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Cell cycle and cell fate interactions in neural development

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Mechanisms coupling cell cycle and cell fate operate at different steps during neural development. Intrinsic factors control the cell proliferation of distinct brain regions and changes of cell fate competence, whereas components of the cell cycle machinery could play a major role in setting the appropriate timing of the generation of different cell types.

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Abbreviations
bHLH basic helix-loop-helix
Cdk cyclin-dependent kinase
Cdki cyclin-dependent kinase inhibitor
NEP neuroepithelial progenitors
Rb retinoblastoma protein
TF transcription factor

Introduction

A major requirement of the vertebrate neural developmental programme is the co-ordination of cell cycle regulation and cell fate determination. Many recent observations have highlighted functional cross-talk between molecules controlling these two processes. Key factors regulating cell cycle progression influence neural cell fate, whereas determination/differentiation factors have a role in regulating the cell cycle [1,2]. Although some of the molecular interactions and pathways have been elucidated, there are still some important questions that remain unanswered. How is cell cycle progression controlled in specific embryonic cell compartments to allow for proper growth of different nervous structures? What are the molecular mechanisms that co-ordinate cell cycle progression and cell determination/differentiation? How do the cell cycle machinery and determination/differentiation factors co-operate to achieve correct timing for the generation of the different cell types? Does the asymmetry of cell division of vertebrate neural progenitors play a role in selecting different neural cell fates?

Recently, several patterning genes were found to control the cell proliferation rate of distinct central nervous system (CNS) structures, and dual function molecules have been described that couple cell cycle and cell fate. Finally, vertebrate genes that control the mode of cell division of neural progenitors, symmetric or asymmetric, have been described. In this review, we focus on intrinsic factors, here defined as intracellular genetic signals that influence a cell regardless of the environment. Among such factors, we consider cell cycle regulators and determination/differentiation factors that control the co-ordination of cell cycle and cell fate in the vertebrate nervous system.

Intrinsic factors control cell proliferation of distinct embryonic neural regions

Emerging evidence suggests that several patterning genes can control cell cycling in specific regions of the embryo, thus contributing to the differential growth of embryonic tissues and organs. Wnts and Sonic Hedgehog are examples of extrinsic cues that regulate the proliferation rate in distinct neural embryonic regions [3,4], while also playing a role in neural induction and the patterning of the CNS. Transcription factors (TFs) that control early patterning of the CNS also support the cell cycle progression of neural progenitors. In *Xenopus* anterior neural plate, high doses of the winged helix TF XBF-1 increase both the number of ectodermal cells devoted to a neural fate and their proliferation [5]. Similarly, the mouse Bf-1 gene controls the patterning and supports proliferation of the cells in the telencephalon, and the Bf-1 mutant mice show accordingly smaller telencephalic vesicles [6]. The homeobox TF Emx2, which specifies early dorsal telencephalic identity and is necessary for proper development of cortical structures, supports clonal expansion of multipotent cortical progenitors in primary cultures without affecting cells of the basal telencephalon [7•]. Finally, *Rx1*, *Six3* and *XOptx2* are necessary for eye formation and their expression is sufficient to drive retinal expansion [8–11]. In overexpression experiments, retinal overgrowth is due to increased cell proliferation rather than to re-specification of non-neural tissue. Notably, *Emx2*, *Rx1*, *Six3* and *XOptx2* show a restricted regional competence, as they can promote cell cycle progression of neural progenitors only in those regions where they are usually expressed. This suggests that co-factors may be required to interact with these TFs in a given developing region to control cell proliferation. This has been shown to be true

for the growth control of the *Drosophila* eye imaginal disc [12•]. In addition, there is evidence that *Emx2* [7•], *Optx2* [11] and *XRx1* (S Casarosa, M Amato, M Andreazzoli, G Gestri, G Barsacchi, F Cremisi, unpublished data) act to control cell cycle progression in single neural progenitors cell-autonomously.

Coupling cell cycle and cell differentiation

Several studies indicate that distinct neural fates may be determined during the final cell cycle of progenitors [1,13], although this may not always be the case [14,15]. Examples of cell determination occurring during the last cell cycle come from neuroblast lineages of *Drosophila* [16••,17], as well as zebrafish and *Xenopus* retinal progenitors [18•–20••]. Interestingly, Ohnuma *et al.* [20••] showed that in retinal progenitors transcription of the proneural genes that induce retinal differentiation is upregulated in the last cell cycle, when transcription of cell cycle activators such as cyclin dependant kinases (cdks) and cyclins has already been downregulated. This would suggest that cell fate is determined in this last, unique cell cycle, and leads us to ask which phase of the final cell cycle is important for cell fate determination? Elegant work by McConnell and Kaznowski [21] demonstrated that laminar fate determination of cortical neuroblasts is established around the S-phase of their last cell division. Conversely, Ohnuma *et al.* [20••] showed that the activity of *Xath5*, one of the proneural genes that specify ganglion cell fate, is affected by p27Xic1, a Cip/Kip cyclin dependant kinase inhibitor (cdki), and by cyclin E1. This suggests that proneural TFs determine retinal cell fates in the G1 phase of cell division. More detailed studies performed in less complex organisms, such as *Drosophila* and *Caenorhabditis elegans*, demonstrate that cell fate determination occurs in phases G1 and G2 [22–24]. Altogether these observations suggest that distinct signals may act in different phases of the cell cycle. Approaches that use genome-wide analysis could help to determine which cell cycle components are affected by cell fate determinants, as illustrated in the study of the gene *glial cells missing* (*gcm*) in *Drosophila* [25•].

As some distinct cell fates are determined during specific phases of the last cell cycle, could there be a molecular mechanism that links cell cycle and cell differentiation? The recent identification of dual function molecules would indicate that this is the case [1]. Homeodomain proteins that specify neuronal cell fates — such as Prospero in *Drosophila* [26], its mammalian homologue Prox-1 [27], and Phox2b in vertebrates [28] — induce exit from the cell cycle. The maternal gene *Tumorhead* has the opposite effect, inhibiting differentiation and promoting proliferation of neural plate cells [29•]. The *Drosophila* zinc-finger protein Tramtrack, which is a key transcriptional repressor inducing glial fate in *Drosophila*, inhibits cell cycle progression by downregulating the S-phase cyclin E [30•]. In addition, cell cycle regulators influence neural cell fate choice and differentiation in addition to and independent of their ability to regulate cell cycle exit [31–33].

Recently, it has been shown that a single cdki, p27Xic1, is required for the differentiation of primary neurons in *Xenopus* from neural progenitors [34••]. Interestingly this effect requires expression of a region of p27Xic1_overlapping with, but distinct from, that required to inhibit overall cdk activity, similar to that required for glial specification in the retina [32]. Moreover, p27Xic1 is required early in the sequence of events of neurogenesis between the early neurogenic factor neurogenin, and NeuroD, the expression of which is associated with cell cycle exit and terminal differentiation. Early results indicate that p27Xic1 may be acting by facilitating the stabilisation of neurogenin protein [34••]. This raises interesting analogies with myogenesis in which the early myogenic basic helix–loop–helix (bHLH) factor myoD, which is subject to ubiquitin-mediated proteolysis, is stabilised by a cdki, p57Kip2. Whereas early work indicated that p57Kip2 could act by inhibiting the cdk-dependent phosphorylation of myoD, triggering differentiation [35], a more recent study indicates that stabilisation of the myoD protein may be via direct binding [36]. In contrast, however, no direct association between p27Xic1 and neurogenin has been demonstrated in *Xenopus* [34••]. The retinoblastoma (Rb) protein, which coordinates entry of myoblasts into S phase, also has a further role in myogenesis. It cooperates with the muscle enhancement factor MEF2 to promote myoD-mediated transcription [37]. Rb-knockout mice show extensive early neural defects [38]. This evidence, along with recent results looking at the effect of Rb loss specifically in the telencephalon [39], indicates that the Rb protein could play an analogous role in neurogenesis and myogenesis, that is, regulating cell death, cell cycle exit and potentially differentiation.

Timing of cell fate generation

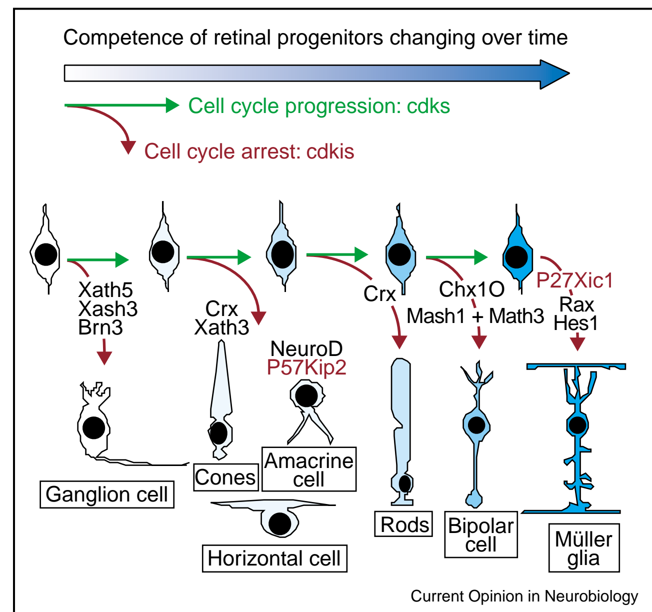
During neural development, different types of neurons and glia are generated from progenitors at specific times in a sequentially conserved order. This process is largely coordinated with the timing of cell cycle exit [40,41]. The competence model proposes that over time neural progenitors pass through intrinsically defined competence states, which correspond to their ability to generate only a defined sub-set of cell types. Whereas extrinsic cues can modulate the number of cells produced at a given time, they cannot specify temporally inappropriate cell fates. An example of such an intrinsic timing programme comes from cortical progenitors [42,43]. In particular, an elegant study of Qian *et al.* [42] showed that isolated stem cells from the embryonic mouse cerebral cortex exhibit a distinct order of cell-type production, which is unaffected by cell culture condition: neuroblasts first and glioblasts

later. Moreover, the accumulation and activation of cdkis is involved in a built-in timing mechanism that helps to determine when oligodendrocyte progenitors stop dividing and differentiate [44•,45]. Nevertheless, the molecular machinery driving the changes in competence is still for the most part undefined.

What level of regulation is involved in the temporal aspect of cell fate determination? Recent observations in *Drosophila* propose that changes in cell fate competence are established by the sequential expression of the TFs Hunchback → Krüpple → Pdm → Castor, which are necessary and sufficient to specify the different cell types generated by different lineages over time [16••]. Notably, although these factors can functionally interact with each other to modulate their own expression, their basal level of transcription and timing of expression are independent of this cross regulation. Therefore, their independence can be seen when one of these genes is mutated, as the expression of the other genes is unaffected. Similar conclusions come from studies on retinogenesis. Several TFs either support or are necessary for the determination of ganglion cells [18•,46–48], amacrine cells [49], photoreceptors [50], bipolar cells [51] and Müller glia [52]. It is largely believed that retinal cell fates are determined by combinations of these TF's (Figure 1). Indeed, distinct retinal proneural molecules have been shown to influence the expression of other proneural genes [47,53]. However, in zebrafish and mice with a mutant proneural gene *ath5* — which is a retinal ganglion cell determinant — ganglion cells are not produced but instead, later types of cells are born and differentiate as they normally would [18•,54]. In addition, overexpression of *Xath5* in *Xenopus* retina cannot change the timing of retinal ganglion cell formation, although it dramatically increases the number of retinal ganglion cells formed [20••]. These observations indicate that cell fates are determined by combinations of TFs, and the timing of sequential cell fate specification is defined by an unknown mechanism. Such a mechanism would be required to regulate both the timing of expression of these TFs and also the competence of neural cells to respond to them.

Different mechanisms that could underlie timing control have recently been suggested. Musashi, an RNA-binding protein that is highly expressed in vertebrate nervous systems, affects *Drosophila* neurogenesis by translational inhibition of Tramtrack69 protein via its direct binding to the 3′ untranslated region of tramtrack69 mRNA [55••]. In *C. elegans*, *lin-4*, (which codes for a microRNA) and *lin-28*, (which codes for an RNA binding protein) were isolated as heterochronic genes, which affect the timing of a developmental event [56]. Moore *et al.* [57•] showed that GSK3β regulates the timing of retinal cell fate by phosphorylation of NeuroD, a proneural factor. Sun *et al.* [58] showed that cell fate choice between a neuron or an

Figure 1



Timing of retinal cell fate generation. In the neural retina, six types of neurons (ganglion cells, cones, horizontal cells, amacrine cells, rods and bipolar cells) and one type of glia (Müller cells) are generated within a schedule timing that is conserved among vertebrates [73]. Each cell type has a specific birthdate (considered as the time of withdrawal from the cell cycle; red arrows), although overlap can occur. The competence of retinal progenitors to generate a specific cell type changes over time (blue gradient) and both extrinsic signals (not shown) and intrinsic cues (different blue levels of progenitor cells) are known to affect the production of the different cell types [73]. Among intrinsic cues, several different TFs (black text) and cdkis (red text) support differentiation of specific cell types (see text for details). Cdk activity drives cell cycle progression (green arrows), and could allow for the timing of changes in cell competence [18•,20••,54], although no direct evidence has been yet reported.

astrocyte is determined as a result of competition between neurogenic and gliogenic transcriptional complexes to attach to the CBP-smad1 protein complex. Thus, translational and post-translational mechanisms, in addition to transcriptional regulation, are probably involved in regulating the timing of neurogenesis.

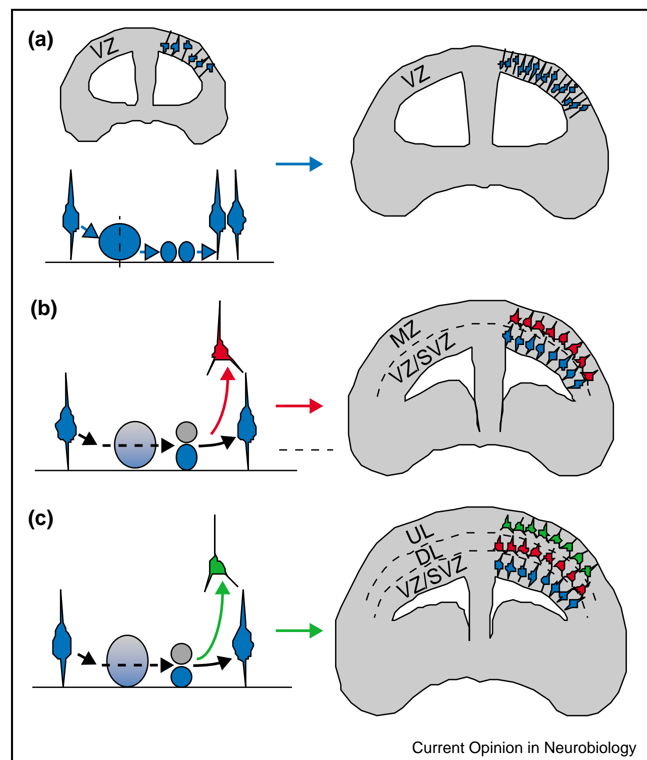
A crucial question to address is whether cell cycle progression is involved in the timing of cell fate determination. Isshiki *et al.* [16••] found that neuroblasts of *string* (*cdc25*) mutants, whose cell cycle is arrested at the G2 → M transition, fail to progess through the normal sequence of gene expression, that is, *Hunchback* → *Krüpple* → *Pdm* → *Castor*. This suggests that a cell cycle-dependent 'clock' is required to drive sequential transcription of these genes. On the other hand, cell differentiation seems unaffected by the number of cell divisions of progenitors. Classical studies of oligodendrocyte differentiation, reviewed by Durand and Raff [45], showed that the intrinsic timer for this process is inde-

pendent of cell division. Harris and Hartenstein [59] found that all types of retinal neurons form in *Xenopus* embryos after cell cycle arrest at S-phase, although they did not study the relative proportions of cell types. Notably, both Harris and Raff's groups suggested that the accumulation of cdkis works as a built-in timing mechanism [32,60]. Thus, although very little is known about how cell cycle regulation contributes to timing, components of the cell cycle machinery (but not the counting of cell divisions) seem to be involved in the timing mechanisms.

Asymmetric versus symmetric cell divisions in the development of layered structures

An asymmetric mode of cell division is a common strategy for the building of stratified neuronal architectures (Figure 2). In these structures, distinct cell types have different cell birthdays and their appropriate neuronal connectivity depends on their distribution in appropriate layers. Several observations indicate that a single progenitor generates different cell types through a series of asymmetric cell divisions in the layered cortex [41,61] and retina [62•,63]. However, despite a fine dissection of the molecular pathway controlling asymmetric divisions in *Drosophila* [64], reports of asymmetric segregation of cell-fate determinants such as Numb protein in vertebrate neural progenitors [62•,65,66], and evidence that such asymmetric segregation can control the cell identity of vertebrate neural progenitors [65,66], few genes have been found that affect the mode of cell division in vertebrates. The PC3/Tis21 gene is a marker of neuroepithelial progenitors (NEP) that are about to shift from symmetric/proliferative to asymmetric/neurogenic divisions, and its overexpression induces cortical NEP to divide asymmetrically *in vitro* [67]. Interestingly, PC3/Tis21 expression overlaps with that of Mnb/Dyrk1A, a kinase involved in neurogenesis, whose mRNA is transiently expressed and asymmetrically segregated in proliferating NEP [68•]. The possible involvement of PC3/Tis21 and Mnb/Dyrk1A in the switch between symmetric and asymmetric divisions forms an attractive hypothesis.

Figure 2



A model of cell divisions during cortical development. In the developing cerebral cortex, the transition from proliferation to neurogenesis is characterised by a progressive shift from symmetric towards asymmetric cell divisions [61]. In a classical model [41], **(a)** early symmetric cell divisions generate two identical daughter cells that expand the pool of cortical progenitors (blue) and allow the growth of the presumptive cortex. **(b)** At the onset of neurogenesis, asymmetric cell divisions cause asymmetric inheritance of cell determinants (grey and blue [61,65]). This allows cortical progenitors to both self-renew (blue daughter cells) and produce post-mitotic daughter cells (grey), without depleting the initial pool of progenitors. **(b, c)** Notably, different types of cells (red or green) are produced at specific times following one another and populate distinct cortical layers. Cell diversity will eventually depend on the time when a cell becomes postmitotic. As corticogenesis proceeds, an increasing number of progenitors divide symmetrically to produce two postmitotic daughter neurons (not shown). Such a model could be extended, with some modifications, to other layered structures such as the neural retina. DL deep layers; MZ, mantle zone; SVZ, sub-ventricular zone; UL, upper layers; VZ, ventricular zone.

Patterning genes also affect the mode of division of cortical NEP. *Pax6* and *Emx2* regulate cell cycling and the rate of progression from symmetric to asymmetric cell divisions in the embryonic cerebral cortex [7•,69,70]. In particular, Heins *et al.* [7•] reported that *Emx2* supports symmetric cell divisions of cortical NEP *in vitro* and is necessary for symmetrically dividing NEP *in vivo*. Notably, PC3/Tis21, *Pax6* and *Emx2* all affect the rate of cell proliferation and the mode of cell division. A functional relationship between the cell cycle machinery and the mode of cell division has recently been suggested for *Drosophila* neuroblasts. In fact, B-type cyclin-mediated cdc2 kinase activity is required to maintain apical and basal crescents of asymmetrically localised components that mediate asymmetric divisions [71••]. However, the molecular mechanism(s) that couples the cell cycle and the mode of cell division remains to be fully elucidated.

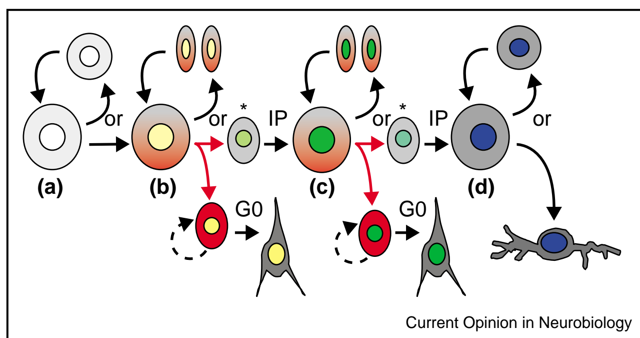
Conclusions

A tight control of cell cycle progression is a pre-requisite for the generation of appropriate cell fates during the many developmental steps that transform proliferating, undifferentiated tissues into fully differentiated and functional brain structures. First, the growth of

distinct CNS regions, such as cerebral cortex and neural retina, appears to be differentially controlled by patterning genes that regulate cell proliferation with a restricted regional competence. Cell diversity is then generated by the asymmetric distribution and inheritance of cell determinants during the mitosis of neural progenitors.

The recent characterisation of dual function molecules has given intriguing insights into the functional link between the control of cell cycle progression and cell commitment/differentiation. Moreover, many observations suggest that cell fate competence is established by the combinatorial activity of specific TFs, the combinations of which change over time. However, the molecular mechanisms coupling changes of progenitor competence and the timing of generation of different cell types are not yet fully elucidated.

Figure 3



Asymmetric cell divisions and cell fate competence. This hypothetical model highlights changes of cell fate competence (as indicated by different colours of nuclei) of neural vertebrate progenitors over time, as related to the mode of cell division. **(a)** A multipotent progenitor self-renews through symmetric divisions (black arrows) during the proliferative phase. **(b)** At the onset of neurogenesis, a progenitor forms that has acquired the competence of an early-type neuron (yellow nucleus). In this progenitor, cell determinants (red), similar to those described in the basal daughter progenitor of *Drosophila* asymmetric divisions, would localise asymmetrically. At mitosis, spindle orientation would decide whether two identical daughters are generated through symmetric division or whether the cell divides asymmetrically (red arrows) to produce one cycling daughter progenitor (asterisk) and one daughter cell that is committed to an early neuron type (yellow nucleus). Evidence from *in vitro* cortical lineages indicates that most neurons are actually produced by an additional symmetric, neurogenic division (dashed arrow) of this committed daughter cell [42]. Notably, in the event of asymmetric division, the cycling daughter progenitor (asterisk) would take some time to produce and localise red determinants during interphase (IP). We speculate that the lack of such determinants would make progenitors sensitive to extrinsic and/or intrinsic signals that can change its competence (shift from yellow to green), although no such evidence has yet been reported. **(c,d)** This process would be repeated over time to generate progenitors with competence for later cell types (e. g. (c) green neuron, (d) blue glia). The need for high cdk activity for asymmetric inheritance of cell determinants has been reported in *Drosophila* [71••]. We hypothesise that increasing concentrations of cdkis (grey cytoplasmic levels), occurring over a given threshold in neural progenitors, would cause the shift from (c) asymmetrically dividing to (d) symmetrically dividing progenitors. In fact, there is evidence that late cortical progenitors that are committed to glial fate divide mostly symmetrically [42]. In this model, (d) a late progenitor with glial competence, which does not localise asymmetric determinants, can self-renew or generate postmitotic glial cells by symmetric divisions.

Finally, fine tuning of the choice between symmetric versus asymmetric modes of cell division seems to be important in the selection of those progenitors that will exit the cell cycle and generate a cell type corresponding to their present competence, as opposed to those that will keep on cycling and change their competence later (Figure 3). Interestingly, there is evidence suggesting that the accumulation of cdkis controls the appropriate timing of generation of different cell types [32,45]. Moreover, cdk activity is necessary to drive asymmetric cell divisions of *Drosophila* neuroblasts [71••]. In addition, recent findings of Lukaszewicz *et al.* suggest that lengthening of the G1 phase is responsible for the onset of differentiative divisions of cortical progenitors [72]. These observations suggest a role of cell cycle effectors in coupling the mode of division of progenitors and cell fate determination (Figure 3). In the future, one important area that remains to be addressed is the co-ordination of extrinsic cues — such as growth factors and components of the Notch pathway — with cell cycle regulation, cell fate determination and the mode of division of neural progenitors.

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