

Temporal fate specification and neural progenitor competence during development

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Abstract | The vast diversity of neurons and glia of the CNS is generated from a small, heterogeneous population of progenitors that undergo transcriptional changes during development to sequentially specify distinct cell fates. Guided by cell-intrinsic and -extrinsic cues, invertebrate and mammalian neural progenitors carefully regulate when and how many of each cell type is produced, enabling the formation of functional neural circuits. Emerging evidence indicates that neural progenitors also undergo changes in global chromatin architecture, thereby restricting when a particular cell type can be generated. Studies of temporal-identity specification and progenitor competence can provide insight into how we could use neural progenitors to more effectively generate specific cell types for brain repair.

Neural progenitors

Multipotent progenitors that give rise to the diverse cell types of the CNS.

Asymmetric cell division

A mitotic division that generates daughter cells that have different cell fates.

Ganglion mother cell

(GMC). The differentiating daughter cell that is derived from the asymmetric division of a neuroblast. This cell will divide once more to generate two neurons or glia.

The complex structure of our brain — and thus its ability to perform impressive cognitive and motor functions — depends on the production of a diverse pool of neurons and glia from a relatively small number of neural progenitors during development. It is well established that spatial patterning cues can produce different types of neural progenitors, and hence different types of neurons and glia, along the rostrocaudal or dorsoventral axes of the CNS¹. It is also known that individual neural progenitors give rise to distinct cell types over time, which increases neural diversity in the CNS². Only recently, however, has there been progress in understanding the molecular mechanisms by which individual progenitors generate a sequence of different cell types — a process called temporal patterning or temporal-identity specification (BOX 1). An understanding of temporal patterning mechanisms is important for multiple reasons: it will illuminate how spatial and temporal cues are integrated to generate specific cell types and how ageing progenitors change competence to produce different cell types over time, and it might help us learn how to direct neuronal differentiation *in vitro* to repair the damaged or diseased brain.

Here, we divide temporal patterning into two processes: the specification of temporal identity (in which changing intrinsic or extrinsic cues act on a neural progenitor to specify a particular cell type) and changes in progenitor competence (the progenitor's response to the changing cues). Here we define 'temporal identity' as an aspect of cell fate that is determined by birth order in a

progenitor lineage, in contrast to 'spatial identity', an aspect of cell fate that is determined by position within the tissue or embryo (BOX 1). For example, 'early-born' temporal identity refers to a neural phenotype that is generated early in the lineage rather than to a particular cell type. Spatially distinct neural progenitors can use the same temporal-identity factor to specify distinct early- or late-born cell fates. We further define a 'temporal window' as the length of time or the number of progenitor cell divisions during which a given temporal identity factor is expressed.

In this Review, we discuss recent advances in our understanding of temporal patterning within the *Drosophila melanogaster* and mammalian CNSs. We highlight key recent results and conserved mechanisms, and discuss several important open questions.

Specifying temporal identity in flies

Temporal specification in embryonic neuroblasts. In the ventral CNS of the *D. melanogaster* embryo, 30 distinct neural progenitors, called neuroblasts, are arranged in a segmentally repeated bilateral pattern and give rise to all neurons and glia of the nerve cord^{3,4} (FIG. 1a). Neuroblasts undergo multiple rounds of asymmetric cell division. With each cell cycle (typically lasting approximately 1 hour), a smaller ganglion mother cell (GMC) 'buds off' and divides once more to generate a pair of neurons or glia (FIG. 1a). The neuroblasts form a layer at the ventral surface of the CNS, and their early-born progeny are displaced by later-born progeny, resulting in a 'laminar' CNS reflecting neuronal birth order⁵. The major advantages of this system

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Box 1 | Essential terms

Listed below are terms used in this Review to describe different aspects of cell-fate specification.

Spatial identity

The aspects of progenitor identity that are determined by its spatial position.

Spatial-identity factor/cue

A molecule that gives positional information to a cell; for example, the *Drosophila melanogaster* Engrailed (En) protein and its orthologues EN1 and EN2 in mammals help to establish regional identities during development of the body plan¹²⁹.

Spatial patterning

The generation of heterogeneous neural progenitors on the basis of their position in the developing nervous system.

Temporal identity

An aspect of progenitor identity that is determined by its birth order.

Temporal-identity factor

A factor that specifies cell fate on the basis of the birth timing of the progeny (for example, early or late) in multiple progenitors. For example, the Hunchback transcription factor specifies first-born temporal identity in multiple progenitor lineages despite their differing spatial identity⁵.

Temporal patterning

The generation of distinct neural progeny in response to developmental stage-specific cues. Temporal patterning cues collectively include any intrinsic or extrinsic factor (such as a temporal-identity factor or switching factor) that contributes to the production of a specific progeny fate on the basis of its birth timing.

Temporal window

The duration (either by time or number of progenitor divisions) in which a particular temporal-identity factor is expressed.

Switching factor

A molecule that promotes the transition between temporal-identity factors; it does not directly specify cell fate. For example, Seven up is required for the Hunchback–Kruppel transition in *D. melanogaster* neuroblasts^{14,26}.

Subtemporal factor

A factor that acts downstream of a temporal-identity factor to subdivide the temporal window into multiple distinct progeny fates. For example: the transcription factors Nab and Squeeze subdivide the last four Castor-expressing divisions of neuroblast 5–6 to specify distinct neurosecretory cells²⁰.

Cell-fate determinant

A factor that specifies a particular cell fate. Such factors are likely to function downstream of the spatial and temporal factors.

Competence

The ability of a progenitor to generate a particular cell fate in response to a spatial- or temporal-identity factor. For example, *D. melanogaster* progenitors are competent to generate first-born neurons in response to Hunchback only early in their lineage^{7,17}.

for studying neurogenesis are that: first, each neuroblast is uniquely identifiable by the presence of specific molecular markers and its position within a grid-like array (for example, neuroblast 7-1 (NB7-1) is always in row 7, column 1 (REFS 3,4)); second, a specific neuroblast always gives rise to a reproducible set of neural progeny in the same birth order^{6–10}; and third, there is minimal neuronal migration^{6,8,9}. These characteristics have allowed the precise study of individual neuroblast lineages and provide a unique platform for identifying and characterizing candidate temporal-identity factors.

Temporal-identity factors were first identified through the observation of laminar expression of the Ikaros family zinc-finger transcription factor Hunchback (Hb), the co-expressed POU domain proteins Nubbin and Pdm2 (from here on referred to together as 'Pdm'),

and the zinc-finger transcription factor Castor (Cas) in the mature CNS¹¹. Hb is expressed in a deep neuronal layer, Pdm in an intermediate layer and Cas in a more superficial layer. Subsequently, it was shown that most neuroblasts sequentially express Hb, the zinc-finger transcription factor Kruppel (Kr), Pdm and Cas as they undergo multiple rounds of cell division⁵. Thus, sequential expression of different transcription factors in neuroblasts leads directly to the laminar expression of transcription factors observed in the mature CNS.

Temporal patterning was first studied in the embryonic NB7-1 lineage, which produces five distinct types of motor neurons (U1–U5) during its first five divisions (FIG. 1b). Hb is necessary and sufficient to specify the earliest-born neural identity in multiple neuroblast lineages, including NB7-1, NB7-3 and NB3-1 (REFS 5,7,10,12–15). Although the specific characteristics of the first-born progeny differ between neuroblast lineages, in each case cells specified with an early-born identity use a discrete neuronal enhancer¹⁶ to maintain active transcription of the *hb* gene, which acts as a molecular marker of their early temporal birth^{17,18}. Kr specifies the second temporal fate in multiple neuroblast lineages^{5,12,13} (FIG. 1b). Both Hb and Kr expression are maintained in neuronal progeny, but their role in postmitotic neurons is not known.

The roles of the later candidate temporal-identity factors Pdm and Cas have been characterized in multiple neuroblast lineages, with different results in each lineage tested. In the NB7-1 lineage (FIG. 1b), Pdm is necessary and sufficient to specify the U4 motor neuron fate, and Pdm and Cas together specify the U5 motor neuron identity¹⁹. In the absence of *cas*, U5 neurons are lost, whereas overexpression of Pdm and Cas together generates extra U5 neurons¹⁹. In the NB3-1 lineage, however, Pdm has no detectable role in directly specifying temporal identity. Instead, Pdm is required to repress Kr: in its absence, Kr expression is extended, resulting in the production of extra Kr-expressing RP3 motor neurons¹⁰ (FIG. 1b). Thus, Pdm acts to specify cell fate in NB7-1 and as a 'switching factor' to regulate the timing of Kr expression in NB3-1. In both NB7-1 and NB3-1 lineages, expression of Cas is required to close the temporal window that specifies late-born neurons, and loss of *cas* results in more of the late-born neuron cell types^{5,10}, indicating that Cas can act as a switching factor in addition to its fate-specifying functions.

The role of Cas is perhaps the most well characterized in the lineage of NB5-6, in which the Apterous transcription factor is expressed in the last four neurons in the lineage²⁰ (FIG. 1b). Cas is expressed by NB5-6 in a broad temporal window that spans ten divisions and includes the last four divisions of the lineage, during which it activates the transcription factor Collier (also known as Knot) and specifies Apterous-expressing neurons. Cas initiates a feedforward transcriptional pathway by activating downstream factors such as Squeeze that help to establish the individual identity of the Apterous-expressing neurons²⁰. Thus, neuroblast temporal windows can be established by one set of factors and be further subdivided by 'subtemporal factors' that act through feedforward and feedback transcriptional regulation to increase neural diversity²⁰.

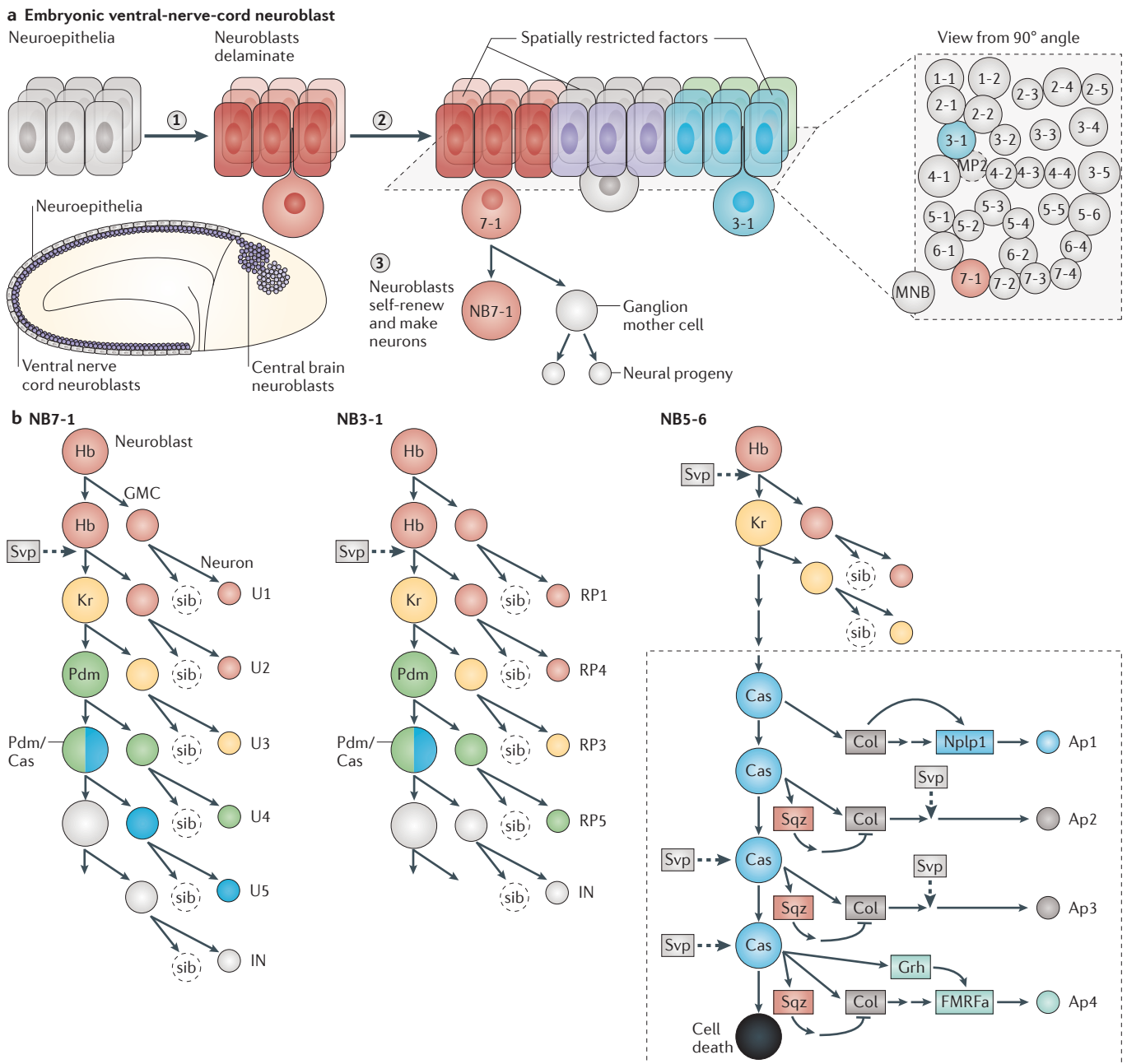


Figure 1 | Neurogenesis in ventral-nerve-cord neuroblast lineages in the *Drosophila melanogaster* embryo. **a** | Neuroblasts are specified and subsequently extruded (delaminated) from the neuroepithelia at the onset of neurogenesis (step 1). Each individual neuroblast can be uniquely identified on the basis of its stereotyped position within the nerve cord and its expression of spatially restricted factors, represented here as different colours (step 2). Neuroblasts are named according to their row and column within the neuroblast ‘grid’ (see inset)^{4,130}. Thus, neuroblast 7-1 (NB7-1; red) is present in the seventh row and occupies the first column position, whereas NB3-1 (blue) is positioned in the third row. Each neuroblast undergoes a series of asymmetric divisions that give rise to a self-renewed neuroblast and a differentiating ganglion mother cell (GMC) (step 3). The GMC often divides again to generate two neural progeny that can adopt distinct fates in a Notch-dependent manner^{131,132}. How the neural progeny maintain temporal identity is unknown. The illustration shows a *D. melanogaster* embryo at stage 9, at the onset of neuroblast delamination. Neuroblasts are shown in

grey along the ventral nerve cord just beneath the epithelial layer. **b** | The lineages of NB7-1, NB3-1 and NB5-6 neuroblasts are shown. Each neuroblast sequentially expresses the temporal-identity factors Hunchback (Hb), Kruppel (Kr), POU domain protein (Pdm) and Castor (Cas) and gives rise to a unique lineage of neural progeny in a stereotyped birth order. The COUP-family nuclear receptor Seven up (Svp) is transiently expressed in neuroblasts to regulate timing of temporal fate-determinant expression^{14,27,133}. At the end of the NB5-6 lineage, Cas activates expression of Collier (Col), a transcription factor that specifies Apterous (Ap) neuron identity in the last four neurons born from the lineage²⁰. Cas additionally initiates a subtemporal transcriptional cascade (inset) by activating Squeeze (Sqz) and Grainy head (Grh). The progressive accumulation of Sqz and Grh as NB5-6 divides results in the generation of distinct Ap neuron subtypes (Ap1 to Ap4)²⁰. At the end of the lineage, NB5-6 undergoes cell death. FMRFa, FMRFamide; IN, interneuron; MNB, median NB; Nplp1, Neuropeptide-like precursor 1; sib, sibling; U1–U5 and RP1–RP5, types of motor neuron.

The transcription factor Grainy head (Grh), is a candidate temporal-identity factor that is expressed after Cas in multiple neuroblast lineages^{21–23}. In the embryonic NB5-6 lineage, Grh is required to specify the last-born neuron that expresses the neuropeptide FMRamide (FMRFa)²⁰, and Grh also has a role in specifying temporal fate in intermediate progenitors of the larval type II neuroblast²⁴ (see below).

Collectively, accumulating evidence indicates that Hb, Kr and Cas are bona fide temporal-identity factors, as they specify the identity of neuroblast progeny on the basis of their birth timing in multiple neuroblast lineages. Analysis of more neuroblast lineages is required to determine whether Pdm or Grh are also multi-lineage temporal-identity factors. In addition to their roles in fate specification, both Pdm and Castor seem to function as switching factors depending on the lineage in which they are expressed, and this might be determined by the activity of lineage-specific spatial-patterning cues.

What regulates the timing of temporal-identity factor expression? Misexpression experiments show that each temporal-identity factor can activate the next factor in the pathway and repress the next but one factor^{5,21}; however, loss of Hb or Kr does not affect the production of later-born cells⁵, suggesting that an independent mechanism contributes to the sequential expression of the temporal-identity factors. The transition from Hb expression to Kr expression requires both neuroblast cytokinesis and the expression of the COUP-family protein Seven up (Svp), which is an orphan nuclear receptor^{14,25,26}. Svp is expressed in two temporal waves in embryonic neuroblast lineages²⁵ and seems to regulate the timing of multiple events, which is consistent with a role as a switching factor (FIG. 1b). Its initial wave of expression represses *hb* transcription to allow the Hb-to-Kr transition, and recent work shows that its re-expression at a later stage in the NB5-6 lineage subdivides the broad, ten-division Cas expression window to allow the production of multiple neural fates^{25,27}. Dissociated embryonic neuroblasts still sequentially express temporal-identity factors as they would *in vivo*^{18,21} and, remarkably, the Kr–Pdm–Cas transitions can occur in cell-cycle-arrested neuroblasts¹⁸, indicating that there is a robust, neuroblast-intrinsic timing mechanism that is independent of cell-cycle progression^{18,21}. However, the molecular nature of the Kr–Pdm–Cas timer mechanism remains unknown.

Temporal specification in larval neuroblasts. Larval type I neuroblasts have lineages that are similar to those of embryonic neuroblasts and also undergo temporal transitions that expand neural diversity; however, the transcription factor cascades that are used differ. Here, we discuss three examples: the anterodorsal projection neuron (adPN) neuroblast²⁸, the four mushroom-body neuroblasts and the optic-lobe medulla neuroblasts. The adPN neuroblast generates a different projection neuron with each cell division. In the adPN lineage, Kr is expressed for just one cell division to specify VA71 neuronal identity, but there is no known role for Hb, Pdm or Cas²⁸. The four mushroom-body neuroblasts, and many other larval neuroblasts, generate multiple neurons

expressing the transcription factor Chronologically inappropriate morphogenesis (Chinmo), followed by a series of smaller neurons expressing Broad-Complex transcription factors^{23,29} (FIG. 2A,B). The protein level of Chinmo in the postmitotic neurons declines as more neurons are produced, and high Chinmo levels specify early-born identity, whereas low Chinmo levels specify late-born identities^{23,29,30}. The Chinmo protein temporal gradient is generated post-transcriptionally as a result of the expression of the *let-7* and *mir-125* microRNAs (which repress Chinmo expression) in the late-born neurons³¹. Intriguingly, Svp seems to reprise its embryonic role as a switching factor in larval neuroblasts, which transiently express Svp just before the switch from the production of Chinmo-expressing neurons to the production of Broad-Complex-expressing neurons; *svp* mutant neuroblasts never make this switch²³. Recent exciting work has revealed a completely novel cascade of temporal-identity factors in the optic-lobe medulla neuroblasts. These neuroblasts give rise to a diverse array of neurons in the visual-processing regions^{32–34}. Two elegant genetic studies recently showed that most optic-lobe neuroblasts sequentially express the transcription factors Homothorax, Eyeless, Sloppy paired, Dichaete and Tailless and contribute approximately 40,000 neurons of more than 70 distinct subtypes to the medulla^{35,36}. These studies give us a glimpse of the complexity of the regulation of temporal fate in a context-dependent manner.

A small subset of larval neuroblasts, called ‘type II’ neuroblasts, have a more complex lineage than the abundant ‘type I’ neuroblasts. Type II neuroblasts sequentially produce intermediate neural progenitors (INPs) that themselves undergo multiple molecularly asymmetric self-renewing divisions to produce a series of approximately six GMCs that terminally divide to generate two neurons or two glia^{37–39} (FIG. 2Ab,c). Thus, compared to type I neuroblasts, which produce GMCs directly and generate approximately 100 neurons per lineage, type II neuroblast lineages contain an amplifying progenitor population that greatly increases their neural output to approximately 600 neurons of over 60 subtypes^{24,37–41}. Where does this neural diversity originate within the type II neuroblast lineages? It has recently been shown that nearly all INPs transition through a cascade of three transcription factors — Dichaete, Grh and Eyeless — as they divide, and these factors specify early-to-late temporal identity within multiple INP sublineages²⁴ (FIG. 2C). In addition to the temporal transitions within INPs, distinct neurons and glia are produced as type II neuroblasts age^{24,41}, suggesting that currently unknown temporal-identity cascades also exist within the parental neuroblast. It is likely that the combinatorial activity of two independent temporal cascades — one within the neuroblast and one within the INPs — is used to generate the neural diversity of the adult brain central complex. How these two temporal axes intersect and how they might be further regulated by spatial patterning cues are fascinating questions for future work.

The parallels between the *D. melanogaster* type II neuroblast lineages and mammalian neural stem cell lineages

Cytokinesis

The final event in the cell-division cycle. Its completion results in the irreversible partition of a mother cell into two daughter cells. It involves cytoplasmic division driven by an actin-based constriction of the contractile ring.

MicroRNAs

Short, non-coding RNAs that inhibit translation of mRNAs in a sequence-specific manner.

Central complex

A region of the fly brain that is involved in multimodal sensory integration.

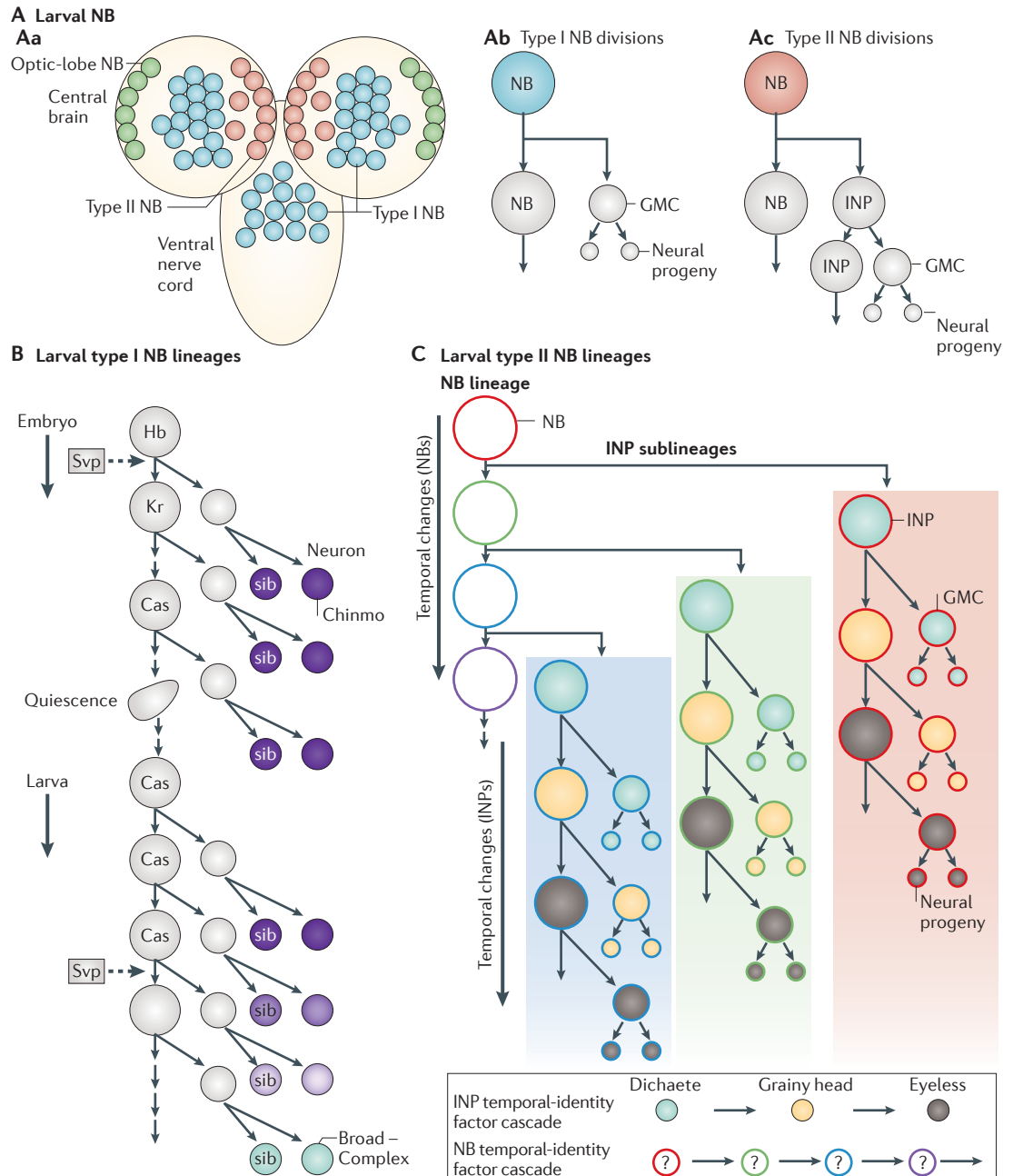


Figure 2 | Neurogenesis in *Drosophila melanogaster* larval central-brain neuroblast lineages. **Aa** | The larval central brain harbours roughly 90 type I and 8 type II neuroblasts (NBs), which are distinct from the optic-lobe neuroblasts (green). **Ab** | Like the embryonic ventral-nerve-cord neuroblasts shown in FIG. 1, type I neuroblasts of the central brain and ventral nerve cord (blue) divide to generate a ganglion mother cell (GMC) that in turn divides to produce two neural progeny. **Ac** | Type II neuroblasts (red) divide to give rise to an intermediate neural progenitor (INP) that undergoes additional ‘neuroblast-like’ asymmetric divisions, thus greatly amplifying the number of neural progeny. **B** | At the end of embryogenesis, neuroblasts stop dividing and producing neurons (quiescence). These neuroblasts become reactivated at the larval stages and resume neurogenesis. Type I neuroblast lineages in the late embryonic and early larval stages express Castor (Cas) and give rise to a series of Chronologically inappropriate morphogenesis (Chinmo)-expressing neurons. The level of Chinmo expression in the postmitotic progeny of larval neuroblasts decreases with each neuron produced, with early-born neurons expressing the highest levels of Chinmo^{23,29,30}. The sibling progeny (labelled ‘sib’) are also regulated by Chinmo protein levels but adopt distinct fates in a Notch-dependent manner^{29,30}. Seven up (Svp) regulates the Hunchback (Hb)-to-Kruppel (Kr) transition in the embryo and is re-expressed in larval neuroblasts to regulate the temporal transition from Chinmo expression to Broad-Complex expression by terminating Cas expression²³. **C** | Type II neuroblast lineages give rise to multiple INP sublineages. These INPs sequentially express Dichaete, Grainy head and Eyeless to temporally specify distinct neural progeny²⁴. Given that neural progeny born early in the neuroblast lineage are different from those born later, it is likely that the neuroblast itself undergoes temporal transitions that are inherited by the INPs (these hypothetical neuroblast temporal-identity factors are depicted by coloured circular outlines).

are intriguing. For example, neural stem cells of the adult subventricular zone (SVZ), the largest germinal zone of the adult mammalian brain, generate INP-like, transit-amplifying progenitors that differentiate into diverse subtypes of olfactory bulb neurons⁴². Additionally, recent work has identified a second population of asymmetrically dividing and self-renewing progenitors in the outer SVZ (oSVZ) of the developing human brain. These progenitors might have greatly expanded cortical size and complexity during evolution^{43,44}. Important areas for future investigation will be ascertaining whether mammalian progenitor or INP lineages undergo temporal-identity transitions and determining how they contribute to the generation of neural diversity.

Specifying temporal identity in mammals

Neural progenitors in the developing mammalian CNS also generate distinct neural progeny in a stereotyped birth order. Here we focus on three examples: the ordered production of retinal cell types, cortical laminar identities and the switch from neurogenesis to gliogenesis. The sequential production of visceral motor neurons and serotonergic hindbrain neurons has been reviewed elsewhere^{2,45,46}.

Temporal-identity specification in the retina. *In vivo* lineage tracing has shown that individual neural progenitors in the vertebrate retina are multipotent and give rise to distinct cell types in a characteristic birth order^{46–49} (FIG. 3). Transcriptome analysis of single retinal progenitor cells from different developmental stages revealed the sequential expression of transcription factors related to the fly temporal-identity factors Hb, Kr, Pdm and Cas⁵⁰. The zinc-finger transcription factor Ikaros is expressed by young retinal progenitors and is required for the specification of early-born cell types⁵¹ (FIG. 3c), a role that is remarkably similar to that of its fly orthologue, Hb⁵. Despite the ordered production of distinct cell types during retinal development, cell lineage-tracing studies of individual progenitors show considerable variability in the number and the composition of neural progeny. Clonally cultured retinal progenitors seem to follow stochastic patterns to decide between self-renewal and differentiation, and the order of retinal cell types produced within each individual progenitor lineage does not necessarily follow the order observed across the whole progenitor population⁵². Retinal progenitors from earlier developmental stages were biased to undergo more self-renewing divisions than those from later stages, however, indicating a cell-intrinsic shift in division mode over time⁵² (reviewed in REF. 53). Alternatively, it is possible that there are multiple progenitor subtypes that each have a different but highly reproducible cell lineage (in a similar way to fly neuroblasts). Consistent with this idea of progenitor heterogeneity is the recent finding that cadherin 6 and oligodendrocyte transcription factor 2 (OLIG2) mark subsets of retinal progenitors that are biased to produce specific types of retinal neurons^{54,55}. How spatial, temporal and stochastic mechanisms might integrate to balance diversity and order is a fascinating area for future study.

Temporal-identity specification in the cortex. The mammalian cerebral cortex provides a most striking example of a system in which radial migration of neuronal progeny on the basis of birth order dictates cortical laminar organization^{56,57}. Ventricular zone (VZ) progenitors in the pseudostratified neuroepithelium initially divide symmetrically to expand the progenitor pool. Elegant time-lapse microscopy studies revealed that radial glia, the progenitors of the VZ, then directly give rise to neurons while undergoing self-renewing divisions, and subsequently give rise to INPs that undergo symmetric neurogenic divisions within the SVZ to produce two more progenitors or two neurons^{58,59}. Postmitotic neurons born from progenitors in the VZ and the SVZ migrate radially to the cortical plate, with later-born neurons climbing past the earlier-born neurons, resulting in six distinct layers formed in an inside-out fashion on the basis of birth order (see REF. 60 for a more in-depth review on cortical development) (FIG. 4a,b).

Although both the VZ and SVZ generate cortical neurons, studies of genes expressed in the VZ (such as paired box gene 6 (*Pax6*) and orthodenticle homologue 1 (*Otx1*)) and SVZ (such as cut-like homeobox 1 (*Cux1*), *Cux2* and subventricular expressed transcript 1 (*Svet1*)) have suggested that VZ progenitors generate deep layer VI–V neurons and that SVZ progenitors generate superficial layer IV–II neurons^{61–64}. *Cux2*, however, is detected in a small subset of progenitors in the VZ of mice as early as embryonic day 10.5, before the formation of the SVZ, which led to the suggestion that *Cux2*-expressing progenitors might be committed from the outset to generating the upper-layer neurons⁶⁴. Consistent with this model, *in utero* fate mapping shows that *Cux2*-expressing VZ progenitors are fate-restricted to give rise to upper-cortical-layer neurons, regardless of niche or birth date, and that superficial versus deep laminar fate is the result of timed cell-cycle exit of progenitors rather than sequential specification⁶⁵. What is not yet clear is whether *Cux2*-negative VZ progenitors can produce deep-layer neurons before beginning to express *Cux2* (FIG. 4c). Other lineage-tracing studies have, however, provided evidence for individual progenitors that give rise to neurons of both the upper and lower layers. For example, *in utero* electroporation of the VZ with a green fluorescent protein (GFP)-encoding retrovirus was used to label the progeny of individual radial glia, and this revealed that ontogenetic radial clones of excitatory neurons span deep and superficial cortical layers⁶⁶. These sibling neurons form synaptic connections that foreshadow mature cortical circuitry, suggesting that a single progenitor can contribute to the columnar microcircuit.

It is clearly important to understand how cell-cycle regulation intersects with temporal generation of neuronal subtypes. Previous studies have shown that loss of *Cux2* in the cortex results in increased proliferation of SVZ progenitors and an overproduction of upper-layer cortical neurons; conversely, overexpression of *CUX2* promotes cell-autonomous cell-cycle exit of neural progenitors *in vitro*⁶⁷. These observations seem to be at odds with the findings that *CUX2*-expressing progenitors

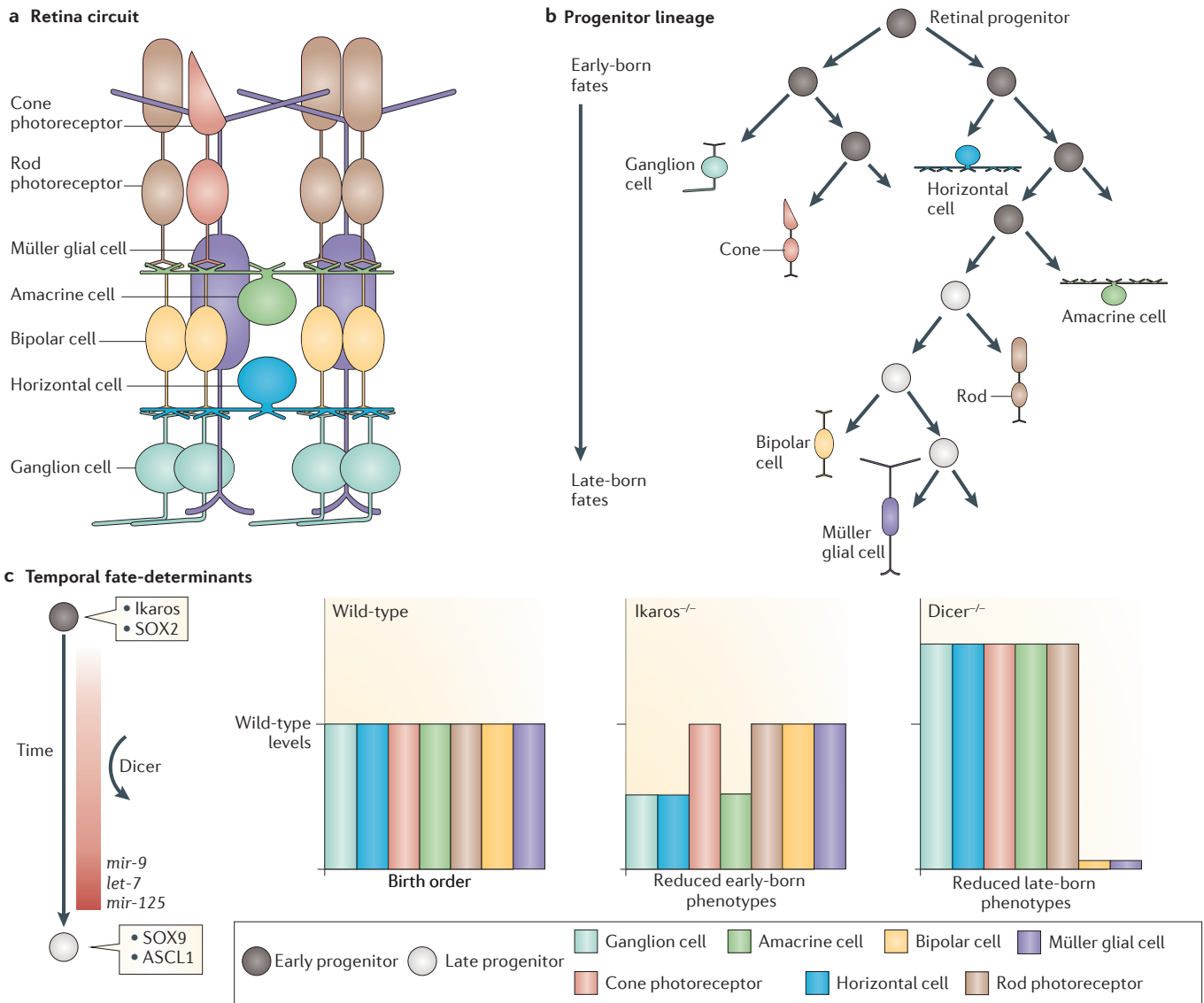


Figure 3 | Temporal fate specification in mammalian retina. **a** | Schematic illustration of the main cell types in the retina and their organization within the retinal circuit. The retina is comprised of six major classes of neurons and one type of glial cell (the Müller glial cell)¹²⁰. **b** | Retinal progenitors give rise to these distinct cell types in an overlapping but sequential order. Ganglion cells are generated first by early retinal progenitors and bipolar cells, and Müller glia are born last from late progenitors. **c** | Several molecular factors are expressed in either early or late progenitors and can determine the temporal phenotypes of the progeny. Dicer is required for the expression of several microRNAs that regulate the temporal transition of the progenitors to produce late-born cell fates⁹⁵. In mouse retina lacking the transcription factor Ikaros, there is a decrease in the number of cells with early-born fates, although the cone photoreceptors are not affected⁹¹. By contrast, in mouse retina lacking Dicer, a key enzyme involved in microRNA processing, there is a loss of the late-born cell fates⁹⁵. ASCL1, achaete-scute homologue 1; SOX, SRY-box containing gene.

remain mitotically active for longer than those lacking CUX2 and that these are the progenitors that generate upper-layer neurons⁶⁵. It is possible that CUX2 might function differently in VZ and SVZ progenitors or might change its function over time. This seems to be the case for the zinc-finger transcription factor Sal-like protein 1 (SAL1; also known as SALL1), which is highly expressed in cortical progenitors but downregulated in differentiating neurons⁶⁸. In *Sall1*-knockout mice, progenitors in the early stages of corticogenesis (predominantly the VZ radial glial cells) prematurely exit the cell cycle

and differentiate into neurons, whereas progenitors at later stages (predominantly the SVZ intermediate progenitors) re-enter the cell cycle without differentiating, resulting in fewer upper-layer neurons⁶⁸. Another regulator of progenitor cell cycle is glycerophosphodiester phosphodiesterase 2 (GDE2; also known as GDDP5), a six-transmembrane protein that is detected throughout corticogenesis in postmitotic neurons⁶⁹. In *Gde2* mutants, progenitors fail to exit the cell cycle until the end of the normal neurogenic period and differentiate en masse into upper-neuron identities at the expense of

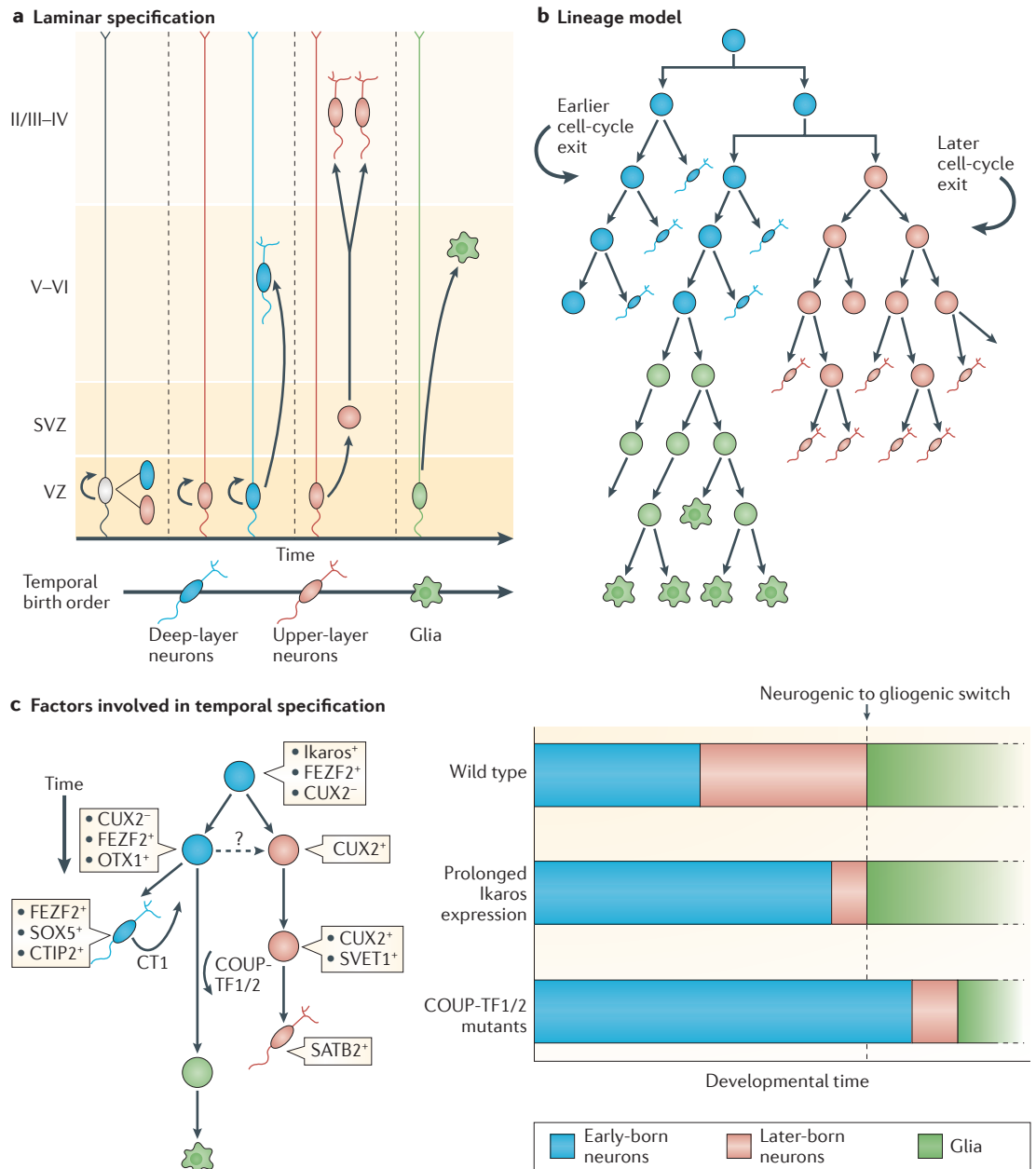


Figure 4 | Temporal fate specification in the mammalian cortex. **a** | The six layers of the mammalian cortex are generated by the sequential production of distinct types of neurons that migrate to progressively more superficial layers in an 'inside-out' fashion⁵⁷. Deep-layer neurons (blue) are born first from the ventricular zone (VZ) radial glia. Subsequently, upper-layer neurons (red) are born from a subset of VZ radial glia as well as the intermediate progenitors in the subventricular zone (SVZ) that are born from the VZ progenitors. Finally, glia (green) are born after the neurogenic period ends. **b** | Progenitors that give rise to deep-layer neurons (blue) exit the cell cycle earlier than the progenitors that primarily give rise to the upper-layer neurons (red). **c** | Factors that function in laminar cell fate are shown on the left. A multipotent progenitor gives rise to more-restricted lineages that preferentially generate deep or superficial cortical neurons^{64,65}. COUP transcription factor 1 (COUP-TF1) and COUP-TF2, as well as extrinsic signals such as cardiotrophin 1 (CT1), act on progenitors to mediate the switch from neurogenesis to gliogenesis^{85,90}. It is currently unknown whether all CUX2-expressing SVZ progenitors that generate upper-layer neurons are derived from the CUX2-expressing VZ progenitors or whether these are separate progenitor pools. Continuous expression of Ikaros in cortical progenitors or loss of both COUP-TF1 and COUP-TF2 results in an expansion of early-born cortical phenotypes at the expense of later-born phenotypes^{80,90,91}. Although prolonged Ikaros expression affects the balance of neuronal fates, it does not affect the timing of gliogenesis⁸⁰, whereas loss of both COUP-TF1 and COUP-TF2 delays gliogenesis in addition to shifting the balance of neuronal fates. CTIP2, COUP-TF-interacting protein 2; FEZF2, Fez family zinc finger protein 2; OTX1, orthodenticle homologue 1; SATB2, special AT-rich sequence-binding protein 2; SOX5, SRY-box containing gene 5; SVET1, subventricular expressed transcript 1.

early-born identities⁶⁹. Thus, GDE2 is an extrinsic regulator of progenitor cell-cycle exit (through feedback from the neuronal progeny) and temporal-identity switching.

Although the role of Sonic hedgehog (SHH) in spatial patterning is well established, increasing evidence implicates SHH in the regulation of the cell cycle and temporal identity. In the *Xenopus laevis* retina, shh regulates the length of the progenitor cell cycle, which in turn regulates the expression of several microRNAs that are important in specifying temporal cell fate⁷⁰. In the chick spinal cord, SHH promotes progenitor pool expansion at the expense of neuronal differentiation, and thus the timing of motor neuron formation⁷¹. The actions of SHH, SAL1 and GDE2 are thus important examples of how extrinsic and intrinsic cues can regulate the timing of progenitor differentiation.

In recent years tremendous progress has been made in identifying factors that are expressed in a cortical layer-specific manner and determining how they specify laminar fates (FIG. 4c). Forebrain embryonic zinc finger protein 2 (FEZF2; also known as FEZL and ZNF312), SRY-box containing gene 5 (SOX5) and COUP-TF-interacting protein 2 (CTIP2; also known as BCL-11B) are transcription factors that are required for the specification of the early-born projection neurons that occupy the deep cortical layers and project to subcortical regions^{72–76}. FEZF2 promotes the specification of deep-layer subcortical projection neurons in part by repressing the chromatin-remodelling protein special AT-rich sequence-binding protein 2 (SATB2)⁷⁵. Conversely, SATB2 promotes superficial-layer, callosal-projection neuron identity by repressing CTIP2 expression^{72,77}. The transcription factors brain-specific homeobox/POU domain protein 1 (BRN1; also known as POU3F3) and BRN2 (also known as POU3F2) also have an important role in generating upper-layer neurons⁷⁸. Recent important work using combinatorial deletion of these laminar-fate-specifying factors uncovered complex, cross-inhibitory genetic interactions among cell-fate determinants in the postmitotic neurons that act to actively repress alternative fates and execute the developmental stage-appropriate transcriptional programme⁷⁹. Interestingly, SATB2 and FEZF2 regulate genes that are implicated in neurological disorders, providing an entry point for the study of complex diseases⁷⁹. Whereas SOX5 and CTIP2 act in postmitotic neurons to promote deep layer-specific phenotypes, FEZF2 is transiently expressed by young VZ progenitors and is maintained in the early-born deep layer V–VI neurons⁷⁶, much like the Hb and Kr temporal-identity factors in fly neuroblasts⁵. Although FEZF2 is necessary and sufficient to induce early cortical neuron phenotypes^{75,76}, it remains unclear whether it functions as a bona fide temporal-identity factor or as a cell-fate determinant for a specific cortical-neuron subtype; further study is needed to ascertain whether it is able to specify early-born phenotypes in multiple neural stem-cell lineages in the cortex or elsewhere. Ikaros, by contrast, appears to fulfil the criteria for being a temporal-identity factor: in addition to its role in specifying early temporal fate in the retina⁵¹, recent work shows that it can induce

early-born neuronal fate in the cortex⁸⁰. When Ikaros expression is genetically maintained in progenitors, there is a sustained increase in the generation of early-born, deep-layer cortical neurons and a decrease in the generation of upper-layer neurons⁸⁰ (FIG. 4c). It remains to be determined how FEZF2 and Ikaros work together to specify early-born temporal identity. In addition to those mentioned above, numerous other transcription factors show cortical-layer-specific expression patterns⁵⁷, and it will be important to understand how these factors fit into the transcriptional network in progenitor and postmitotic neurons to establish sharp boundaries of temporal identities.

Temporal switch from neurogenesis to gliogenesis. One transition in cell fate that is observed in multiple regions of the developing CNS is the switch from neurogenesis to gliogenesis^{81,82}. Previous studies have found that neurogenins, which are proneural basic helix–loop–helix (bHLH) proteins, can promote neurogenesis and inhibit gliogenesis⁸³; conversely, the gliogenic factor SOX9 is required for the timely neuron-to-glia switch⁸⁴. Extrinsic mechanisms also have an important role: signalling by the cytokines ciliary neurotrophic factor (CNTF), leukaemia inhibitory factor (LIF) or cardiotrophin 1 (CT1) induces astrogenesis by activating the glial gene glial fibrillary acidic protein (*Gfap*)^{85–88}. CT1 is secreted by newly born cortical neurons, indicating that the onset of gliogenesis involves feedback regulation⁸⁵ (FIG. 4c). Other signalling pathways, such as the Notch and bone morphogenetic protein (BMP) signalling pathways, also promote gliogenesis (reviewed in REF. 89). These results suggest that neurogenic and gliogenic cell-fate programmes are closely interconnected via multiple cell-intrinsic and -extrinsic mechanisms. Intriguingly, recent work has shown that the COUP transcription factor 1 (COUP-TF1; also known as COUP-TFI) and COUP-TF2 (also known as COUP-TFII) nuclear receptors, which are orthologues of *D. melanogaster* Svp, function as a ‘timer’ that switches progenitors from neurogenesis to gliogenesis. They are transiently expressed in neural progenitors near the end of the neurogenic phase, and their loss prolongs neurogenesis at the expense of gliogenesis⁹⁰. COUP-TF1 has also been implicated in the switch from early-born to late-born cortical neurons⁹¹. Thus, Svp and the COUP-TF1 and 2 proteins seem to have conserved roles as switching factors in both *D. melanogaster* and mammalian neural progenitors (FIG. 4c).

MicroRNAs in temporal fate specification. MicroRNAs have recently been added to the repertoire of factors that contribute to temporal fate specification (FIG. 3c). Conditional deletion of Dicer, a key microRNA-processing enzyme, in progenitors results in an increase in early-born neuronal phenotypes and a decrease in late-born phenotypes in both cortex and retina^{92–95} (reviewed in REF. 96). Similarly, loss of Dicer results in a loss of late-born glia in the spinal cord but leaves early-born motor neurons intact⁹⁷. Ikaros, like its *D. melanogaster* orthologue Hb, promotes early-born cell fate in both the cortex

and the retina. In the retina, Ikaros mRNA is expressed throughout development, whereas its protein is detected only in the early progenitors⁵¹, suggesting that it is regulated post-transcriptionally, perhaps through microRNA function. In *Caenorhabditis elegans*, in which microRNAs have a well-documented role in regulating the timing of developmental transitions⁹⁸, the *let-7* family of microRNAs must degrade *hunchback-like (hbl)* RNA, the *C. elegans* orthologue of *D. melanogaster hb*, to allow the transition from the L2 to the L3 larval stage⁹⁹. Whether Ikaros and Dicer-mediated regulation of temporal identity are part of the same or parallel pathways, and whether other members of the Ikaros family function in temporal fate specification are still unknown. Recent work has further shown that microRNAs function at multiple stages of neuronal differentiation. When Dicer is deleted in postmitotic neurons of the cortex, there is a reduction in dendritic branching, but the laminar organization is normal¹⁰⁰. In *D. melanogaster*, the *let-7* and *mir-125* microRNAs are expressed in some larval neuroblast lineages by late-born neurons and are required for the specification of late neuronal temporal identities by decreasing the levels of Chinmo protein expression³¹. Thus, microRNAs seem to have an important role in temporal fate specification in both the progenitor and the postmitotic progeny in multiple organisms.

The above studies pave the way for several research avenues. Perhaps the two most important will be to investigate how spatial cues and multiple temporal cues are integrated to generate neural diversity and how temporal-identity factors govern neuronal terminal differentiation. An attractive possibility is that spatial-patterning factors in neural stem cells establish a chromatin state that determines which target genes become transcriptionally active in response to downstream temporal-patterning factors, and such chromatin states can subsequently be inherited by the postmitotic progeny.

Changes in progenitor competence

In addition to the transcriptional changes that neural progenitors undergo to specify distinct temporal cell fates, the competence of neural progenitors to specify particular fates also changes during development. While gradually losing the ability to specify earlier-born cell fates (competence restriction), neural progenitors acquire the competence to make later-born cell types. Thus, a given neural cell type can be specified during a limited time window. Investigating how competence is regulated is crucial to our understanding of brain development and ongoing efforts to generate specific neural cell types from induced pluripotent stem cells.

Changing progenitor competence in *D. melanogaster*.

The best-characterized model for studying progenitor competence is the embryonic NB7-1, for which there are markers for each of the neuronal progeny (U1–U5) specified by temporal-identity factors^{3,5,7,12} (FIG. 1b). Pioneering work showed that although Hb normally specifies U1 and U2 fates during the first two neuroblast divisions^{6,8,9}, transient pulses of ectopic Hb expression in NB7-1 at later stages can induce extra U1 and U2 neuron generation

until the fifth division⁷ (FIG. 5a). Similarly, competence to respond to Kr and specify the U3 fate is lost after the fifth division, showing that NB7-1 has an early competence window to respond to both Hb and Kr^{12,13}.

What closes the early competence window? It was initially thought that sequential expression of the temporal-identity-factor genes restricts neuroblast competence, as early work suggested that continuous expression of Hb can prevent Cas expression and extend the competence window⁷. However recent work has revised this conclusion¹⁷. It has been shown that Hb cannot extend neuroblast competence and that temporal fate specification and competence are regulated independently (FIG. 5b). This study revealed that neuroblasts lose competence to specify early-born fate by undergoing a developmentally regulated reorganization of the genome that repositions the *hb* genomic locus on the nuclear lamina¹⁷, a gene-silencing hub^{101–103} (FIG. 5c). As described above, NB7-1 normally specifies *hb*-transcribing, early-born-identity neurons for the first two divisions, and yet remains competent to specify early-born fate for an additional three divisions. This suggests that the *hb* gene locus, although transcriptionally turned off at the second neuroblast division, undergoes a subsequent transition to a permanently silenced state at the fifth division (FIG. 5b,c). It is well known that genes occupy nonrandom subnuclear positions that can affect their transcriptional states¹⁰⁴. By using *in vivo* DNA fluorescent *in situ* hybridization (FISH), it was shown that the repositioning of the *hb* locus to the nuclear lamina coincided with the end of the competence window at the fifth division, three cell divisions after the end of *hb* transcription¹⁷ (FIG. 5c). Genetic disruption of the nuclear lamina reduced *hb* gene–lamina association and increased the probability of NB7-1 producing an extra *hb*-transcribing neuron, indicating that the nuclear lamina is essential for permanently silencing the *hb* gene¹⁷.

How is neuroblast competence regulated? Previous work had identified the co-expressed and partially redundant Distal antenna (Dan) and Dan-related (Danr) proteins, which are members of the Centromere protein B (CENP-B)/transposase family of proteins¹⁰⁵, as regulators of embryonic neuroblast temporal identity²⁵. Their expression in neuroblasts is transient and is rapidly downregulated coincident with *hb* gene movement to the lamina and the end of the early competence window¹⁷ (FIG. 5c). Although prolonged expression of Dan in neuroblasts has no effect on the timing of *hb* transcription or number of early-born neurons, the *hb* genomic locus fails to move to the nuclear periphery in these cells, and the early competence window to specify the early-born identity is extended (FIG. 5d). The extension in competence is revealed only when expression of the temporal-identity factor Hb is also prolonged in the neuroblast together with Dan. This shows that progenitor competence and temporal identity are regulated by two distinct mechanisms: Dan regulates competence and Hb specifies temporal identity. Interestingly, Dan is expressed in neuroblasts in two phases: first in newborn neuroblasts and then again in late embryonic neuroblasts²⁵. The temporal-identity factor Kr is also expressed twice in the NB7-1 lineage, once during the

Nuclear lamina

A network of intermediate filaments and membrane-associated proteins of the nuclear envelope. Genes associated with the nuclear lamina compartment are often in a silenced or repressed state.

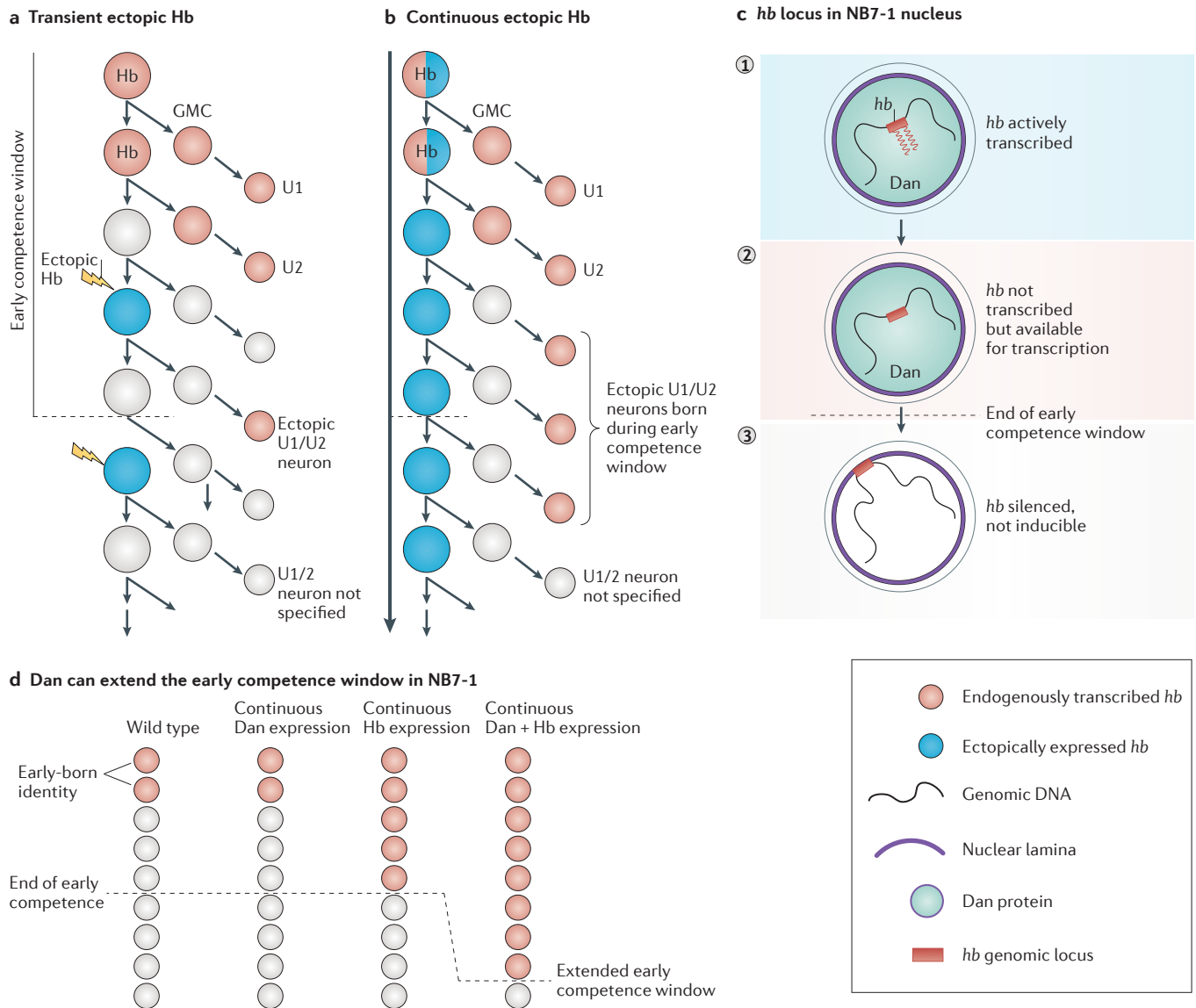


Figure 5 | Reorganization of the neuroblast genome regulates competence transition in *Drosophila melanogaster* embryos. **a** | The Hunchback (Hb) temporal-identity factor is expressed in neuroblast 7-1 (NB7-1) of the fly embryonic nerve cord for the first two divisions (red), giving rise to the U1 and U2 early-born motor neurons, which maintain Hb expression⁵. A transient pulse of ectopic Hb (blue) can induce the neuroblast to produce an ectopic U1/U2 neuron up to the fifth division⁷. These first five divisions are called the ‘early competence window’. After this window ends (dashed line), NB7-1 is no longer competent to respond to ectopic Hb and cannot specify early-born neuronal fate. **b** | If ectopic Hb (blue) is continuously expressed in the neuroblast, only the postmitotic progeny born during the early competence window, and not those born after, will activate endogenous *hb* transcription (red)¹⁷. **c** | During the first two divisions when *hb* is actively transcribed, the *hb* genomic locus is positioned in the nuclear interior (stage 1). During the subsequent three divisions, the *hb* gene is transcriptionally inactive but is still positioned in the nuclear interior and is amenable for activation in the progeny (stage 2). At the end of the early competence window, the *hb* locus becomes repositioned to the nuclear lamina, where it is permanently silenced and is no longer inducible (stage 3). The nuclear factor Distal antenna (Dan; green) is expressed in the neuroblast during the early competence window, and its downregulation is required for *hb* gene repositioning to the lamina¹⁷. **d** | Dan can extend the NB7-1 early competence window. Continuous expression of Hb alone results in the specification of early-born identity only during the early competence window. Continuous expression of Hb and Dan together results in prolonged NB7-1 competence to specify early-born identity¹⁷.

first Dan window (where it specifies U3 motor neuron) and again during the second Dan window when interneurons are being generated¹². An attractive model is that Dan is used to generate two competence windows, allowing the same temporal-identity factor to

act on a distinct genome architecture or epigenetic landscape to produce different outcomes in each competence window. Further experiments are needed to determine the molecular mechanisms of Dan function, its target genes during its early and late phase of

expression and how its expression is regulated. The near synchrony with which Dan is downregulated in neuroblasts¹⁷, which delaminate at different times and have widely varying lineage lengths^{3,5}, suggests that a global extrinsic signal might have a role in regulating Dan expression and neuroblast competence.

Neuroblast competence is also regulated by the Polycomb repressive complexes (PRCs), which promote heritable gene silencing during development¹⁰⁶. Overexpressing Kr in a genetic background of reduced expression of Polyhomeotic or Suppressor of zeste 12 (which are components of PRC1 and PRC2, respectively) led to greater numbers of ectopic Kr-specified fates compared to overexpressing Kr alone¹³. Conversely, overexpressing Polyhomeotic suppressed this effect. Determining the relationship between Dan- and PRC-regulated competence will give further insight into the mechanism of progenitor competence.

Recent evidence from *C. elegans* suggests that changes in genome architecture might be a common characteristic of competence transitions. It was recently shown that decompaction of chromatin at the *lys-6* microRNA locus is required to make only one of two bilateral neurons competent to transcribe *lys-6* at a later developmental stage — a crucial event in establishing the left–right asymmetry of gustatory neurons in *C. elegans*^{107,108}. What is emerging is the importance of understanding how cell type-specific and developmental stage-specific changes in genome architecture intersect with the timely expression of key cell-fate determinants to specify the correct cell fate. Another fascinating direction for future research would be to determine whether type II neuroblasts and/or INPs also undergo competence transitions. Reorganization of the type II neuroblast genome, as observed in embryonic neuroblasts¹⁷, could explain how the same set of temporal-identity factors in INPs can give rise to distinct neural identities as the parental type II neuroblast ages over time²⁴. Perhaps regulation of genome architecture is a general mechanism that allows a limited group of transcriptional regulators to generate cellular diversity in a spatially and temporally controlled manner.

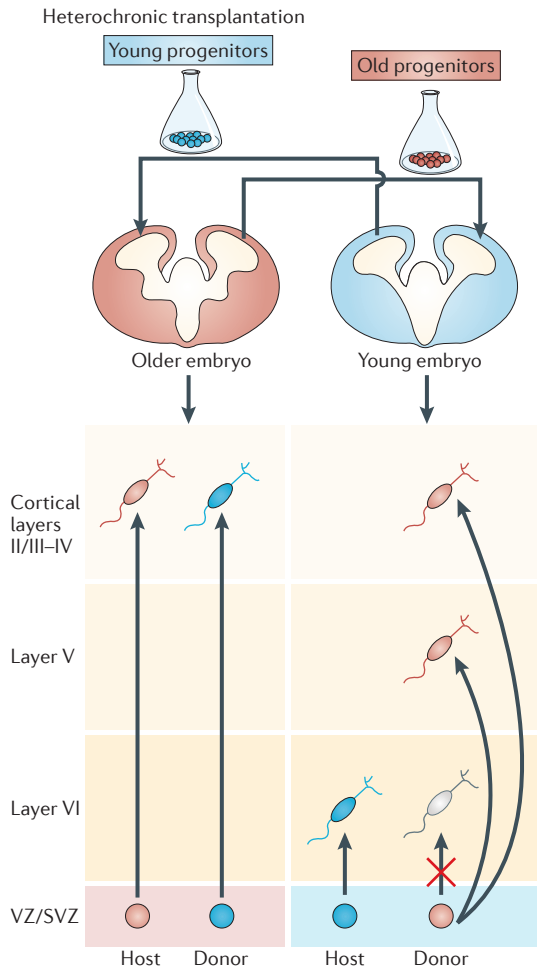
Changing progenitor competence in mammals. The first examples of changes in competence states of neural progenitors during development came from studies in the mammalian cortex and retina. A series of elegant studies used heterochronic transplantation experiments in the ferret to expose young or old neural progenitors to the opposite host environment and probe for their ability to generate host-appropriate laminar fates^{109–111}. Early cortical progenitors, which normally produce deep layer V and VI neurons, were competent to produce the later-born, superficial layer II–IV neurons when transplanted into an older embryo, but not vice versa¹⁰⁹ (FIG. 6a). Remarkably, the early cortical progenitors were competent to follow the older host programme and produce late-born neurons only when the progenitors were transplanted prior to undergoing S phase¹¹². By contrast, older progenitors were not competent to generate deep-layer, early-born neurons even if they had undergone one or

more rounds of cell division in the younger host environment¹⁰⁹. Interestingly, however, when layer IV progenitors were heterochronically transplanted into a younger host environment in which layer VI neurons were being made, the donor progenitors were no longer competent to produce the early-born layer VI neurons but were still able to give rise to layer V neurons¹¹¹. Because the donor progenitors were isolated after layer V neuron production had already ceased, the results suggest that competence to specify temporal identity persists for a limited time after the generation of that cell type and is not governed by counting cell divisions. This result is strikingly reminiscent of *D. melanogaster* neuroblasts, which remain competent to specify early-born identity for several divisions after these cell types cease to be produced and highlight a fundamental property of neural stem cells: that is, competence transitions are not temporally aligned with cell-fate transitions.

The identification of mammalian temporal-identity factors has facilitated the investigation of mammalian neural-progenitor competence. When Ikaros is ectopically expressed in older retinal progenitors *in vivo*, it can induce production of early-born neuronal identities, such as horizontal and amacrine cells, and suppress the production of the late-born Müller glia⁵¹. However, Ikaros misexpression cannot generate early-born ganglion cells *in vivo*, suggesting that some but not all early progenitor competence can be restored⁵¹. Similarly, when *lin28* mRNA, a late retinal-progenitor microRNA target, is ectopically expressed in early progenitors, there is an increase in the BRN3⁺ early-born ganglion cell type; however, there is no increase when *lin28* is expressed in late progenitors, which suggests that there is a limited competence window in which early-born fate can be specified⁹⁵. In the cortex, ectopic expression of FEZF2 in late cortical progenitors induces the production of neuronal progeny with the characteristics of early-born neurons⁷⁶. However, the neurons still migrate to superficial layers and can make callosal projections, which are characteristics of late-born neurons, suggesting that older progenitors are not fully competent to specify early-born identity. More recently, competence to respond to FEZF2 was studied in postmitotic neurons, revealing that ectopic expression of FEZF2 in postmitotic, upper cortical neurons can reprogram them to adopt characteristics of deep-layer neurons, including the expression of the appropriate molecular markers, axonal projection patterns and physiological phenotypes^{113,114}. Interestingly, these postmitotic neurons could switch phenotypes only for a brief time window after their terminal mitosis, which suggests that there is a progressive restriction in competence as postmitotic neurons age. Work in *D. melanogaster* has previously shown that late-born postmitotic neurons are not competent to adopt early-born neuron characteristics on misexpression of Hb⁷, but it is not clear whether loss of competence occurs immediately upon neuronal birth or whether there exists a short period of competence after the terminal mitotic division. It will be important to determine what aspects of competence become restricted over time in the progenitor versus the progeny.

Polycomb repressive complexes (PRCs). Multiprotein complexes that remodel chromatin to establish epigenetic silencing.

a Competence for laminar fate specification



b Gliogenic competence

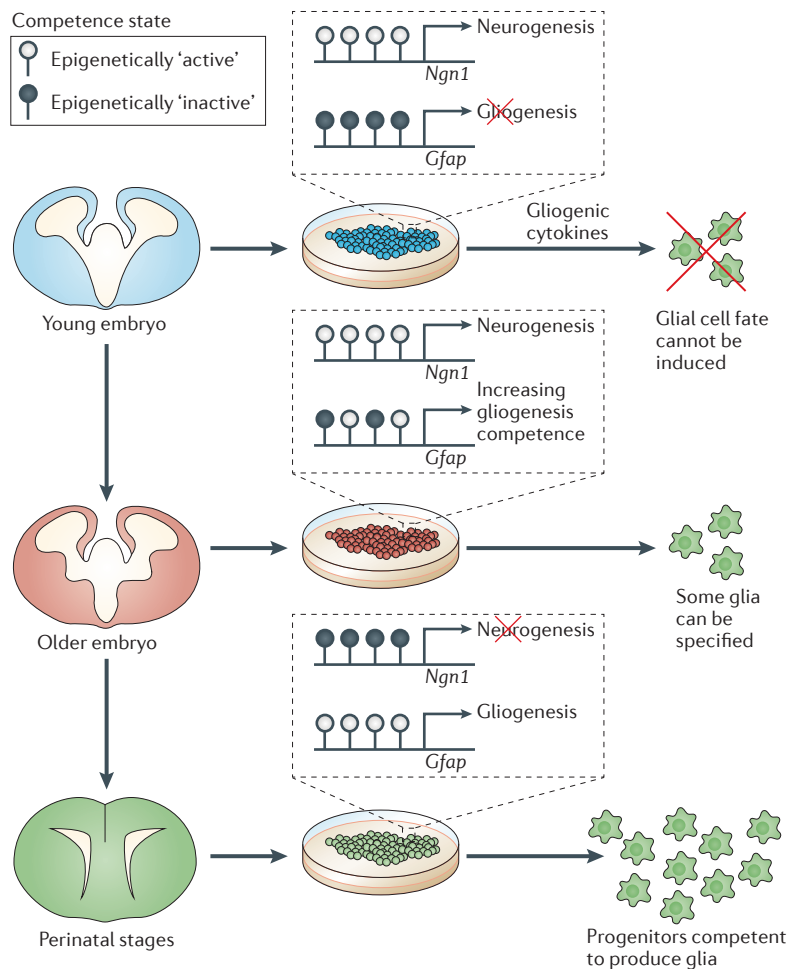


Figure 6 | Competence transitions during mammalian neurogenesis. a | Cortical progenitors lose competence to specify early-born neuronal phenotypes over time. In heterochronic transplantation experiments — in which neural progenitors are isolated from one developmental stage in the donor and then placed in a similar environment but at a different developmental stage in the host — early progenitors (blue) that are transplanted into an older host can give rise to later-born phenotypes. However, older progenitors (red) that are transplanted into a younger embryo do not give rise to early-born (layer VI) phenotypes¹¹¹. **b** | Changes in chromatin structure at proneural and gliogenic genes contribute to the neurogenic to gliogenic competence transition in the embryo. In early progenitors, regulatory DNA sequences of key gliogenic genes (such as glial fibrillary acidic protein (*Gfap*)) are hypermethylated and silenced. In older progenitors, these DNA regions become hypomethylated and subsequently competent for transcriptional activation. Neural progenitors cultured from older embryos (red and green embryos) are thus competent to respond to gliogenic signals and give rise to glia (green cells). As progenitors acquire competence to produce glia, they also lose competence to produce neurons because proneural genes, such as neurogenin 1 (*Ngn1*; also known as *Neurog1*), undergo Polycomb-mediated silencing^{126–128}. SVZ, subventricular zone; VZ, ventricular zone.

Culturing neural progenitors *in vitro* has provided information on the role of extrinsic cues in regulating progenitor competence. Pioneering work showed that rat retinal progenitors dissociated *in vitro* generate progeny on the same schedule as they do *in vivo*. Moreover, when co-cultured with progenitors from a different developmental stage, they can respond to diffusible extrinsic signals and alter the proportions of progeny subtypes produced, but not the timing^{115,116}. In fact, when old progenitors are cultured in an excess of young progenitors (or vice versa), changes in environmental signals can bias the relative proportion of cell fates generated but cannot induce

them to specify cell fates outside their normal temporal window^{117–119}; this suggests that retinal progenitors pass through multiple competence stages over the course of their lineage (reviewed in REF. 120). Interestingly, ectopic expression of *Ikaros* in late-stage retinal progenitors can induce transcription of the early-born ganglion cell marker *Brn3* only when progenitors are cultured *in vitro*, but not *in vivo*⁵¹, which suggests a role for extrinsic signals in terminating progenitor competence to specify early-born fate. In a similar way to retinal progenitors, mammalian cortical progenitors can sequentially produce neurons *in vitro* with gene expression that is appropriate for their

birth order, and they even lose competence to specify earlier-born neuronal fates¹²¹. Together, observations from retina and cortical studies implicate a coordination of cell-intrinsic timing mechanisms and cell-extrinsic signals that regulate competence.

The switch from neurogenesis to gliogenesis occurs in multiple regions of the developing CNS and reflects an important competence transition. Neurogenic cortical progenitors cultured on embryonic brain slices make neurons but can switch to making glia when cultured on postnatal brain slices¹²². Consistently, ectopic expression of CNTF *in vivo* can induce neurogenic progenitors to precociously produce glia⁸⁵. These results suggest that at least a subset of neurogenic progenitors is competent to make glia when exposed to the proper extrinsic signals. However, early cortical progenitors have limited competence to generate glia, as suggested by the lack of glia produced during early corticogenesis despite the presence of gliogenic cytokines^{123,124}, and young cortical progenitors were less competent to produce glia than older progenitors when exposed to gliogenic cytokines in culture¹²⁵ (FIG. 6b). This difference in gliogenic competence is due at least in part to the highly methylated status of the *Gfap* promoter during the neurogenic phase, as revealed by the finding that neural progenitors lacking DNA methyltransferase 1 (*Dnmt1*) can precociously generate GFAP⁺ astrocytes in response to LIF^{126,127}. COUP-TF1 and COUP-TF2, which are transiently expressed just before the onset of gliogenesis, are needed to release silencing chromatin modifications at promoter regions of glial genes, thus allowing progenitors to become gliogenically competent⁹⁰. The PRC has also been found to silence the proneural bHLH genes to end neurogenesis¹²⁸, highlighting the role of epigenetic mechanisms in the competence switch. Interestingly, a recent study observed that the switch from neurogenesis to gliogenesis involves a change in progenitor competence that is independent from the progenitor temporal-identity transition. During cortical neurogenesis, sustained expression of Ikaros, an early-born temporal-identity factor, dramatically affects the fate of the neuronal progeny but does not affect the timing of gliogenesis⁸⁰ (FIG. 4c) — neurons adopt the early-born, deep-layer

phenotypes at the expense of the later-born, upper-layer phenotypes, but gliogenesis still begins on time. These results are strikingly similar to those observed in *D. melanogaster* neuroblasts, in which maintained expression of Hb produces ectopic neurons with early-born identity only during the early competence window. Similarly, Ikaros seems to be able to produce extra early-born neurons in the cortex, but only during the neurogenic competence phase.

Conclusions and future perspectives

We are only scratching the surface of understanding how neural stem-cell temporal identity and competence are regulated during development, and the work highlighted above reveals new research avenues that would provide insight into this important process. It is clear that the specification of cell fate involves a complex interplay among the expression of temporal-identity factors, spatial information and the competence state of the progenitor. As we have discussed, changes in competence are not necessarily restricted to cell-fate decisions during neurogenesis but can be studied in a broader context of developmental transitions. It is still unclear how mechanisms that regulate competence transitions intersect with changes in progenitor temporal identity. Do unique genome architectures allow the same transcription factors to regulate different target genes that are appropriate for the cell type and stage? How is neurogenic competence maintained indefinitely in adult neural progenitors? Are these adult progenitors competent to make neural cell types outside their normal repertoire?

What can we learn from studying temporal fate specification and the regulation of progenitor competence to develop more effective tools for tissue repair? How could we start with a pluripotent stem cell and induce the specification of a particular cell type? Understanding the mechanism (or mechanisms) that regulate progenitor competence might help to increase the efficiency of somatic-cell reprogramming, and understanding how multiple competence windows are established might increase the accuracy of directed cellular reprogramming. These are important and exciting directions for future research.

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Competing interests statement

The authors declare no competing interests.