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Cell Cycle Regulation & Interneuron Production

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Abstract

The regulation of progenitor proliferation in developing brain has been extensively studied in the cerebral cortex, but relatively little is known about progenitor divisions in ventral germinal zones. Recent observations pertinent to interneuron genesis in the ventral forebrain, especially in the medial ganglionic eminence (MGE), indicate similarities to cerebral cortical neurogenesis and hint at some interesting differences between ventral and dorsal telencephalon progenitors. Proliferation within the ganglionic eminences (GE) is discussed from the vantage point of neural precursor cell cycles, especially G1-phase, and current models of neurogenic divisions in cortex that may apply to ventral forebrain as well.

In regard to brain development, one tends to think of the cell cycle as a molecular engine churning out the cellular bricks needed for brain construction. Cell divisions during brain morphogenesis are modulated by both intrinsic and extrinsic influences that play on cell cycle parameters like the duration of the cycle and its phases, and the timing of exit. But the rules of the molecular program and the role of cell cycle timing in the genesis of neurons are still incompletely understood. Much of the molecular-level cell cycle information currently in hand derives from oncological studies that have probed how cells may evade their proliferation constraints to become malignant. Here we discuss some recent studies that examine regulation of cell cycle protein levels and sub cellular specializations like centrosomes and mitotic spindles in forebrain neural progenitors that link proliferation behavior with outcomes including numbers and cell types. The medial ganglionic eminence (MGE) is becoming particularly interesting in this context, because disruption of normal cell cycle progression is associated with changing the balance of interneuron types generated from this ventral forebrain region.

Cell cycle components

The major molecular features of the cell division cycle have been known for some time (Sherr, 1993, 2000). All that is required for a cell to proliferate is for DNA to be replicated faithfully and the fully executed copies of the genome to be segregated equally into two daughter cells that may then repeat the process. Molecular safeguards must be in place to ensure that the genome is completely copied before mitotic division (cytokinesis) begins and that the steps required for precisely equal distribution of DNA to daughter cells occur in proper order. Indeed, early embryonic divisions are largely comprised of oscillating mitoses (M phase) and DNA synthesis (S phase). As embryogenesis advances, added phases intervene, so that Gap1 (G1 phase) spans the progression from completion of M into S and Gap2 (G2 phase) separates the end of S phase and entry into M. Progression through these phases is driven by cyclin dependent kinases cdk1, cdk2, cdk4 and cdk6 that require non-catalytic partners, called cyclins, in order to form enzymatically active holoenzymes. Each of these holoenzymes and their interacting proteins govern the advancement of the cell cycle

through particular transitions or restriction points, encompassing the mid G1-restriction (governed by cyclins D-cdk4/6, that regulate the retinoblastoma protein RB), the G1/S transition (cyclin E-cdk2), the S-phase checkpoint (cyclin A/B-cdk1/2), the mid G2-restriction (cyclin A-cdk1) and the G2-M transition (cyclins B-cdk2-cdc25).

G2 phase is primarily important as the final opportunity to arrest cell cycle progression in order to allow for DNA repair of double stranded breaks after insults like exposure to ultraviolet light or X-irradiation (Abraham, 2001). DNA repair proteins – most acting through the PI3-kinase family kinases (PIKK) including ATM (ataxia telangectasia mutated) and ATR (AT and Rad3 related) kinases – can be activated in G1 or S, but they make a final stand against DNA damage in G2. The many examples of gene mutations identified in tumor cells that abrogate this checkpoint or impede G2-M progression reflects the critical roles these molecules play in maintaining genome integrity in proliferating cells. Regulation of the G2-M transition and M-phase progression is clearly critical for brain neurogenesis, since at least a half-dozen of the mutant genes associated with human microcephaly are involved in G2-M checkpoint or mitotic spindle or cytokinesis regulation (Ross, in press).

It is G1 phase regulation, however, that stands out as a focal point of neural development and brain construction, if only because it is in G1 that the cell commits to a round of division and it is only in G1 that cells respond to mitogenic factors in the cellular environment. At the conclusion of M phase and cytokinesis, progenitor daughter cells must either exit the cycle to enter a resting, Go, state or prepare to advance through G1. Progression through G1 requires the continual presence of mitogenic factors that promote the formation and action of D-type cyclins D1, D2 or D3, each partnered with cdk4 or cdk6 (Sherr, 1995). Once past the G1 restriction, withdrawal of mitogens will not prevent completion of the cycle (Sherr, 2000). The cyclin D-cdk4/6 holoenzymes act on RB in both phosphorylation-dependent and independent ways to release the suppressive action of RB on downstream transcription factors such as E2Fs that are required for DNA synthesis and induction of cyclins E and A (Sherr, 2000; Baker et al., 2005). The time required to transit through G1 is dependent in large measure on the complex interaction of D-type cyclins with cyclin dependent kinase inhibitors (cdki's) in the Kip (p27-Kip1, p57-Kip2), Cip (p21) and inhibitor of cdk4 or INK4 protein (p16, p15, p18, p19) families (Sherr, 2000). Especially in neural progenitors, p27 has a major influence on G1 in that cyclins D require binding of p27 (or p21 if present) in order to assemble the cyclin D-cdk holoenzymes (Cheng et al., 1999). Cyclins D in turn sequester p27, preventing it from inhibiting cyclin E-cdk2 that would block the passage from G1-into S. Thus, levels of D-type cyclins and Kip/Cip cdkis create a sort of 'set-point' in which the relative levels of cyclin or inhibitor protein expression will determine G1-phase length and the ability to clear the G1-S transition.

Cell cycle regulation in cerebral cortex

Most of the information currently available regarding cell cycle dynamics in developing brain has come from studies of cerebral cortical development, and has been extrapolated to some extent to the ventral forebrain. Regulation of cortical cell cycle parameters change over the course of the neurogenic epoch—or in the mouse from gestational day 11–17 (GD11–17) (reviewed in(Caviness et al., 1995; Caviness et al., 2008). Cumulative BrdU incorporation studies showed that over this period, the total length of the neural progenitor cell cycle increases from approximately 8 h at GD11 to 18 h by GD16 and this lengthening is almost entirely accounted for by prolongation of G1-phase (Caviness et al., 1995). Subsequent evidence has supported the hypothesis that this G1 lengthening increases the likelihood that daughter cells will exit the cell cycle upon completion of a division. For example, use of pharmacological inhibitors that prolong but do not block G1 increase the numbers of progenitors in cortical slice culture that exit the cycle (Calegari and Huttner,

2003; Calegari et al., 2005). Conversely, shortening G1 by overexpression of cyclin D1 or cyclin E delays cell cycle exit (Lange et al., 2009; Pilaz et al., 2009). Absence of cyclin D2 in $-/-$ knockout embryos is associated with prolongation of G1 phase and shortening of S-phase that leaves the total cell cycle time in the cortical VZ essentially unchanged (Glickstein et al., 2009). Interestingly, studies using GFP-Tis1 transgenic animals to enable visualization of progenitors as they undergo neurogenic divisions indicated that as progenitors transition from proliferative to neurogenic divisions, their G2 phase becomes prolonged as well (Calegari et al., 2005).

Detailed studies of the proliferative behavior of cortical progenitors led to the hypothesis that cerebral cortex is constructed through a coordinated progression of symmetric and asymmetric divisions in which early progenitors (GD11–14) undergo symmetric divisions giving rise to two stem cell daughters (still in cycle) (Haydar et al., 2003). By GD14.5, a roughly equal number of divisions are symmetric stem and asymmetric neurogenic divisions (divisions giving rise to one progenitor that reenters the cycle and one presumed postmitotic daughter that moves to the SVZ to become a neuron or glial cell). From GD15–17, a steadily increasing proportion of divisions result in two daughters that are postmitotic neurons or glia (symmetric neurogenic divisions), thereby depleting the progenitor pool.

The hallmark of these symmetric and asymmetric divisions is still somewhat unsettled. One definition that is widely accepted hinges on division outcome, so that symmetric divisions give rise to two equivalent progenitors (radial glial cells, RGCs) or two postmitotic cells (neurons or glia), while asymmetric divisions yield one self renewing progenitor (RGC) and one daughter that moves to the SVZ to either exit the cycle or become an intermediate progenitor cell to divide again symmetrically (Haubensak et al., 2004; Noctor et al., 2004; Hevner et al., 2006). Another definition of symmetric vs. asymmetric division hinges on the orientation of the mitotic cleavage plane, so that radial glial progenitors with a cleavage plane perpendicular to the ventricular surface are said to divide symmetrically, while those with a horizontal cleavage plane, parallel to the ventricular surface, divide asymmetrically and the angle of cleavage that falls between those two extremes determines whether the division will be symmetric or asymmetric (Chenn and McConnell, 1995; Haydar et al., 2003; Fish et al., 2006). Other investigations provided evidence that the cleavage plane orientation is more predictive of precursor type than daughter cell fate (Noctor et al., 2004; Konno et al., 2008; Noctor et al., 2008). That is, radial glia with vertical cleavage planes in the cortical VZ undergo both symmetric (stem) and asymmetric, self-renewing divisions depending on their developmental stage, while abventricular divisions in the SVZ undergo horizontal cleavage plane divisions that nevertheless give rise to symmetric daughter cells. What appears to be more relevant is the dynamic segregation of cell polarity proteins like mammalian partition protein mPar3 in the RGC (Bultje et al., 2009). Those investigators found that asymmetric inheritance of mPar3 was equally probable among RGCs with vertical or horizontal cleavage planes. However, the axis of mPar3 asymmetry was linked to (i.e., perpendicular to) the orientation of the cleavage plane. Moreover, the inheritance of the older mother centriole is a marker indicating which daughter of an RGC asymmetric division will remain as a renewed RGC (Wang et al., 2009). Thus the older mother centriole may provide a sort of organizing center for distribution of proteins or response to local signals (Wang et al., 2009) leading to induction of gene expression in one of two sisters as has been described for neurogenin 2 and Tbr2 (Ochiai et al., 2009).

Studies in the cortex are particularly advantageous because intermediate progenitors of the SVZ can be antigenically identified by the expression of T-box transcription factor Tbr2 (aka EOMES or eomesodermin) (Hevner et al., 2006; Pontious et al., 2008). Recently, these Tbr2 positive intermediate progenitors have been shown to display two morphologies (Kowalczyk et al., 2009). The Tbr2+ cells displayed not only the more familiar multipolar

cells in the cortical SVZ, but also a short radial morphology of cells that divide at or near the ventricular surface (Kowalczyk et al., 2009). Thus, most Pax6+ radial glia are thought to undergo asymmetric divisions, while a subpopulation of short radial glial precursors appear to be Tbr2+ intermediate progenitors dividing symmetrically, and intermediate progenitors of the SVZ are thought to all undergo symmetric divisions (reviewed in (Kriegstein et al., 2006; Fish et al., 2008). This expansion of progenitors through symmetric division in the SVZ has been credited with the evolutionary enlargement of cortical size. Intermediate progenitor, SVZ divisions are also credited with generating the greater number of neurons in the superficial layers contrasted with deep layers of the cerebral cortex (Molnar et al., 2006; Kriegstein et al., 2006). A complementary view is that the intermediate neural progenitor divisions amplify radial unit precursors and enable the regulation of neuronal output across lamina and cortical areas (Pontious et al., 2008). This later view is consistent with the demonstration that late-born neurons that would have to come from the intermediate progenitors appear to selectively synapse on earlier born, deeper pyramidal neurons that are clonally related, deriving from the same mother cell (Yu et al., 2009).

Proliferation in the ganglionic eminences (GE)

The MGE is the origin of as much as 60-80% of interneurons in the mouse cortex and gives rise primarily to GABAergic interneurons that express parvalbumin (PV+) or somatostatin (SST+) (reviewed in (Wonders and Anderson, 2006). It is anticipated that progenitors in the MGE will display proliferative behavior that is similar to cerebral cortex, though direct evidence has yet to be reported (Figure 1). Cumulative BrdU labeling studies analogous to those conducted for cerebral cortex characterized the rate and pattern of cellular output of the lateral ganglionic eminence (LGE) (Bhide, 1996; Sheth and Bhide, 1997). Those investigations demonstrated the progressive lengthening of G1 with embryonic age (Bhide, 1996). They also identified the GE SVZ (or secondary proliferative population) and the VZ as two distinct cytokinetic striatal compartments, thought to contribute approximately equivalent proportions to the overall cellular output from the GE (Sheth and Bhide, 1997). Moreover, the shift of location of terminal neurogenic divisions from the VZ to the SVZ occurs at earlier embryonic ages in the ventral forebrain (by GD11.5 in the LGE) than in the cortex (by GD 14) (Bhide, 1996; Sheth and Bhide, 1997). More detailed studies of proliferation in the GEs have been hampered by the present lack of specific markers of SVZ cells, for which protein expression of the Dlx1/Dlx2 or Brn4 transcription factors may be candidates (Eisenstat et al., 1999; Long et al., 2009). Another challenge for more classical histological studies of proliferation in the MGE/LGE is the comparatively greater admixing of proliferative zone cells with tangentially migrating, postmitotic neurons than in cerebral cortex (Wonders et al., 2008). The alternative approach, devising *in utero* methods for fluorescently labeling isolated progenitors in the MGE for timelapse video imaging has proven to be more challenging than for cerebral cortex. Nevertheless, experiments pursuing direct visualization of progenitor proliferation in the MGE and outcomes of divisions there will be required in order to compare and contrast progenitor divisions in dorsal vs. ventral forebrain.

Cyclins D in cortical development and interneuron specification

As discussed above, G1 phase regulation has become a focal point of interest in view of its pivotal position both in governing cell cycle length as cerebral progenitors advance their differentiation programs and the ability of cells in G1 to sense and respond to local changes in growth and differentiation factors. Beyond its gatekeeping function to proliferation, the regulation of neural progenitor divisions may under certain circumstances also influence fate outcomes – and certainly influences the relative representation of neuronal types in brain tissue. For example, in the absence of the G1-active cyclin D2, only half the normal number

of cerebellar granule neurons are generated and cerebellar cortical stellate interneurons fail to appear, despite preserved representation of Purkinje neurons, basket and Golgi interneurons (Huard et al., 1999). This lack of NO synthase expressing stellate interneurons is associated with ablation of the normal activation of cerebellar cortical hyperemia during sensory stimulation, confirming the importance of stellate interneurons for cerebellar blood flow regulation (Yang et al., 2000).

In forebrain, cyclins D1 and D2 are expressed in distinct progenitor niches, with cyclin D1 predominating in VZ and cyclin D2 in SVZ of cerebral cortex and ganglionic eminences (Glickstein et al., 2007a; Glickstein et al., 2007b; Glickstein et al., 2009). While cyclin D1 is expressed both in proliferating progenitors and selected postmitotic neurons in the mouse, cyclin D2 is found only in progenitors that are proliferating (Glickstein et al., 2007a; Koeller et al., 2008). In adult cerebral cortex, mice that lack cyclin D2 display microcephaly, with significant reductions in cortical surface area and thinning of the cerebral wall (Glickstein et al., 2007a). In addition, cyclin D2^{-/-} cortex reveals a 30% to 40% reduction in the density of PV⁺ interneurons, with no change in the density of SST⁺ interneurons whose progenitors also proliferate in the MGE (Glickstein et al., 2007b). The cD2^{-/-} PV deficit in adult mice is associated with cortical hyperexcitability on EEG telemetry and evidence of GABA deficit in whole cell voltage clamp recordings in cortical slices.

Examination of cell cycle indicators in the cD2 deficient MGE has shown a marked decrease in SVZ proliferation by S-phase (BrdU), M-phase (PH3) and Ki67 immunolabeling. This is accompanied by an increased rate of cell cycle exit in the cD2^{-/-} MGE at GD14.5, along with increased immunohistochemical labeling of p27 and decreased phosphorylated-RB, compared with wildtype siblings (Glickstein et al., 2007b). Importantly, BrdU birthdating experiments demonstrated that PV⁺ interneuron output from the MGE was reduced both at early and late gestational timepoints, while in contrast SST⁺ interneuron densities generated early or late were unchanged from wild type. Therefore, the reduction in PV interneurons in adult cD2^{-/-} mouse cortex did not result from a premature depletion of the MGE progenitor pool that would limit the generation of PV interneurons (Glickstein et al., 2007b), which have been said to be generated over a longer period than SST interneurons (Miyoshi et al., 2007). The early vs. late birthdating outcomes in cD2^{+/+} and cD2^{-/-} mutants are consistent with experiments indicating that the SST and PV interneurons within a given cortical layer were born at the same time in the MGE (Wonders et al., 2008). From these studies, we hypothesized that, in contrast to those that give rise to SST⁺ interneurons, progenitors of PV⁺ interneurons require cell divisions in the MGE SVZ in order to generate a full complement of PV⁺ cells (Glickstein et al., 2007b).

Niches and gradients affecting proliferation within the MGE

Several features of MGE development suggest the presence of growth and differentiation signals that may compete to exert a countercurrent of influences allowing for precise coordination of interneuron type specification and generation of appropriate relative numbers. For example, explants taken from the dorsal MGE produce predominantly SST⁺ interneurons *in vitro*, in contrast to explants from the ventral MGE that produce mostly PV⁺ interneurons (Wonders et al., 2008). These PV⁺ and SST⁺ lineages both depend upon the expression of transcription factor Nkx2.1 and presumably diverging downstream events (Xu et al., 2010; Gulacsi and Anderson, 2006; Butt et al., 2008; Du et al., 2008). Thus, dorsal-ventral differences in signaling systems may be required to play upon proliferation and transcription factor expression and so fine-tune specification cascades. Sonic hedgehog signaling is required to maintain this Nkx2.1 expression as progenitors proliferate and progress along their differentiation path (Xu et al., 2010; Xu et al., 2005). Conditional mosaic inactivation of Smoothed receptor indicates that, in contradistinction to the

presumptive Hh ligand gradient, Shh signaling is high in the dMGE and low in the vMGE (Xu et al., 2010; Wonders et al., 2008). Interestingly, despite the known critical importance of Shh to promote proliferation in other settings, MGE progenitor divisions continue, albeit reduced, even in the setting of conditional inactivation of Shh signaling (Xu et al., 2010; Machold et al., 2003; Xu et al., 2005). MGE progenitor proliferation is strikingly affected however, by levels of Wnt dependent beta-catenin signaling (Gulacsi and Anderson, 2008). Just as Shh signaling regulates cortical proliferation and size (Xu et al., 2005; Komada et al., 2008), it appears that the actions of Shh and Wnt signaling serve to coordinate dorsal ventral patterns of specification and proliferation in the MGE (Gulacsi and Anderson, 2008).

In addition to dorsal-ventral signaling gradients, the vascular compartment in MGE may provide medio-lateral influences on MGE progenitor pools. Just as studies in adult neurogenesis have indicated the importance of a vascular niche in regulating proliferation and stem cell differentiation, recent investigations have provided evidence for the coordinate regulation of neural patterning and angiogenesis during cortical development (Vasudevan et al., 2008). At the same time, vascular development provides a niche in which Tbr2+ intermediate progenitors in cerebral cortex proliferate in close proximity to forming neurovasculature (Javaherian and Kriegstein, 2009; Stubbs et al., 2009). Moreover, overexpression of VEGF disrupted the organization of forming cortical arterioles and in turn disrupted the placement of Tbr2+ intermediate progenitors in the SVZ (Javaherian and Kriegstein, 2009). This relationship between angiogenesis and progenitors in embryonic cortex is analogous to the proliferative niches provided by vasculature for adult SVZ and adult hippocampal progenitors (Palmer et al., 2000; Riquelme et al., 2008; Shen et al., 2008). In contrast to hypotheses involving adult niches, the observation that Tbr2+ cells associate with vascular endothelial cells before the formation of a vessel lumen suggested that the relationship between angiogenesis and stem cell does not depend upon access to growth factors provided by the circulation (Javaherian and Kriegstein, 2009). A similar relationship between intermediate progenitor proliferation and angiogenesis is likely to occur in the MGE as well. This is supported by the observation that brain endothelial cells express transcription factors appropriate to their neural progenitor neighbors, including *Dlx1/5* and *Nkx2.1* expression in ventral forebrain vascular endothelium (Vasudevan et al., 2008).

Summary

How then can we think of MGE proliferation in the context of current understanding of interneuron specification and differentiation? A temporal-spatial model is emerging for the contribution of progenitor proliferation to interneuron generation in the MGE (Figure 2). Interkinetic nuclear migration is not in the same lock-step in the ventral telencephalon as it is in embryonic cortex (i.e., S-phase nuclei labeled by a 30 min BrdU pulse are more evenly distributed across the MGE-SVZ). There is nonetheless a distinctive VZ in the MGE that can be distinguished from SVZ based on cytokinetic behaviors and protein expression of transcription factors like *Dlx* and cell cycle proteins cyclin D1 (in VZ) and cyclin D2 (in SVZ). Available data discussed above indicate dorsal-ventral influences operating in ventral forebrain that favor SST+ neurogenesis in the dMGE and PV+ neurogenesis in the vMGE and these appear to be modulated by the effects of Wnt and Shh signaling. In parallel, medial-lateral (or temporal) regulatory effects orchestrate cell divisions that use cyclin D1 in the VZ and cyclin D2 in the SVZ of the MGE, and cyclin D2 dependent divisions in the SVZ are particularly important for the generation of PV+ interneurons. The fact that a pure lineage population has not been identified in the MGE suggests the presence of “undecided” progenitors in the VZ that have not yet committed to a PV+ or SST+ specification path and that are sensitive to local influences including, though not limited to, Shh and Wnt.

Certainly it will be critically important to follow the proliferation of individual MGE progenitor clones in experiments similar to the ones performed in cerebral cortex. There are several scenarios that could fit existing observations (Figure 3). In one schema (Fig 3A), progenitors may become fate restricted early in MGE development so that RGCs destined to become SST+ interneurons divide, mostly asymmetrically, to produce a precursor that most often becomes postmitotic while the other reenters the cycle as an RGC. RGCs giving rise to PV+ interneurons renew the RGC and generate an intermediate progenitor that then divides symmetrically in the SVZ. A minority of the RGC divisions must also be symmetric (Fig 3B) in order to expand the population as the brain size increases, and these may or may not be multipotential. In another scenario (Fig 3C), an RGC capable of generating SST+ and PV+ interneurons may divide asymmetrically and those that exit the cycle before dividing again could be destined to become SST+ interneurons while those that divide as intermediate progenitors in the SVZ are more likely to become PV+ interneurons.

Exciting advances have been made in the dorsal telencephalon toward elucidating the rules governing cortical progenitor proliferation and differentiation. However, there are many more gaps in the puzzle of neurogenesis in the ventral forebrain. Direct investigation of the MGE is especially interesting since we now know that manipulation of proliferation can change the relative representation of particular neurons in cortex, and that will have measurable impact on cortical function. Such studies promise to significantly further our understanding the ways different progenitor pools use the relationship between proliferation and fate determination.

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Model of MGE Progenitor Divisions

(Based on MGE appearance and analogy with cortex)

Figure 1. Temporal-spatial progression of neurogenic divisions in the MGE

Neural progenitor divisions in the VZ are expected to be mostly asymmetric, producing either a neuron or intermediate progenitor cell (IPC) and renewing the radial glial cell (RGC). A minority of the RGCs divisions are likely to be symmetric giving rise to two RGCs. IPCs that move to the SVZ divide symmetrically to produce two neurons or two glial daughters that migrate to cerebral cortex or striatum.

Figure 2. Neurogenesis in the MGE

Interneuron progenitors reflect both dorso-ventral and temporal, medial-lateral, gradients. Progenitors that generate SST+ (red) interneurons predominate in the dorsal MGE (dMGE), while PV+ (green) interneuron precursors originate mostly in the ventral MGE (vMGE). Their fate depends in part on Shh signaling that is more robust in the dMGE than vMGE. Beta-catenin dependent Wnt signaling throughout the MGE is critical for progenitor proliferation. At the same time, progenitors in the VZ use G1-phase active cyclin D1 (cD1) to proliferate while symmetric SVZ neurogenic divisions use cyclin D2 (cD2), reflecting a temporal progression from radial glial to intermediate progenitor divisions.

Figure 3. Model progenitor divisions in the MGE

Radial glial cell progenitors (triangle) and interneuron precursors (circles) are shown as having mitotic potential (thin black rim) or as postmitotic cells (thick red rim). **A.** RGCs that are already determined to become somatostatin (SST+, pale yellow) or parvalbumin (PV+, green) expressing precursors, divide asymmetrically in the VZ. SST+ interneuron precursor daughters are initially able to divide again, but most become postmitotic and migrate away from the MGE. PV+ interneuron precursor daughters become intermediate progenitors and divide symmetrically, primarily in the SVZ, before exiting the cycle and migrating away. **B.** RGCs are multipotential (pale green) and can give rise to either SST+ or PV+ interneuron types. A minority of RGCs divide symmetrically to expand the RGC population. **C.** Multipotential RGCs divide asymmetrically in the MGE. RGC daughters that exit the cycle, primarily in the VZ, become SST+ interneurons, while those daughters that become intermediate progenitors to divide once more symmetrically, mostly in the SVZ, will yield PV+ precursors that exit the cell cycle and migrate