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Principles of neural stem cell lineage progression: Insights from developing cerebral cortex

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Abstract

How to generate a brain of correct size and with appropriate cell-type diversity during development is a major question in Neuroscience. In the developing neocortex, radial glial progenitor (RGP) cells are the main neural stem cells that produce cortical excitatory projection neurons, glial cells, and establish the prospective postnatal stem cell niche in the lateral ventricles. RGPs follow a tightly orchestrated developmental program that when disrupted can result in severe cortical malformations such as microcephaly and megalencephaly. The precise cellular and molecular mechanisms instructing faithful RGP lineage progression are however not well understood. This review will summarize recent conceptual advances that contribute to our understanding of the general principles of RGP lineage progression.

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Introduction

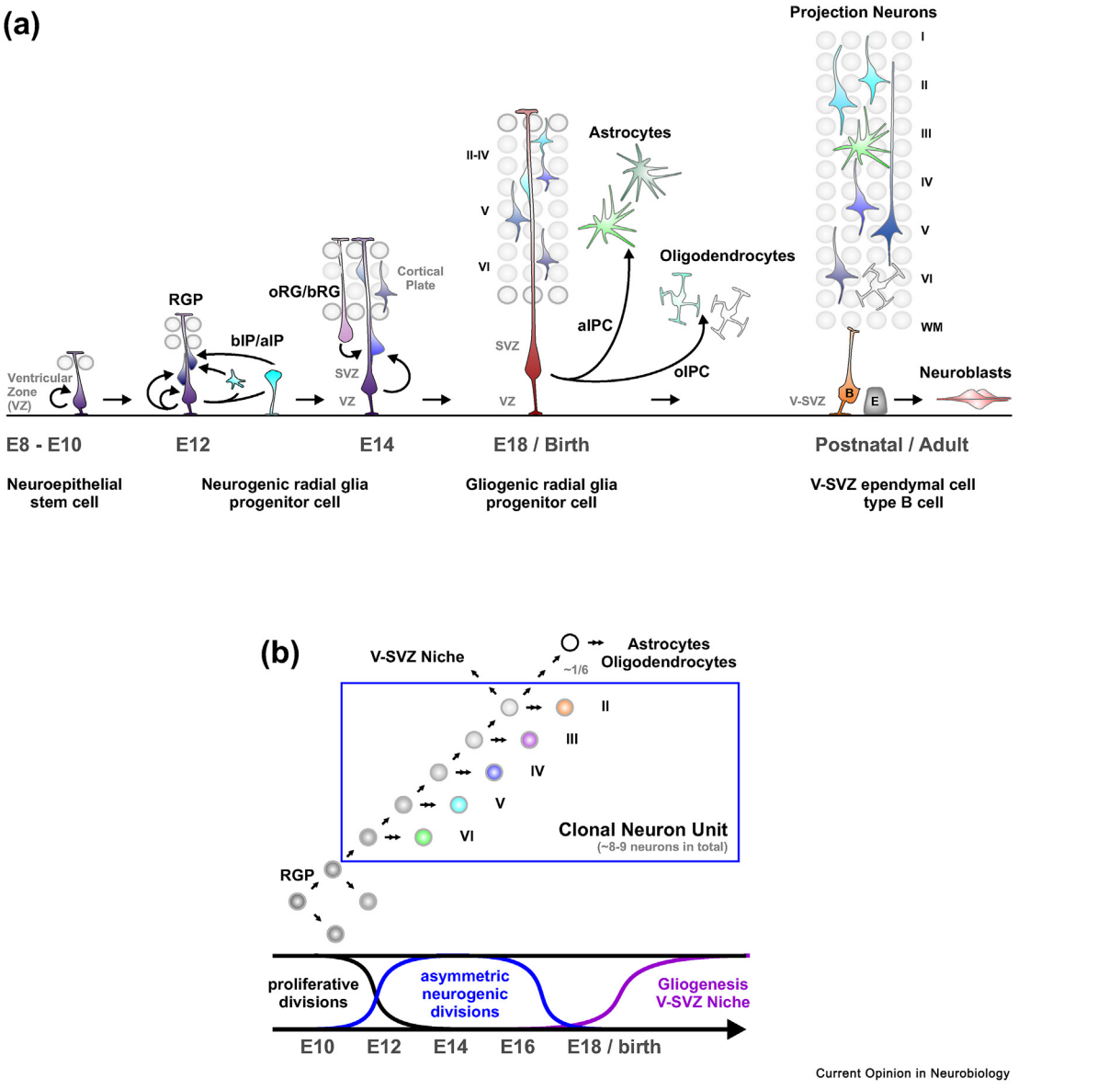
The cerebral cortex is composed of a vast number of neuronal and glial cell types assembling into cortical circuits that account for cognitive abilities. Based on global gene expression, remarkable heterogeneity among cortical cell types has been described [1–6], albeit the precise physiological relevance of transcriptomic cortical cell-type diversity remains to be established at the microcircuit level [1,7,8]. However, the identity of distinct neuronal classes is to a large extent genetically hard-wired [9,10]. The cellular and molecular mechanisms generating cortical cell-type diversity are not well understood but precisely regulated developmental programs appear critical for establishing the full spectrum of cortical cell fates [11–16]. At the quantitative level, tightly orchestrated regulatory processes regulating neural stem cell (NSC) proliferation behavior ensure the generation of a cerebral cortex, and more generally of an entire brain, of correct relative size and with appropriate cell density. The programs controlling the generation and maturation of postmitotic neurons by NSCs need to be executed flawlessly. Impairments in cortical neurogenesis lead to alterations in the cortical cytoarchitecture, which is thought to reflect the underlying cause of cortical malformations such as microcephaly or megalencephaly, and other neurodevelopmental diseases including autism, intellectual disability, and epilepsy [17–19].

During development, the cortical cell wall emerges from neuroepithelial stem cells (NESCs) which initially amplify their pool but then transform into radial glial progenitor (RGP) cells [20,21]. RGPs are the major neural progenitors and their proliferation dynamics along temporal lineage progression determine the final number of projection neurons in the mature cortex [20–22]. RGPs also produce transient amplifying progenitors, such as apical [23,24] and basal [25,26] intermediate progenitors (aIPs and bIPs), and outer SVZ radial glial progenitors (oRGs aka basal RGs or bRGs) [27–30]. While this review mainly focuses on RGP lineage progression in mouse, the reader may consult other excellent reviews that discuss RGP lineage progression in evolutionary context and humans [22,31]. RGPs also give rise to glia intermediate progenitors (astrocyte intermediate progenitor cells, aIPCs; oligodendrocyte intermediate progenitors, oIPCs), and establish the adult stem cell niche [32] (Figure 1a). This review will focus on recent studies and discuss emerging concepts that contribute to our understanding of the cellular and molecular principles regulating RGP proliferation behavior and lineage progression in the course of cortical development.

Quantitative framework of radial glial progenitor cell progression

The RGP-mediated generation of cortical projection neurons, followed by glial cells, and the establishment of the postnatal stem cell niche follows a temporally

Figure 1



Model and quantitative framework of neural stem cell lineage progression in developing mouse cerebral cortex. (a) Neuroepithelial stem cells amplify their pool at early E8-E10 stages before they transit to RGPs. RGPs initially amplify their pool as well but then assume neurogenic properties and sequentially produce excitatory projection neurons either directly or via IPs (bIP/aIP) and/or oRGs/bRGPs. Once neurogenesis is completed RGPs become gliogenic and produce astrocytes and/or oligodendrocytes via aIPCs or oIPCs, respectively. A subset of RGPs will establish the postnatal stem cell niche in V-SVZ with ependymal cells (E) and type B stem cells (B) that generate olfactory bulb-destined neuroblasts. I-VI indicates cortical layers, WM, white matter. S/VZ, sub/ventricular zone. Elements of this figure have been adapted with permission from Figure 8 in [39]. **(b)** RGPs initially undergo symmetric proliferative amplification divisions. The neurogenic potential of individual RGPs, as they switch from symmetric proliferative division to asymmetric neurogenic division, is overall predictable with an output of about 8–9 neurons per individual RGP. Upon completion of neurogenesis a sizeable fraction of RGPs (~1 in 6) proceed to gliogenesis whereas others establish the postnatal stem cell niche.

ordered process [32]. However, it is still not entirely clear what the precise quantitative (i.e. numbers of cells) and qualitative (i.e. cell type) output of individual cortical RGPs is, and how such output is modulated across time. Owing to clonal analysis of individual RGPs, a systematic framework of the lineage relationship of RGPs, nascent projection neurons and glia is emerging [33–35]. In particular, recent lineage tracing experiments employing the MADM (Mosaic Analysis with Double Markers) technology provided quantitative information, at true single-cell level, about RGP division patterns across spatiotemporal axis. MADM relies on

the reconstitution of two fluorescent marker genes (i.e. GFP and tdT) by Cre recombinase-mediated induction of interchromosomal recombination [36,37] during mitotic stem cell division. The outcome of such MADM events in particular G2-X events, with interchromosomal recombination in G2 phase of the cell cycle and X segregation of recombined chromosomes to different sister cells during mitosis, are informative. As such, G2-X MADM events in dividing RGPs result in differential cell labeling of the two nascent daughter cells, and their subsequent cell lineages. Thus, MADM events in conjunction with temporally controlled CreER can provide exact information on birth dates of RGP clones, their cell division patterns, and clonal architecture at single-cell level [37,38].

Given the exquisite single-cell resolution and quantitative nature of the MADM approach systematic clonal analysis was pursued in the developing neocortex. The results from these analyses revealed that RGPs progress in their lineage in a highly stereotyped manner. At early stages (E10-E12) RGPs predominantly divide symmetrically and with a predicable rate of cell-cycle rounds. Subsequently, RGPs switch relatively sharply around E12 to asymmetric cell division producing IPs and/or neurons with a total output of about 8–9 projection neurons per individual asymmetric clone [39–41]. Once neurogenesis is completed, about 1/6 RGPs turn gliogenic, producing aIPCs and/or oIPCs which then generate astrocytes and/or oligodendrocytes, respectively, at predictable rates [40,42,43], while others establish components (ependymal cells and type B stem cells of the postnatal stem cell niche) [39,44]. Interestingly, the relative fractions of aIPCs and oIPCs derived from individual RGPs appear relatively stable. In contrast, the total numbers of a/oIPCs and subsequent numbers of glial cells per clone, originating from single RGPs, are subject to high variability [39,42,43]. Altogether, MADM-based lineage tracing efforts have led to an inaugural quantitative and highly stereotyped framework of RGP lineage progression at the single-cell level. In a broader context, the overarching clonal analysis has also revealed the ontogenetic principles of neocortical projection neurons and glia, besides providing concrete evidence for a progressive temporal competence model of RGP proliferation (Figure 1b).

RGP lineage heterogeneity versus RGP cell type and cell state diversity

The above conceptual framework indicates a predictable unit of clonally-related projection neurons to be produced once RGPs switch from symmetric proliferative to asymmetric neurogenic division mode. On average the unit is composed of about 8–9 neurons located in both superficial and deep layers. However, the RGP-derived neuron units appear more heterogeneous if criteria beyond simple neuron number (i.e. clone size), such as laminar position, are considered. The majority of clonal units show projection neurons in all layers (canonical units) but sizeable fractions of units show variable clonal compositions. For example, about 15% of clones comprise projection neurons in all but layer 5 (i.e. skip layer 5) [41]. Currently the underlying molecular and cellular mechanisms resulting in heterogeneous RGP lineages are unknown. A few major, not mutually exclusive, scenarios could be taken into account. First, programmed cell death could play an instructive role by eliminating specific projection neuron types and thereby contributing to RGP lineage heterogeneity. Second, variable IP production and/or IP proliferation dynamics could specifically amplify RGP output at defined temporal windows, and thereby within specific layers. Third, variable RGP proliferation behavior and/or dynamics due to dynamic RGP cell state transitions during temporal lineage progression. Forth, the co-existence of different RGP 'subtypes' which bear distinct inherent cellular output potentials could result in lineage heterogeneity.

While the role of programmed cell death in RGP lineage diversification remains to be addressed recent data provides evidence that IP-mediated indirect neurogenesis could actually contribute not only to increased neuron numbers across distinct layers, but also to projection neuron diversity and potential connectivity. In effect, fate-mapping experiments revealed that apical IPs (aIPs) produce transcriptionally defined glutamatergic cortical projection neurons when compared to neighboring neurons born from different progenitor pools [8]. By using patch clamp recordings and optogenetic experiments, Ellender and colleagues could further demonstrate that aIP-derived neurons exhibit systematic biases in both their intralaminar monosynaptic connectivity and their postsynaptic partners in the deeper cortical layers [8]. Along the same lines, Huilgol and colleagues used genetic intersectional and subtraction fate-mapping approaches to trace direct RGP-mediated and indirect basal IP (bIP)-mediated neurogenesis [45]. First they confirmed earlier studies reporting that both direct and indirect IP-mediated neurogenesis generates all major (intratelencephalic, IT; pyramidal tract, PT; and corticothalamic, CT) projection neuron classes throughout all cortical layers. Yet, indirect neurogenesis appeared to amplify and perhaps even diversify projection neuron types within each class, but with substantial contribution to IT class. Interestingly, projection neurons derived from direct and indirect neurogenesis, respectively, showed distinct overall axonal projection patterns at the population level. Altogether, the above data indicate that the sequential patterns of direct versus indirect RGP-mediated neurogenesis could contribute to lineage heterogeneity on the basis of axonal projection pattern. However, both of the above analyses were performed at the population level and the relative contribution of direct versus indirect neurogenesis at individual RGP/clone level remains to be established.

Based on mathematical modeling a limited number of progenitor subtypes could in theory account for the observed diversity of clone architectures [41]. However, up to date, the evidence for distinct RGP types is relatively scarce. Numerous recent studies using single-cell RNA-sequencing (scRNAseq) approaches have some potential to address RGP cell diversity based on transcriptome and/or epigenetic modifications. The majority of up-to-date available sequencing datasets are derived by Drop-Seq protocol/10X Chromium platform, e.g. [2,46,47] or by using Smart-seq protocols [48]; and isolating cortical cells in time course starting at E9 [48], E10 [2,47], or E12 [46] until late embryonic and early postnatal stages, all revealed relatively similar temporal cell differentiation trajectories. In effect, cortical projection neurons appeared to share molecular trajectories that originate from one common progenitor branch in respective statistical lineage inference models. Furthermore, UMAP (uniform manifold approximation and projection) and t-SNE (t-distributed stochastic neighbor embedding) for dimension reduction of single-cell data sets did not indicate separate clusters of RGPs when adjusted for cell-cycle effects. Comparative scRNAseq analysis between early human (Carnegie stage 12–22, corresponding to gestational weeks 6–10) and mouse (E9/E10) further indicated that initially uniform stem cells give rise to neuronal and glia heterogeneity [49]. One recent study that focused specifically at late neurogenic stage E15, observed two types of RGPs and five types of IPCs in their t-SNE analysis [50] based on transcriptomic signatures. However, the observed RGP diversity at E15 may well be attributed to highly dynamic transcriptional states and potentially transcriptional priming (accumulation of untranslated mRNAs preceding expression of the respective protein) [50,51]. Thus all the above transcriptome analysis did not really provide conclusive evidence so far for distinct RGP (sub)-types. It is however important to mention that the measured transcriptome in developing RGPs only provided a snapshot at a particular developmental time point. Thus environmental influence at any given developmental stage may render the RGPs into a particular cell state, with a transcriptional signature that could dominate over the one associated with defined cell-type characteristics. The above analyses investigated lineages based on temporal patterns (i.e. histories) of gene expression in single-cell data sets and atlases. However, it is important to note that inference of lineage in above studies was based on computational methods [52,53] and confirmation of cell lineage *in vivo* at true cellular level awaits future studies. Yet, recent efforts using *in vivo* barcoding in combination with scRNAseq showed great potential to delineate lineage and genetic identity [54,55]. These approaches will also enable the better evaluation of lineage convergence and divergence on the basis of single-cell transcriptomes.

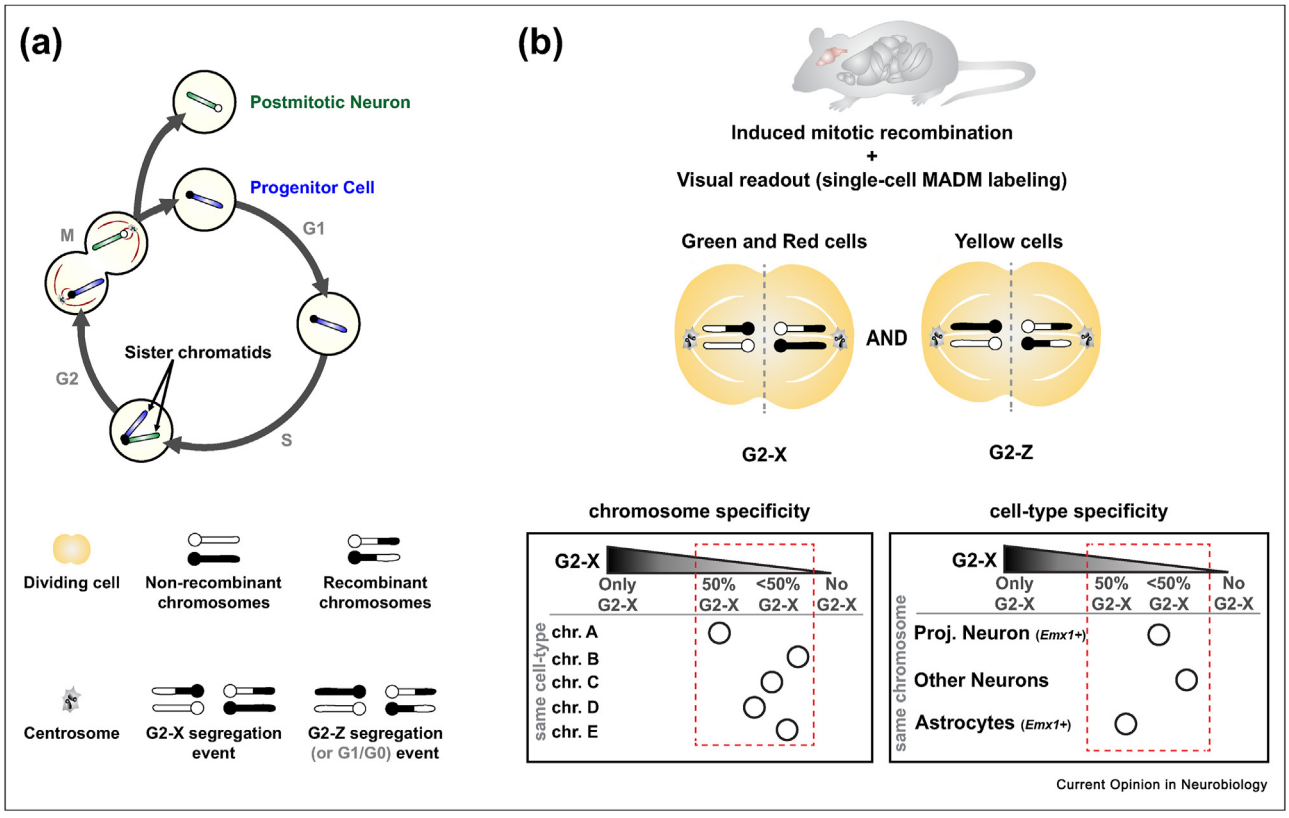
Interestingly, recent fate mapping experiments [56] did indicate the putative co-existence of distinct projection neuron lineages depending on whether they originated from *Lhx2*$^{+}$ and/or *Fezf2*$^{+}$ RGPs. Importantly, both RGP^{Lhx2} and RGP^{Fezf2} showed multipotency and produced projection neurons across all cortical layers. To probe the lineage relationship of RGP^{Lhx2} and RGP^{Fezf2} intersectional-subtraction (IS) strategies were applied. Such IS experiments revealed three different lineages and thus imply distinct RGPs based on the differential expression of *Lhx2* and *Fezf2*. RGP$^{Lhx2+/Fezf2-}$ were twice as abundant as RGP$^{Lhx2+/Fezf2+}$, and RGP$^{Lhx2-/Fezf2+}$ were only present as a very sparse population. Although both RGP$^{Lhx2+/Fezf2-}$ and RGP$^{Lhx2+/Fezf2+}$ generated cortical neurons across all cortical layers the RGP$^{Lhx2+/Fezf2-}$-derived projection neurons extended callosal but no subcortical axons whereas RGP$^{Lhx2+/Fezf2+}$-derived projection neurons extended subcortical but no callosal projections. Thus the presence/absence of *Fezf2* in RGP^{Lhx2+} appears to instruct the axonal projection pattern. The above data also indicate that the vast majority of RGPs express *Lhx2* which could define an early RGP ground state. While the above fate-mapping experiments provided intriguing new insights and hypotheses to test in the future, it will be important to assess the scale of RGP progenitor diversity also by using independent methods. Furthermore, it will be intriguing to mechanistically decipher the cues that regulate the onset of *Fezf2* in just a subset of cortical RGPs and thereby contributing to RGP lineage diversification.

It is important to note here that the degree of RGP lineage diversity obviously depends on the degree of cell-type diversity within the cluster of clonally-related cells. But what is a cell type and how shall we define it [57]? In the past decades mainly the criteria of cortical layer position, molecular, morphological, physiological, and functional properties have been taken into account. In recent years single-cell approaches with a heavy sequencing component have however revolutionized the characterization of cortical projection neuron cell types and current estimates range in the order of several dozens of distinct transcriptomic projection neuron cell types (t-types) in the adult neocortex. The cellular and molecular mechanisms that instruct the generation of cortical RGP-mediated cell-type diversity remain however still mostly unclear. Whether and how certain t-types contribute in a physiologically relevant manner to RGP lineage diversification/heterogeneity will be also an important issue to address in future studies.

Role of non-random sister chromatid segregation in proliferating RGPs

In order to generate stereotyped projection neuron units, RGPs divide asymmetrically to self-renew and sequentially produce neurons/IPs. The mechanisms

Figure 2



General model of non-random sister chromatid segregation and summary of *in vivo* evidence in *Emx1*$^+$ lineage. (a) Hypothetical model of non-random sister chromatid segregation during self-renewing stem cell division. During S phase in cell-cycle, chromosomes are replicated. Based on data in ES cells and *Drosophila* germline the two sister chromatids, although identical on the DNA sequence level, would acquire distinctive epigenetic marks (before mitosis) that then instruct passively or actively their biased segregation into either the postmitotic cell or the renewing progenitor cell, respectively. **(b)** By using the MADM system, sister chromatid segregation patterns can be monitored based on the MADM cell labeling paradigm that is created through recombination of MADM chromosomes [see text and [36] for details]. If MADM events result in green and red labeled cells (G2-X event) the recombinant chromosomes segregate away from each other. In yellow cells however the chromosomes segregate together into the same cell (G2-Z). If sister chromatid segregation in *Emx1*$^+$ lineage were completely random, one would expect equal ratio of G2-X and G2-Z segregation patterns. However, distinct chromosomes during projection neuron production in *Emx1*$^+$ lineage in mouse show biased ratios, indicating non-random segregation. For identical chromosomes, the segregation patterns appeared to also be influenced by cell type. As such, astrocytes in *Emx1*$^+$ lineage show very distinct segregation bias than projection neurons for the same chromosome. Part of the figure is adapted and modified with permission from Figure 7 in [36].

associated with asymmetric RGP cell division have been studied extensively and involve the non-equivalent distribution of cell-fate determinants including mRNA, protein complexes, and/or intracellular organelles such as mitochondria or centrosomal components [21,58–60]. Previous work using embryonic stem cell (ESC) cultures *in vitro* [61,62] or analyzing the developing *Drosophila* germ cell niche *in vivo* [63] have provided evidence that support an intriguing model. In this model, non-random mitotic sister chromatid segregation in asymmetric stem cell division would play a key role. The model postulates that during cell division the newly replicated sister chromatids, although supposed to be chemically identical, differentiate unevenly by epigenetic means and selectively segregate to either daughter cell [64–66]. The distinctiveness of the two sister chromatids would then contribute to the differential cell-fate acquisition of the renewing stem cell and the differentiating postmitotic cell, respectively (Figure 2a). However, experimental *in vivo* evidence in mice supporting the non-random sister chromatid segregation model was thus far lacking.

In order to distinguish and trace sister chromatids during cell division the ESC culture studies used induced mitotic recombination followed by genotyping based on restriction-site sensitivity [61,62]. The approach of tracing recombinant chromosomes upon cell division is in principle identical to the MADM strategy [36,37], except that recombinant MADM chromosomes express fluorescent markers that ease the visual tracing *in vivo*. Recently all 19 mouse autosomes have been engineered to contain MADM recombination cassettes and thus provide also an experimental platform to

systematically trace recombinant sister chromatid segregation *in vivo* based on the differential fluorescent daughter cell labeling [36]. In other words, RGP clones that contain red and green cells indicate that recombinant sister chromosomes (i.e. one expressing GFP and the other tdT) segregated away from each other and did not end up in the same cell, whereas yellow MADM-labeled cells contain both recombinant chromosomes (GFP/tdT double positive). Thus quantification of the relative numbers of red, green, and yellow MADM-labeled cells can serve at least as a proxy of whether (recombinant) sister chromosomes segregated away from each other or ended up together in the same cell [36] (Figure 2b). If sister chromatid segregation would be completely random one would expect identical relative ratios of red/green over yellow numbers over large populations of individual clones. However, when MADM was induced to systematically (but separately) trace the segregation pattern of all 19 autosomes in *Emx1*$^{+}$ RGP lineages we could show that sister chromatid segregation in mitotic RGPs exhibit high degree of chromosome specificity and thus non-random modus [36]. Furthermore, the pattern of sister chromatid segregation in neurogenic RGPs was different than the one in gliogenic RGPs for most analyzed chromosomes. Therefore the chromosome-specific segregation pattern may also depend on the cell type to be generated by the proliferating RGP (Figure 2b). It will be important, in future studies, to analyze whether such mechanisms also occur at the finer scale of lineage progression, i.e. whether at every subsequent asymmetric neurogenic division during unit production non-random sister chromatid segregation may be observed. More generally it will be crucial to probe the molecular mechanisms and the actual physiological relevance of asymmetric chromatid segregation in RGP lineage progression.

Interplay of cell-autonomous gene function and tissue-wide mechanisms in RGP lineage progression

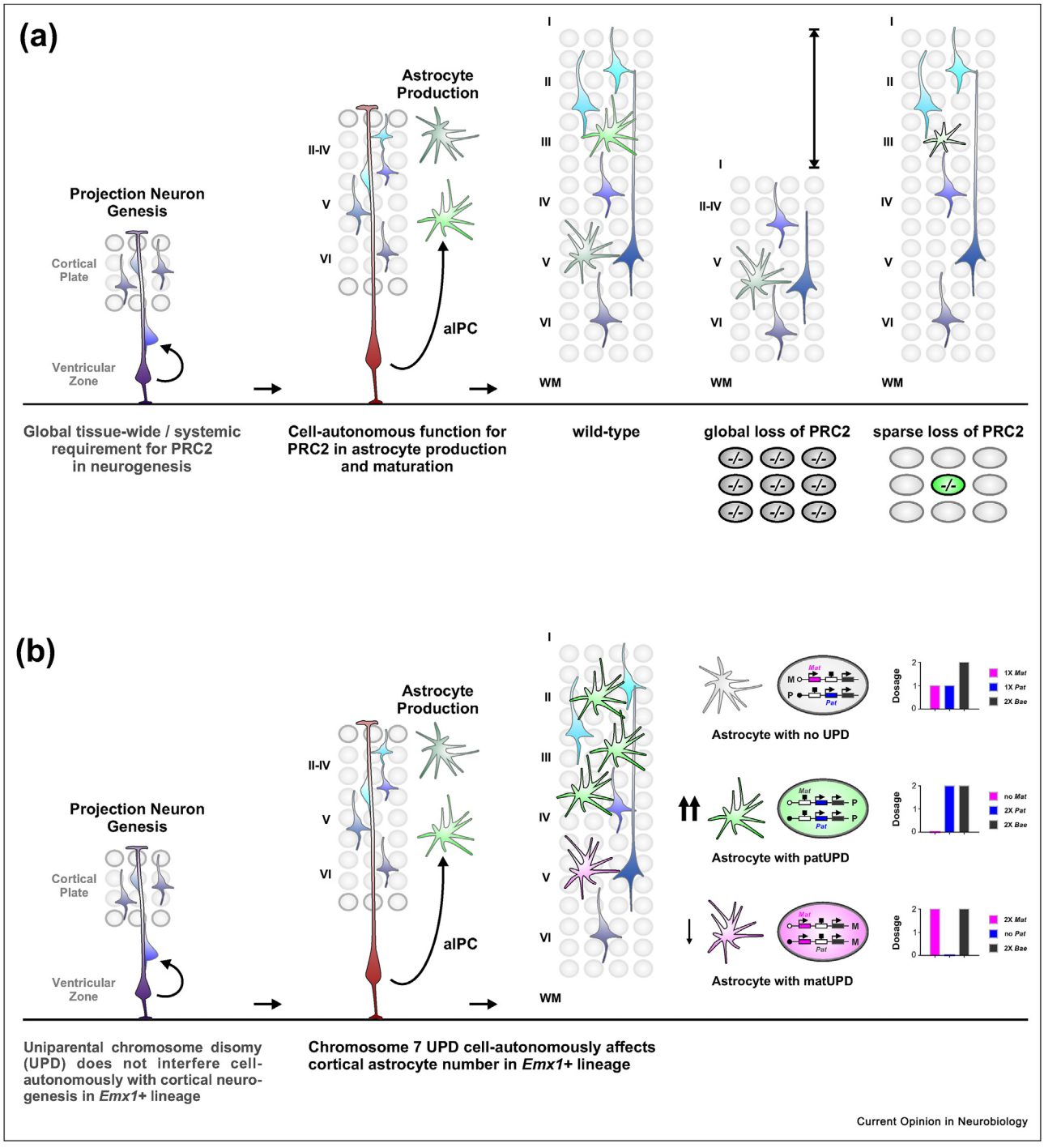
The molecular mechanisms instructing the orchestrated RGP lineage progression in the developing cerebral cortex are not clear. Unlike invertebrate stem cell niches where intrinsic properties such as sequential cascades of single transcription factors play key roles [9,67–69], RGP lineage progression appears to involve a fine interplay of cell-autonomous and tissue-wide cues [39]. Although a large catalogue of genes has been implicated in faithful RGP lineage progression, the true cell-autonomous gene functions and how they interact with more global tissue-wide mechanisms are not understood. An important class of regulatory cues for RGP lineage progression includes enzymes and protein complexes that regulate epigenetic modifications [70,71]. In particular the Polycomb repressive complex 2 (PRC2) — mediating posttranslational chromatin modifications — plays a critical role in proliferating RGPs during neurogenesis and glia generation [15,72,73]. More specifically, conditional knockout of the essential PRC2 component EED results in dramatic microcephaly indicating a deficit in RGP proliferation and/or lineage progression, potentially due to accelerated temporal progression with shortened neurogenic time window [15]. The function of PRC2 in gliogenic RGPs is less clear since some studies indicated precocious gliogenesis [73] whereas others implied a delay in glia production [72]. In any case, the true cell-autonomous function of PRC2 in RGP lineage progression is not understood. To this end, recent efforts have established genetic MADM paradigms to conditionally delete PRC2 either sparsely in single RGPs/clones or across the entire developing cortical tissue including all RGPs [74]. Perhaps surprisingly, the detailed analysis provided conclusive evidence that PRC2 function in neurogenic RGPs is not cell-autonomously required. Rather, PRC2-dependent mechanisms appear to operate at the global tissue level (Figure 3a). Mechanistically, deregulated gene expression upon PRC2 loss of (repressive) function was very different upon sparse or global ablation. Deregulated gene expression in global (but not sparse) PRC2 knockout also included cell-cycle regulators and components that control apoptosis programs. Thus the tissue-wide genetic state and cellular landscape, including PRC2 expression, fulfills essential regulatory functions during neurogenic RGP lineage progression. While PRC2 function is not cell-autonomously required for neurogenic RGP proliferation dynamics, the production (and maturation) of cortical astrocytes critically depends on PRC2-dependent transcriptional regulation [74] (Figure 3a).

How the global loss of PRC2 leads to microcephaly is not entirely clear but could involve secondary 'synthetic' downstream effects culminating in disrupted RGP cell cycle due to primary deregulated gene expression. Interestingly, human patients that suffer from Weaver syndrome carry mutations in components of the PRC2 complex resulting in loss of gene repression [75]. Paradoxically however these patients do not show microcephaly, as observed in mice that lack PRC2 activity, but rather macrocephaly and polymicrogyria. Thus species-specific mechanisms that could result in distinct tissue-wide synthetic effects may play an important role in phenotypic manifestation upon PRC2 loss, and even more generally in RGP lineage progression in human versus mice.

Role of genomic imprinting in RGP lineage progression

In the developing cerebral cortex, most genes are expressed from both parental chromosomes. However, a subset of genes is regulated by genomic imprinting that leads to preferential silencing of either the maternal or paternal allele [76,77]. Expression of the correct

Figure 3



Role of tissue-wide mechanisms and genomic imprinting in RGP lineage progression. (a) Distinct sequential functions of PRC2 in RGP lineage progression. During cortical projection neuron production PRC2 is required at the global tissue level. During cortical astrocyte production and maturation PRC2 has a cell-autonomous role. The genetic and cellular state of the tissue is critical since global tissue-wide loss of PRC2 results in drastic microcephaly. In contrast, sparse loss of PRC2 results in decreased numbers of astrocytes and affects astrocyte morphology, i.e. smaller size and reduced branching. **(b)** Role of genomic imprinting in RGP lineage progression. Genomic imprinting is not cell-autonomously required for cortical projection neuron generation since uniparental chromosome disomy (UPD) does not interfere with embryonic neurogenesis. In contrast, UPD of chromosome 7 specifically and cell-autonomously affects cortical astrocyte numbers. Astrocytes with paternal UPD (patUPD) – two copies of the paternal chromosome – show increased numbers in comparison with astrocytes with maternal UPD – two copies of the maternal chromosome. Schematics on the right illustrate how UPD affect gene dosage. On top, astrocyte with no UPD is illustrated with regular expression of imprinted (*Mat*, maternally expressed, pink; *Pat*, paternally expressed, blue) and biallelically expressed genes (*Bae*, black). Astrocytes with patUPD show twofold expression of paternally expressed genes and no expression of maternally expressed genes; whereas astrocytes with matUPD show no expression of paternally expressed genes and twofold expression of maternally expressed genes.

imprinted gene dose is however essential for cortical development and deregulation of imprinting is associated with the pathogenesis of neurodevelopmental diseases [78–80]. The imprinted *Cdkn1c* gene (maternally expressed) has been implicated in macrocephaly phenotypes [81] thus implying a role in regulating RGP lineage progression. However, recent analysis at true single-cell resolution revealed that *Cdkn1c* fulfills no cell-autonomous growth-inhibitory role in cortical neurogenesis [82]. These results were quite unexpected as they implied major non-cell-autonomous tissue-wide or even systemic *Cdkn1c* functions in RGP lineage progression and cortical tissue growth [82]. Importantly, these results again emphasize the importance of systematic dissection of cell-autonomous gene function and their interaction with global tissue-wide mechanisms. By using conditional deletion paradigms in combination with single-cell labeling, a novel growth-promoting function in RGPs was also discovered. Mechanistically, *Cdkn1c* promotes RGP and nascent projection neuron survival. Despite that this growth-promoting *Cdkn1c* function is highly dosage-sensitive it is not subject to imprinting [82].

More generally, the prevalence of imprinting and cell-autonomous impact in RGPs during lineage progression is essentially unknown due to the lack of suitable assays affording single-cell resolution. To this end, the genetic MADM paradigm recently offered a new assay to systematically probe the role of imprinted genes in cortical neurogenesis and glia production. The key properties enabling such analysis were: 1) the cell-type-specific generation and visualization of uniparental chromosome disomy (UPD) — somatic cells that contain two copies of the maternal or paternal chromosome — for the assessment of dosage-sensitive imprinted gene function; and 2) the sparseness of UPDs to probe cell-autonomy [83,84]. UPDs exhibit imbalanced expression of imprinted genes, either overexpressing or silencing the particular imprinted gene. Thus, in combination with single-cell labeling, such an experimental platform offers a unique approach to systematically probe cellular imprinting phenotypes in *Emx1*$^{+}$ RGP lineage. Interestingly, systematic analysis of UPDs of all 19 mouse autosomes revealed no prominent neurogenesis phenotype [36,83] implying no major cell-autonomous role for genomic imprinting, across the genome, in neurogenic RGPs (Figure 3b). In contrast, the detailed assessment of cortical astrocyte production in cells carrying chr7 UPD revealed a novel function of imprinting in the regulation of aIPC and/or subsequent astrocyte survival (in a *Bax*-dependent manner) (Figure 3b). At the mechanistic level, high-sensitivity RNAseq indicated that only a small number of imprinted genes on chromosome 7 associate with large deregulated gene networks implicated in growth and apoptosis [83]. More generally, the above findings indicate that correct expression of imprinted gene dosage is critical not only for postnatal and adult neurogenic stem cell niches [85], but also for RGP lineage progression at stages when astrocytes are generated.

Conclusions and perspectives

Progress in the last years has provided exciting insights into the molecular and cellular principles of RGP lineage progression in the developing cerebral cortex. The amazing and ever-evolving technological advancements in single-cell biology in general, and especially in single-cell transcriptomics and epigenomics have provided deeper insights at the individual-cell level [2,86]. In combination with the general frameworks obtained from population and single-cell genetic mutant analysis, the field has now tools at hand that will enable to decipher the molecular and genetic mechanisms driving RGP lineage progression at much higher resolution and true single-cell level.

Systematic clonal analysis has yielded an inaugural quantitative model of RGP lineage progression at the individual progenitor level. On the basis of this model, future efforts using genetic gain- and loss-of-function, shall successively provide a conclusive mechanistic framework and decipher the degree and physiological relevance of RGP lineage heterogeneity. How genetically heterogeneous populations of RGP-derived neurons assemble into canonical functional cortical circuits [87] and how cell type diversity may tune information transformation in such microcircuits is an outstanding question that should be addressed.

The here-described lineage framework in mouse neocortex may also serve as a blueprint for future investigations in other species and in human cortical organoids. Even without the complete clonal history in the human context, crucial information on equipotency, proliferative potential, and fate behavior may be recovered from clonal analysis [88].

Concerted efforts along the above-indicated issues will also enable better understanding of the critical cellular transitions of RGPs that often are the spot where mutations in humans affect the global neocortical growth process. It became clear that the complex developmental principles driving temporal RGP lineage progression critically depends on the interplay of cell-autonomous gene function and global tissue-wide mechanisms. Thus, the investigation of gene function in sparse cellular ensembles, such as in cases of somatic mosaicism, as opposed to germline mutations affecting the entire organism should gain attention. Ultimately, such genetic analyses with single-cell resolution have the potential to reveal the underlying pathogenic mechanisms associated with neurodevelopmental diseases [89]. More generally, our knowledge of the general mechanisms instructing RGP lineage progression may

provide a possible foundation for putative stem cell-based directed brain tissue regeneration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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- • of special interest
- •• of outstanding interest

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