenotypes, suggesting that *Nkx2.1* is required for the initial specification of these cell types. By contrast, bipolar CR-positive interneurons were plentiful in cortical cultures from *Nkx2.1* nulls, consistent with their origin in the *Nkx2.1*-negative dCGE.

SHH maintains Nkx2.1 and interneuron fate. The requirement for *Nkx2.1* in the specification of MGE-derived interneuron subgroups provides an anchor point for the search for interneuron fate-specifying factors that act upstream and downstream of this gene. Initial patterning of *Nkx2.1* expression in the ventral telencephalon involves the coordinated actions of the signalling molecules fibroblast growth factor 8 (FGF8) and sonic hedgehog (SHH)[68,69]. The transcription factor SIX3 might confer competence of the telencephalic tissue to respond to SHH by inducing *Nkx2.1* (REF. 70), and the repression of bone morphogenetic protein (BMP) signalling is also required for normal patterning of the *Nkx2.1* domain[71].

Although the patterning role of SHH is largely complete by E11.5 (REFS 72,73), some targets of SHH signalling, including NKX2.1, remain dependent on SHH for their maintenance in MGE progenitors well into the age range of cortical interneuronogenesis (in mice, roughly E12.5–E16.5). Reductions in SHH signalling in MGE progenitors, essentially all of which normally express *Nkx2.1*, result in both a large reduction in NKX2.1 detectability despite continued progenitor cycling and a reduction in the ability of these progenitors to generate PV- or SST-containing interneurons[56]. Remarkably, both the levels of NKX2.1 and the generation of SST-containing interneurons are rescued by the restoration of SHH signalling[56]. This result suggests that interneuron specification remains plastic during the age range of neurogenesis; therefore, interneuron generation could be altered by a variety of environmental conditions that effect the signalling of SHH, FGF8, BMPs and possibly other factors[46,74].

Interestingly, the mechanism by which SHH maintains *Nkx2.1* expression might differ from that involved in initial patterning. The substantial rescue of failed ventral telencephalic patterning in *Shh*$^{-/-}$;*Gli3*$^{-/-}$ double mutants suggests that SHH primarily contributes to patterning by inhibiting the formation of the GLI3 repressor[75]. However, a reduction in the expression of *Nkx2.1* in the MGE by the addition of the SHH signalling inhibitor cyclopamine still occurs in slices prepared from *Gli3*-null mutants[76].

Generation of interneuron diversity in the MGE. Whereas the presence of *Nkx2.1* distinguishes the origins of most PV- and SST-containing interneuron subgroups from that of the vertically orientated CR-positive

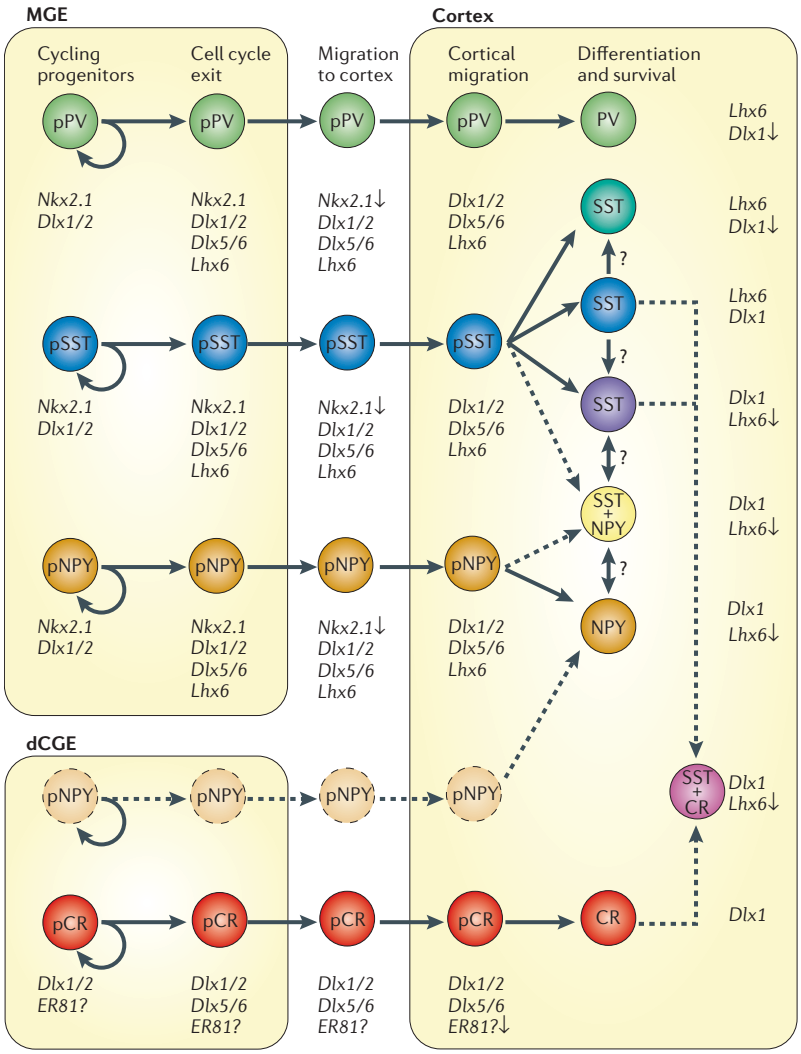

Figure 3 | **The transcriptional regulation of cortical interneuron specification and differentiation.** Transcription factor expression profiles throughout specification, migration and differentiation/survival of cortical interneurons deriving from the medial ganglionic eminence (MGE) and the dorsal caudal ganglionic eminence (dCGE). Most, if not all, interneuron progenitors from the MGE express *Nkx2.1* and *Dlx1/2*. As they leave the cell cycle, *Dlx5/6* and *Lhx6* also begin to be expressed. When they begin to migrate to the cortex, *Nkx2.1* expression is quickly downregulated (↓), while the expression of the *Dlx* genes is maintained. Finally, upon differentiation, parvalbumin (PV)-positive and a subset of somatostatin (SST)-positive interneurons maintain *Lhx6* expression and downregulate *Dlx1*, while other SST-positive along with neuropeptide Y (NPY)-positive interneurons maintain *Dlx1*. Calretinin (CR) progenitors from the dCGE also express *Dlx1/2*, but not *Nkx2.1*. In addition, the transcription factor *ER81* might have a role in CR interneuron specification. Upon differentiation in the cortex, CR-positive interneurons maintain expression of *Dlx1*. In the case of dCGE-derived NPY-containing interneurons, it is unclear whether they represent a genetically separate subgroup of progenitors from those found in the MGE (indicated by dashed lines). Similarly, it is unknown whether Martinotti cells, in which CR and SST are colocalized, derive from an *Nkx2.1*-positive lineage. p, a progenitor stage that does not yet express the denoted subgroup marker.

subgroup, little is known about the differential specification of PV- and SST-containing subgroups within the *Nkx2.1* lineage. One possibility, which would be analogous to the neuronal fate determination in the spinal cord[77], is that MGE-derived interneuron subgroups originate from distinct lineages that are separated on the dorsoventral axis. In fact, *Nkx6.2*, a transcription factor that contributes to oligodendrocyte generation in the ventral spinal cord[78], is selectively expressed in the dorsal-most region of the MGE[79], and is downregulated in CNS-specific *Shh* nulls that also have a large reduction of PV- and SST-containing cortical interneurons[56]. The same region expresses *Gli1* (REF. 80), a positive effector of SHH signalling, and hedgehog-interacting protein (*Hip*) (REF. 81), a gene that encodes a hedgehog binding protein[82], both of which tend to be strongly expressed in regions of elevated SHH signalling. Indeed, ongoing studies suggest that PV- and SST-containing interneuron subgroups arise primarily from distinct lineages that are segregated on the dorsoventral axis of the MGE (C.P.W. and S.A.A., unpublished observations).

As with the differential specification of MGE-derived interneuron subgroups, transcriptional regulators that effect interneuron development downstream of *Nkx2.1* are beginning to be appreciated. Chief among these are *Lhx6* and *Lhx7*, members of the LIM homeodomain family of transcription factors that are strongly expressed in the postmitotic mantle zone of the MGE and preoptic region[83]. In the spinal cord, LIM homeodomain genes regulate the specification of subgroups of motor neurons[84]. Both LHX6 and LHX7 are undetectable in the telencephali of *Nkx2.1* nulls[18]. LHX7 is required for the specification of cholinergic neurons, including the cholinergic interneurons of the striatum[85,86], but null mutants for *Lhx6* have not yet been reported. However, *Lhx6* is expressed in MGE-derived interneurons migrating to the cortex[14,87], and in most PV-containing and many SST-containing cortical interneurons in mature mice[88]. Transfection of MGE cells in slice culture with an RNA interference construct targeting *Lhx6* resulted in a reduction of interneuron migration into the cortex, but no alteration in the levels of GABA[89]. Ongoing studies suggest that although LHX6 is dispensable for the maintenance of GABA levels, it is sufficient for the rescue of PV and SST in transplanted, *Nkx2.1*$^{-/-}$ cells (T. Du and S.A.A., unpublished observations), suggesting that it promotes both cortical migration and the later aspects of MGE-derived interneuron differentiation.

Transcriptional control of interneuron maturation

Aside from the potential role for BMP in directing interneuron differentiation, indicated by *in vitro* assays[74], little is known about the postmitotic regulation of cortical interneuron differentiation and survival. A recent paper by Cobos *et al.*[88] made an important advance in understanding the genetic regulation of postnatal cortical interneuron development. By combining immunohistochemistry and *in situ* hybridization, the authors demonstrated that although the genes *Dlx1* and *Lhx6* are initially expressed in most cortical interneurons as they migrate from the ventral telencephalon, their expression becomes limited to specific subgroups during postnatal maturation (FIG. 3). Specifically, PV-containing interneurons maintain *Lhx6* expression but downregulate *Dlx1*, whereas CR-containing interneurons maintain *Dlx1* expression postnatally. The SST subgroup is slightly more complicated, as some express only *Lhx6*, others continue to express only *Dlx1*, and yet a third group

Table 2 | **Transcriptional regulation of cortical interneuron fate***

Mutant (gene)	Max. age analysed	Cortical interneuron phenotype	Other telencephalic phenotypes	References
Dlx1	P20–P60	Delayed ↓ GABA+; SST+; NPY+; CR+	nd	88
Dlx1/Dlx2	P0	Ncx: 75% ↓ GABA+; Hip[125]: ~100% ↓ GABA+	Striatal dysgenesis; ↓↓ GABA+ in olfactory bulb	9
Nkx2.1	E19.5	50% ↓ GABA+; no SST+; NPY+[20]	Normal MGE replaced by 'LGE-like' tissue	18
NsCre:*Shh*$^{Fl/Fl}$	P12	↓GABA+; ↓SST/PV+ in layers 5–6; ↓↓SST+; PV+ in layers 2–4	↑ excitatory neurons (*Tbr*+); ↓ interneurons in striatum	56
Mash1[126]	P0	↓ GABA+	Dysgenesis of striatum and ventral pallidum	126
Pax6	P0	↑ ventral → dorsal migration; cortical ectopias expressing *Dlx1/2*, *Gad67* and GABA[128]	Abnormal cortical migration, patterning and thalamic connectivity	127
Tailless	Adult	↓ CR+; ↓ SST+	↓ rhinencephalon; ↓ dentate	129
Emx2/Pax6	E16.5	↑↑ GAD65/67	Cortex re-specified as basal ganglia	130
Arx	E12.5–P3	↓ ventral → dorsal migration; no NPY+	Ectopic expression of *Nkx2.1* in the VZ of LGE and Ctx; ↓ cortical proliferation	96
Npas1/Npas3	Adult	↓ reelin in cortical interneurons (not uniform across cortex)	Abnormal social recognition; impaired pre-pulse inhibition	106

*This table lists mutations with effects specific to cortical interneuron fate specification. ↓, decrease in; ↑, increase in; +, -positive; CR, calretinin; Ctx, cortex; E, embryonic day; GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; Hip, hippocampus; LGE, lateral ganglionic eminence; MGE, medial ganglionic eminence; Ncx, neocortex; nd, not determined; NPY, neuropeptide Y; Ns, Nestin; P, postnatal day; PV, parvalbumin; *Shh*, Sonic hedgehog; SST, somatostatin; VZ, ventricular zone.

expresses both genes. It is not known whether this is indicative of three separate subclasses of SST interneurons, or whether neurons only transiently express both *Lhx6* and *Dlx1* before downregulating one or the other. It is also important to note that NPY-containing interneurons, a subgroup that partially overlaps with SST, postnatally express *Dlx1* but not *Lhx6* (FIG. 3). Although the role for continued *Lhx6* expression remains unclear, the postnatal expression of *Dlx1* is crucial as *Dlx1* mutants show a selective loss of CR-, NPY- and SST-containing interneurons, beginning around the fourth postnatal week. The transplantation of GFP-expressing MGE progenitors from *Dlx1* mutants into wild-type neonatal cortex further showed that this cell loss is due to a cell-autonomous requirement for *Dlx1* and is preceded by decreased dendritic length and branching. These findings could be the first to describe a transcription factor mutation that cell-autonomously alters the postnatal development of cortical interneurons.

Interneuronopathies

Progress in identifying the regulators of cortical interneuron fate and function is likely to advance our understanding of the causes of various diseases and help with the development of new treatments (TABLE 2). Although mutations of *Shh* and *Six3* have been linked to familial cases of holoprosencephaly, the phenotypes associated with these mutations in humans are far more varied. Studies of families that have mutations in *Shh* and *Six3* have revealed that microcephaly is a common attribute of a syndrome that only includes holoprosencephaly in the most severely affected individuals[90,91]. These individuals with microcephaly in the absence of holoprosencephaly can also suffer from behavioural abnormalities[92,93], including hyperactivity and seizures, that might result from interneuron deficits.

In the case of seizure disorders, mutations in *Dlx1* lead to seizures that develop after sexual maturation in mice, and although *Dlx1* has not been identified as a disease gene in humans, a small percentage of patients with autism (a condition that is frequently accompanied by seizures) have been reported to show rare variations in *Dlx2* and *Dlx5* (REF. 94). A mutation of *Arx*, which appears to be regulated by *Dlx1* and *Dlx2* (REF. 95), results in the abnormal development of interneurons in mice and severe infantile seizures in humans[96]. Humans with single copy mutations in *Nkx2.1* develop a moderate movement disorder that was associated in one study with a reduction in the number of striatal interneurons[97,98], although seizures have not been reported and there is a potential confounding effect of hypothyroidism on interneuron maturation.

The abnormal development and function of cortical interneurons have also been implicated in the pathobiology of major neuropsychiatric illnesses, including autism[99–101], schizophrenia[102,103] and anxiety disorders[104]. Several mouse models have been generated in which the abnormal development or function of interneurons is thought to produce behavioural abnormalities similar to those seen in humans with schizophrenia[105–107]. In some of these models, allelic variations in *Ncam1*, *ErbB4* and *neuregulin 1* might contribute to the risk of developing

Holoprosencephaly
A developmental disorder caused by the failure of the forebrain to divide into bilateral hemispheres.

Box 2 | **Specification of the GABA phenotype**

Although the presence of GABA (γ-aminobutyric acid) is a feature common to the cortical interneuron subgroups discussed here, the GABA phenotype is not specific to interneurons. In fact, the overwhelming majority of subpallial neurons use GABA as a neurotransmitter, and recent evidence suggests that most of those that do not — the cholinergic population — derive from the GABA-positive lineage[121].

Genetic studies in mice have implicated roles for *Dlx2* and *Mash1* in specifying the GABA phenotype in the developing telencephalon. *Dlx2*, when overexpressed in the developing mouse cortex, can induce expression of both *Gad65* and *Gad67* (REF. 122), which encode the two enzymes that are responsible for the synthesis of GABA. Similarly, the misexpression of *Mash1* in the dorsal telencephalon results in the ectopic expression of *Dlx1* and *Gad67* (REFS 123,124). Although these studies demonstrate that *Dlx2* and *Mash1* are each sufficient to induce the GABA phenotype, the genes that are required for the specification of this phenotype in the telencephalon remain unknown.

schizophrenia[105,108,109]. In schizophrenia, multiple studies point to an abnormality in the chandelier interneuron axo-axonic synapse in the prefrontal cortex[110]. Whether this abnormality is causative, contributory or is an epiphenomenon of the disease is not known. However, as we unravel the mechanisms by which interneurons make specific connections[107,111], and by which this process is regulated at the transcriptional level, new pathologies and treatments of important neuropsychiatric illnesses are likely to be uncovered.

Conclusions and perspectives

So, where do we stand in the process of understanding the molecular regulation of cortical interneuron fate determination? Compared with the spinal cord, in which there have been tremendous advances in understanding the spatial, temporal and molecular differences that account for the observed neuronal subtypes[77], we are perhaps ten years behind. In fact, even the molecular determinants that specify the GABA-utilizing fate remain to be esablished (BOX 2). However, the temporal and spatial origins of cortical interneurons and their migratory pathways are relatively well described, particularly in rodents. The differential spatial origins correlate with differences in the localization of a few fate-affecting proteins and with the fate determination of neurochemically and physiologically distinct interneuron subgroups. Moving forward, these findings are being extended by methods that permit the systematic study of interneuron fate determination. For example, neurochemical aspects of interneuron subgroup fate determination are maintained *in vitro* when interneuron progenitors are plated over a feeder layer of cortical cells. This technique provides a relatively high-throughput way to study the molecular regulation of subgroup fate determination and the cortical influences on interneuron differentiation[23,56]. Of particular interest is whether aspects of interneuron subgroup physiology and connectivity can be meaningfully studied using the interneuron progenitor–cortical culture method.

More importantly, techniques have been developed to study interneuron fate determination *in vivo*. The most elegant method currently available is that of homotopically transplanting genetically labelled interneuron progenitors through *in utero* transplantation[19,21]. This methodology can also be performed with genetically altered progenitors, which, in addition to transplanting interneurons directly into the cortical plate[88], will provide crucial data on the cell-autonomous regulation of interneuron development. *In utero* electroporation of marker genes[112], a technique that will probably be extended to gain- and loss-of-function studies, provides yet another tool for the *in vivo* examination of the effects of embryonic manipulations on fate determination. Meanwhile, genetic differences in mature cortical interneuron populations are beginning to be elucidated[113], enhancing our ability to perform specific labelling of subgroups of cortical interneurons in the adult[114–116]. The field is poised to bridge the gap between the molecular control of interneuron fate determination and the molecular basis of interneuron connectivity and physiology.

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Competing interests statement
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DATABASES
The following terms in this article are linked online to:
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Dlx1 | DLX2 | *ER81* | *FGF8* | *Gli3* | *Gsh2* | *Lhx6* | *Lhx7* | *Nkx2.1* | NPY | SHH | SIX3 | *Vax1* | VIP
OMIM: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
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