

Cell cycle and cell fate in the developing nervous system: the role of CDC25B phosphatase

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Received: 31 March 2014 / Accepted: 4 September 2014 / Published online: 27 September 2014
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Abstract Deciphering the core machinery of the cell cycle and cell division has been primarily the focus of cell biologists, while developmental biologists have identified the signaling pathways and transcriptional programs controlling cell fate choices. As a result, until recently, the interplay between these two fundamental aspects of biology have remained largely unexplored. Increasing data show that the cell cycle and regulators of the core cell cycle machinery are important players in cell fate decisions during neurogenesis. Here, we summarize recent data describing how cell cycle dynamics affect the switch between proliferation and differentiation, with an emphasis on the roles played by the cell cycle regulators, the CDC25 phosphatases.

Keywords Neurogenesis · Cell cycle · CDC25 phosphatase · Proliferation/differentiation · Spinal cord

Introduction

Neural stem cells are self-renewing multipotent progenitors generating neurons and glial cells in the embryonic and adult vertebrate nervous system. A fundamental issue in developmental biology and in nervous system homeostasis is to understand the molecular mechanisms governing the balance between their maintenance as proliferating progenitors versus their differentiation into post-mitotic neurons. Alterations in this balance could lead to premature differentiation and

therefore in the depletion of the pool of progenitors, leading to neurodevelopmental disorders such as microcephaly. Alternatively, it could result in deregulated proliferation that may trigger stem cell-related brain tumorigenesis following oncogenic transformation.

Accumulating data suggest that the cell cycle can play a major role in regulating the balance between proliferation and differentiation. Notably, the length of the G1 phase has been shown to play a major role in controlling this balance in neurogenesis, hematopoiesis (Lange and Calegari 2010) and in cell fate decisions in mammalian embryonic stem cells (Coronado et al. 2013), including human embryonic stem cells (hESC) (Pauklin and Vallier 2013; Sela et al. 2012). Other modifications of cell cycle kinetics that have been associated with neurogenesis include shortening of the G2 phase (Agathocleous et al. 2007; Locker et al. 2006; Peco et al. 2012; Saade et al. 2013) and changes in S phase length (Arai et al. 2011; Le Dreau et al. 2014; Olivera-Martinez et al. 2014; Saade et al. 2013).

The aim of this review is to give an overview of our current knowledge about the influence of cell cycle regulation on cell fate decisions in the central nervous system, focusing mainly on the developing spinal cord. After a brief description of the cell cycle machinery, we will give a short overview of embryonic neurogenesis using the developing spinal cord as a paradigm. We will then address the impact of cell cycle exit on subsequent neuronal differentiation and discuss how cell cycle phase duration can influence fate choices of neural cells. Finally, we will present recent advances concerning the role of a particular family of cell cycle regulators, the CDC25 phosphatases.

A snapshot of the core cell cycle machinery

The cell cycle is subdivided into four successive phases, beginning with a Gap phase (G1), followed by the phase of

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DNA replication (S), a second Gap phase (G2) and finally the division itself or mitosis (M), which gives rise to two daughter cells (Fig. 1a). The G1 phase is critical, because it is the phase at which the cells will make the decision to enter another round of cell division, or to exit the cell cycle in response to extracellular cues (Harashima et al. 2013). Progression through the cell cycle is regulated by the action of Cyclin:Cyclin-dependent kinase (CDK) complexes (Fig. 1a). Extracellular signals are linked to the cell cycle machinery by D-type cyclins (D1, D2, D3) that govern progression through G1. Cyclin D molecules assemble with cyclin-dependent kinases CDK4/6 to phosphorylate key substrates, including the tumor suppressor retinoblastoma protein (pRb). Phosphorylation of Rb promotes the release of E2F transcription factor, inducing the expression of S-phase related genes such as Cyclin E and Cyclin A. Activation of the CyclinE-CDK2 complex allows the cell to cross the restriction point in late G1, committing it to enter into S phase. Progression through S- and G2- phases results from the sequential activation of the CyclinA-CDK2 and Cyclin A-CDK1 complexes, respectively and entry into mitosis is promoted by the activation of the CyclinB-CDK1 complex (Fig. 1a). Importantly, the activity of CDK complexes is modulated by phosphorylation. They are kept inactive by the phosphorylation of two residues

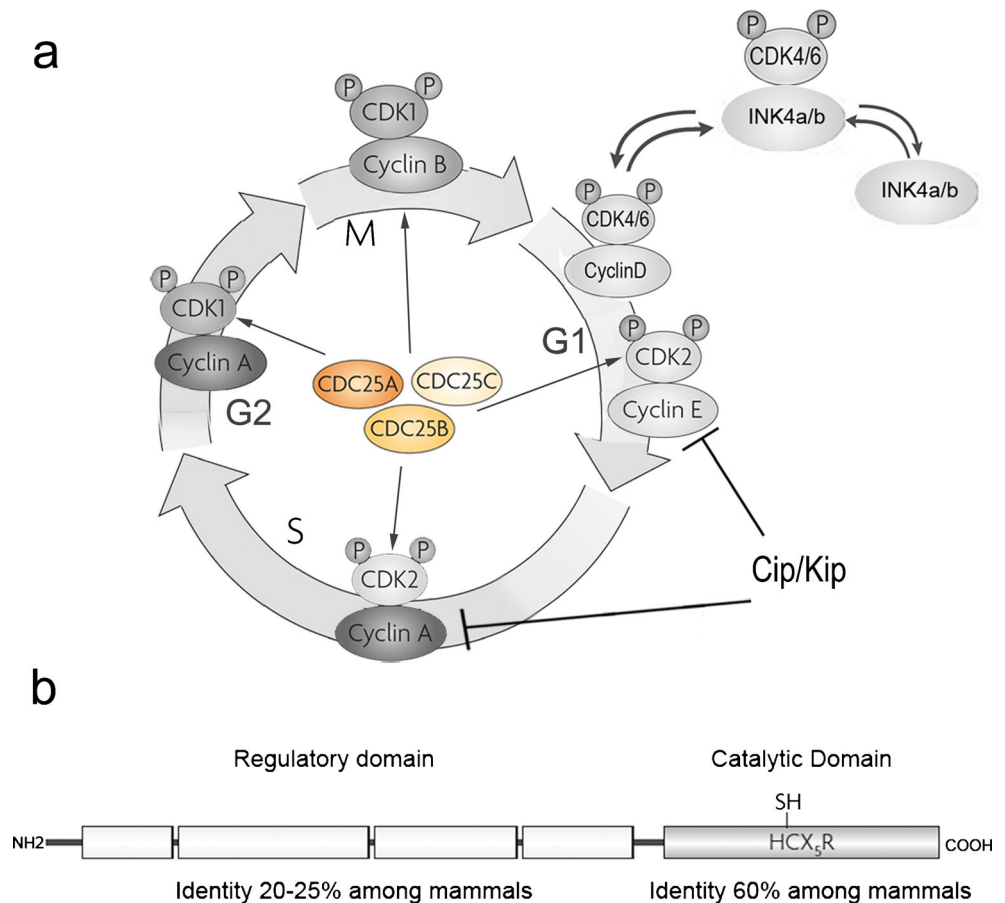
(Threonine 14 and Tyrosine 15 on CDK1) by the WEE1 and MYT1 kinases. When CDK activity becomes required for cell cycle progression, the dual specificity CDC25 phosphatases dephosphorylate specifically Threonine and Tyrosine on CDKs, thereby activating the CDK-Cyclin complexes (Fig. 1a).

Cell cycle arrest relies on two classes of G1 cyclin-CDK inhibitors, the INK4 and Cip/Kip families (Sherr and Roberts 1999). They operate in distinct fashions: the INK4 family members (p15, p16, p18 and p19) specifically bind monomeric CDK4/6 to prevent Cyclin D activation; Cip/Kip family members (p21^{Cip1}, p27^{Kip1}, p57^{Kip2}) form inactive complexes with CDK2-cyclin E and CDK2-cyclin A (Fig. 1a). The basic cell cycle machinery is, therefore, well known (Harashima et al. 2013) but how this interacts with the programs of neuronal specification and differentiation that are necessary to build a functional nervous system is still poorly understood.

The developing spinal cord as a paradigm to understand the interplay between cell cycle machinery and cell fate acquisition during neurogenesis

The developing spinal cord (neural tube) is a pertinent model to decipher the role of the cell cycle during the course of

Fig. 1 A simplified schematic representation of the cell cycle. **a** Progression in the mitotic cell cycle is controlled by the activity of CDK-Cyclin complexes and balanced by CDK cyclins inhibitors (see text for details). **b** Scheme of the CDC25 phosphatase proteins. The N-terminal regulatory domains of CDC25 phosphatases are subjected to alternative exon splicing (*boxes*), generating multiple isoforms. The highly conserved catalytic domain of CDC25 is shown in *gray*. The catalytic cysteine residue is also indicated



neurogenesis because of its well-defined anatomical structure and because the diversity of progenitor's populations is less complex than in the developing cortex (Fig. 2). Cortical progenitors are composed of radial glial cells or apical progenitors (AP), located in the ventricular zone and dividing at the ventricular (apical) surface and of basal progenitors (BP) located in the subventricular zone (SVZ) that divide at a distance from the ventricle (Gotz and Huttner 2005). In the neural tube, there is only one type of progenitor that divides apically (Fig. 2). Furthermore, we have a substantial knowledge of the signaling molecules and transcription factors governing the specification and differentiation of spinal neurons. (for reviews, see Briscoe and Therond 2013; Cohen et al. 2013; Le Dreau and Marti 2013).

The organization of the developing spinal cord

In vertebrate, the spinal cord develops from a caudal stem zone containing immature neural progenitors maintained in the undifferentiated cell state under the control of FGF signaling (Akai et al. 2005; Bertrand et al. 2000; Wilson et al. 2009). Neural progenitors leaving the stem zone form the neural tube, a monolayered, pseudostratified neuroepithelium composed of neural progenitor cells proliferating asynchronously (Fig. 2a). These cells display an elongated shape, with cytoplasmic connections to both the apical and basal surfaces (Fig. 2b).

The nuclei occupy a specific position within the neuroepithelium depending on the phase of the cell cycle: Mitotic nuclei are confined to the apical side, S phase nuclei are located basally, nuclei of the Gap phases (G1 and G2 phases) are in-between and moving in opposite directions (Fig. 2b). This oscillatory nuclear movement, termed interkinetic nuclear migration (INM), is a common feature of neural progenitor cells in the developing nervous system (for review, see Kosodo 2012; Taverna and Huttner 2010;

Willardsen and Link 2011). As a consequence of the apical motion occurring in G2, cells entering into mitosis are located at the apex. The mechanics of INM has been particularly well studied in the developing mouse brain and zebrafish retina. In these organs, it was recently proposed that nuclear motion in G1 and S is stochastic, or driven by a crowding effect, whereas the rapid apical motion in G2 is strongly directed (Kosodo et al. 2011; Leung et al. 2011; see also S. Laguesse et al., this issue). The molecular mechanisms driving this directed, basal to apical nuclear migration are beginning to be elucidated, highlighting the role of the dynein/microtubule motor system in brain development (Hu et al. 2013; Kawauchi et al. 2013) and actomyosin in the zebrafish retina (Leung et al. 2011). The molecular mechanisms driving the nuclear motion in the developing spinal cord, remain to be explored (Ahlstrom and Erickson 2009).

After they exit mitosis, spinal neural progenitors committed to neuronal differentiation undergo a form of cell subdivision that abscises apical cell membrane and mediates cell detachment from the ventricle (Das and Storey 2014). These cells subsequently locate transiently at the periphery, prior to migrating to the differentiating field (mantle zone) (Fig. 2c). In the neural tube, differentiation progresses from ventral to dorsal (Fig. 2c) and among the first neuronal populations to differentiate are the motor neurons generated from a specific pool of ventral progenitors followed by numerous subtypes of interneurons (Cohen et al. 2013; Le Dreau and Marti 2013).

Mode of division and spindle orientation

Three types of cell division have been described for spinal progenitors: symmetric proliferative division that generates two progenitors (PP, self-expanding), asymmetric neurogenic division that gives rise to a progenitor and a neuron (PN, self-replacing) and a terminal symmetric neurogenic division that produces two neurons (NN, self-consuming) (Morin et al.

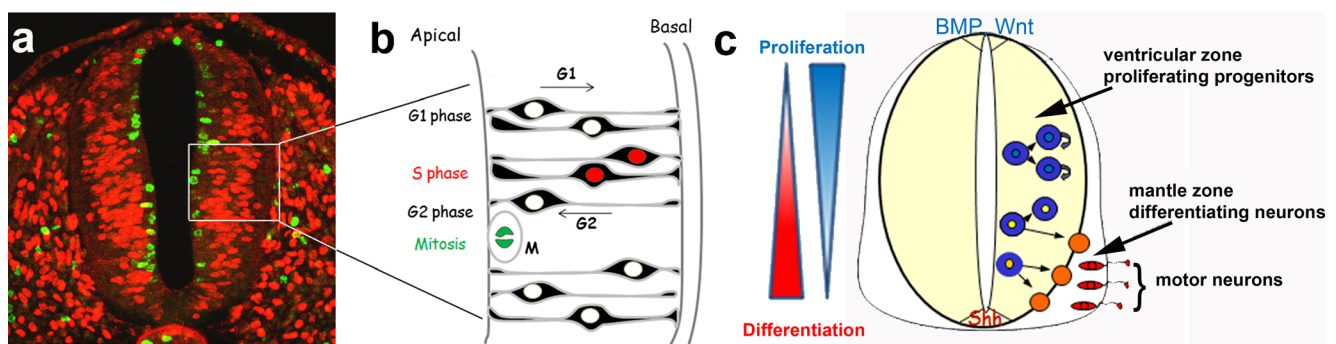


Fig. 2 The developing spinal cord, a useful model to study the interplay between the cell cycle and neurogenesis. **a** Cross-section illustrating the intensive proliferation occurring in E2.5-days old chicken neural tube. Mitotic cells are revealed using an antibody against phosphorylated Histone H3 (green) and S phase cells are shown by BrdU incorporation (red). **b** Schematic representation of interkinetic nuclear movements. **c**

Schematic representation of a spinal cord showing the two opposite gradients of proliferation and differentiation. Proliferating progenitors performing PP, PN or NN divisions are confined to the ventricular zone while differentiating neurons migrate to the periphery and constitute the mantle zone

2007; Wilcock et al. 2007). How the choice between proliferative (PP) and neurogenic (PN and NN) divisions is controlled remains poorly understood. One popular hypothesis proposes that the precise orientation of the axis of division plays a role in controlling symmetric versus asymmetric cell fate choice in the developing CNS (Chenn and McConnell 1995; Lancaster and Knoblich 2012). Planar spindle orientation would favor symmetric fate acquisition, by allowing the symmetric inheritance of fate determinants and subapical attachments between sister cells, whereas an oblique axis of division would promote an asymmetric fate. Even though correlations between oblique divisions and neurogenesis have been described both in wild-type and mutant situations, whether mitotic spindle orientation plays an instructive role in controlling the fate of the progeny, or whether both phenomena are regulated in parallel by shared upstream regulators, remains to be determined (reviewed in Peyre and Morin 2012). For example, it was shown recently that misexpressing the adaptor protein *Inscuteable* in the chicken neural tube, shifts the spindle towards oblique orientation at the expense of planar divisions and simultaneously causes accelerated neurogenesis (Das and Storey 2012). In contrast, randomization of mitotic spindle orientation through loss of function of members of the LGN complex (the core player of the spindle orientation machinery) did not cause fate determination defects in daughter cells in the neural tube. The latter experiment, however, indicated an essential role for planar divisions in the organization of the tissue through the maintenance of neural progenitors in the ventricular zone. This in turn appears to be crucial for the long-term balance between proliferation and differentiation (Morin et al. 2007).

Morphogens and the control of cell proliferation in the developing spinal cord

Spinal progenitors are subjected to opposing gradients of secreted signals along the dorso-ventral axis: Sonic Hedgehog (Shh) diffusing from ventral sources and bone morphogenetic protein (BMP) and Wnt proteins emanating from dorsal sources (Fig. 2c). These signals act as morphogens, inducing the specification of different neuronal subtypes, at a distance and in a concentration-dependent manner. Because recent comprehensive reviews (Briscoe and Therond 2013; Cohen et al. 2013; Le Dreau and Marti 2013) have been written on that topic, we will focus on the link between these signaling pathways, the cell cycle machinery and the mode of divisions.

Wnt and Shh signals have been shown to promote proliferation and survival of neural progenitors (Ueno et al. 2006). Within the neural tube, a dorso-ventral gradient, principally of Wnt1 and Wnt3a, has been proposed to organize the growth of the developing spinal cord (Megason and McMahon 2002). The Wnt- β -catenin pathway positively regulates proliferation by promoting G1 to S progression and negatively regulates

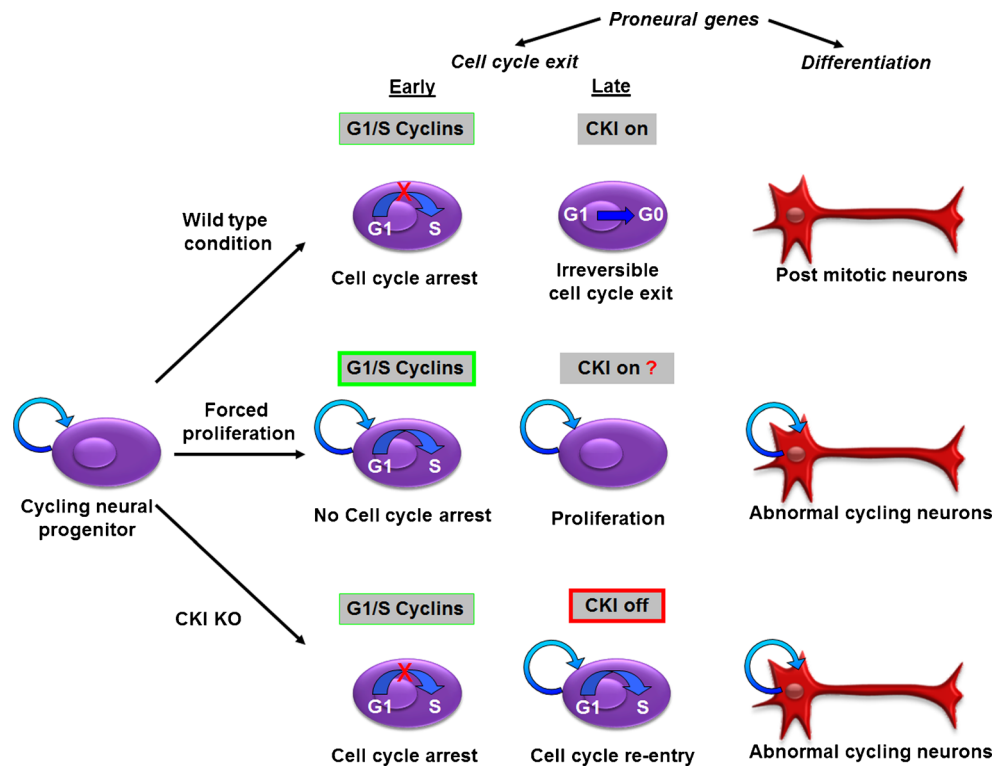
cell differentiation by inhibiting cell cycle exit and maintaining the progenitor fate (Alvarez-Medina et al. 2009; Megason and McMahon 2002), and counteracting Wnt activity is sufficient to trigger cell cycle exit (Martinez-Morales et al. 2010). In chick and mouse, numerous studies have shown that activation of the Shh-Gli pathway also increases progenitor proliferation and consequently neural tube growth (for a review, see Ueno et al. 2006). In the neural tube, Shh activity controls both G1- and G2- phase progression (Alvarez-Medina et al. 2009). Interestingly, while the action of Shh on cell specification is restricted to the ventral neural tube, its effect on proliferation extends further dorsally (Cayuso et al. 2006). Indeed, proliferation control involves cross-talk between these two pathways. In the dorsal neural tube, in the absence of Shh signaling, repressor forms of Gli3 inhibit Wnt/ β -catenin signaling (Ulloa et al. 2007) and in the ventral neural tube, Shh activity regulates the expression of Tcf3/4 DNA binding proteins, thereby controlling the Wnt effect on proliferation (Alvarez-Medina et al. 2009).

Recently, it has been shown that the signaling pathways involved in patterning the spinal cord also affect the mode of division of spinal progenitors. Using an elegant strategy to identify progenitors performing PP versus PN and NN divisions, the group of Elisa Marti showed that Shh and BMP pathways promote self-expanding divisions, respectively of motor neuron and interneuron progenitors (Le Dreau et al. 2014; Saade et al. 2013). Maintaining Shh activity high promotes self-expanding divisions; a reduction in Shh activity is required to switch to neurogenic divisions (Saade et al. 2013). At the mitotic phase, high, intermediate and low levels of the BMP effectors SMAD1/5 correlate with PP, PN and NN divisions, respectively and SMAD1/5 inhibition leads to a reduction of PP divisions in favor of NN divisions (Le Dreau et al. 2014). Hence, these signaling pathways, in addition to controlling the neural tube patterning, maintain a pool of immature progenitors that will be progressively used to produce the different neuronal subtypes during spinal cord morphogenesis.

Cell cycle exit and neuronal differentiation

Cell cycle exit and neuronal differentiation occur coordinately during neurogenesis and links between the core cell cycle machinery and the transcriptional program controlling generic neurogenesis have been identified. Cell cycle exit is controlled by proneural genes that also regulate neuronal fate acquisition and differentiation (Bertrand et al. 2002) and it was thought until recently that induction of Cyclin-dependent kinase inhibitors (CKI) triggered cell cycle exit (Farah et al. 2000; Novitsch et al. 2001) (Fig. 3). Accordingly, overexpression of NEUROG2 in the chick neural tube leads to premature cell cycle arrest in NEUROG2 misexpressing cells (Mizuguchi et al. 2001; Novitsch et al. 2001), these NEUROG2 expressing

Fig. 3 Cell cycle exit is not a gate for neuronal differentiation. During neurogenesis, cell cycle exit is coordinated with neuronal differentiation through the action of proneural genes. 1) Proneural transcription factors, at least NEUROG2, trigger cell cycle arrest by rapidly repressing G1/S cyclins. Later, the activation of CKI by proneurals locks irreversibly the cell cycle arrest. 2) When neural progenitors are experimentally forced to proliferate, proneural genes still activate the neuronal differentiation program in these cells. 3) In CKI knock-out conditions, cell cycle exit is not stabilized in post-mitotic neurons and they re-enter the cell cycle. Both situations result in the presence of abnormal cycling neurons



cells accumulating high levels of p27Kip1 and p57kip2 (Gui et al. 2007; Novitsch et al. 2001). However, this observation was made in differentiated neurons, making it difficult to conclude which molecular event initiates proliferation arrest of neuronal precursors. To clarify this issue, we characterized molecular events occurring shortly after NEUROG2 expression and we showed that one of the first functions of the proneural NEUROG2 is to specifically repress the expression of G1/S cyclins, to impede S phase re-entry of neural progenitors, prior to CKI induction (Lacomme et al. 2012).

As differentiation in the nervous system is tightly coupled with cell cycle withdrawal, how the timing of cell cycle exit affects cell fate choice and neuronal differentiation has been an attractive issue. In the developing spinal cord, experimentally maintaining progenitor cycling, by forcing CyclinD1/D2 expression, initially promotes progenitor cell proliferation at the expense of neuronal differentiation, as observed 24 h after electroporation (Lobjois et al. 2004). However, this phenotype is transient and 1 day later neural cells overexpressing CyclinDs, although still proliferating, migrate in the differentiation field and differentiate as neurons (Lobjois et al. 2008). Moreover, maintaining neural progenitor cycling is not sufficient to block NEUROG2-induced neuronal differentiation. Thus, cells co expressing CyclinD1 or CyclinE and NEUROG2 express neuronal traits while still proliferating (Lacomme et al. 2012). Forcing neural progenitors to cycle, therefore, does not alter their differentiation potential but rather results in aberrant neurons that initiate their differentiation while still incorporating thymidine analogues (BrdU)

and showing mitotic figures (Fig. 3). Conversely, it was proposed that cell cycle exit was triggering neuronal differentiation (Cremisi et al. 2003). However, recent observations challenge that idea. For example, overexpression of the transcription factor Gata2, involved in the production of a specific subtype of spinal neurons, the V2 interneurons, reduces proliferation of spinal progenitors but does not increase neuronal differentiation (El Wakil et al. 2006). More recently, it has been shown that reducing proliferation by decreasing the CyclinD1 level is not sufficient to promote neuronal differentiation (Lacomme et al. 2012; Lukaszewicz and Anderson 2011). These examples suggest that cell cycle exit is not sufficient to cause neuronal differentiation. Altogether, these studies support the notion that, in the developing spinal cord, cell cycle exit can be uncoupled from differentiation and is not the gateway in timing neuronal differentiation.

It is important for homeostasis to maintain the cell cycle OFF once neurons are differentiated and cell cycle re-entry has been associated with neurodegenerative disorders such as Alzheimer disease (Herrup and Yang 2007). As mentioned in the previous section, cell cycle arrest during neurogenesis is initiated through the repression of CyclinD/CDK complexes (Lacomme et al. 2012) but this first step is not sufficient to ensure an irreversible cell cycle exit of differentiated neurons and subsequent activation of CKI expression is required to maintain the cell cycle OFF in these cells. Indeed, cell cycle re-entry of neuronal cells has been observed in numerous mouse mutants, in which one or more cell cycle regulators are inactivated. For example, ectopic mitotic cells expressing

neuronal markers have been observed in cortices of pRB knock-out mouse embryos (Clarke et al. 1992; Jacks et al. 1992; Lee et al. 1992, 1994), or when the CKI of the INK4 and Cip/Kip families are concomitantly inactivated (Cunningham et al. 2002; Cunningham and Roussel 2001; Zindy et al. 1999). In the developing spinal cord, removing the p57^{Kip2} function leads to cell cycle re-entry of newborn neurons (Gui et al. 2007). p57^{Kip2} acts through antagonizing the cyclinD1 function and, consequently, forced expression of p57^{Kip2} in the neural tube leads to premature cell cycle arrest. The importance of keeping high levels of p57^{Kip2} in postmitotic neurons has been underlined in a recent work concerning the transcription factor Scratch 2 (Rodriguez-Aznar et al. 2013). In this study, the authors showed that Scratch 2 knockdown using antisense morpholinos in the zebrafish embryo induces post-mitotic neurons to re-enter mitosis by increasing the expression of the microRNA miR-25, which in turn represses p57^{Kip2} expression. Hence, these data show that in the CNS, even if cell cycle exit is not indispensable for differentiation, once cells have stopped cycling, it is important to lock in proliferation arrest by using CKI to avoid cell cycle re-entry of differentiated neurons (Fig. 3). Some exception to this rule has been observed in the peripheral nervous system. Indeed, in sympathetic ganglia, neuroblasts differentiate from neural crest (NC)-derived progenitor cells and continue to divide while expressing a variety of neuronal markers (Rohrer and Thoenen 1987). It has been shown that, in mouse stellate ganglia, the initial expression of neuronal markers at E10.5 coincides with a transient withdrawal from the cell cycle but most neuroblasts then re-enter the cell cycle at E11.5 (Gonsalvez et al. 2013). These observations show that, in the peripheral nervous system, cell division of differentiated cells is a way of amplifying the sympathetic ganglia neuronal populations.

How cell cycle kinetics impacts cell fate decision

While cell cycle exit is not sufficient to trigger neuronal differentiation, increasing data suggest that controlling cell cycle kinetics and in particular cell cycle phase duration, is of major importance for cell fate decisions. Numerous advances in analyzing the impact of cell cycle kinetics have been made while studying retinogenesis and corticogenesis (Fig. 4). In mouse cortical precursor cells, a clear link between cell cycle kinetics and the propensity of cells to differentiate has been established. By studying the influence on the cell cycle parameters of two extracellular signals, basic fibroblast growth factor (bFGF) and neurotrophin 3 (NT3), respectively known to promote proliferation and differentiation of neuroblasts, Lukaszewicz and colleagues showed that bFGF promotes proliferative divisions by decreasing G1 phase duration whereas NT3 lengthens G1 phase to promote differentiating divisions (Lukaszewicz et al. 2002). In the amphibian or fish retina, Hedgehog signaling converts slowly-dividing

stem cells into fast-cycling, transient amplifying, progenitors that display shorter G1- and G2-phases and are closer to exiting the cell cycle and differentiation (Agathocleous et al. 2007; Locker et al. 2006). However, in these studies, it is difficult to separate the effect due to changes in cell cycle phase length from the effect of trophic factors on differentiation. To overcome this problem, several studies have addressed the question by directly manipulating cell cycle length through misexpression of cell cycle regulators and have analyzed the impact of these manipulations on the ratio between proliferation and differentiation. In the developing mouse cortex, overexpressing either cyclinD1, CyclinE or the heterodimer CDK4/cyclinD1 reduces G1 phase length and leads to inhibition of neuronal differentiation while increasing the progenitor pool. Lengthening the G1 phase by blocking the CDK4/cyclinD1 function displays the opposite effect (Calegari and Huttner 2003; Lange et al. 2009; Pilaz et al. 2009). These results converge to show that, in the cortex, G1 lengthening promotes neuronal differentiation. The situation may not be so clear in the developing spinal cord, as experiments in which cyclinD1 levels are reduced never lead to premature differentiation (see previous section) (Lacomme et al. 2012; Lukaszewicz and Anderson 2011).

Another study dedicated to determining cell cycle parameters in specific classes of neural stem-like (apical progenitors, AP) and fate-restricted progenitor cells (basal progenitors) of the cortex showed that G1 lengthening reflects a change in the ratio between APs and BPs, in coherence with the more differentiated state of BPs. Furthermore, this study revealed that the key difference between proliferating and neurogenic neural progenitor cells is the S phase duration, since self-expanding neural progenitors have on average a 3.3-fold longer S phase than those committed to the neurogenic lineage (Arai et al. 2011). In the developing spinal cord, cell cycle parameters were measured during motor neuron differentiation, revealing a global acceleration of the cell cycle in the neurogenic phase, due to a shortening of the S and G2 phases but without modification of the G1 phase (Saade et al. 2013). We have shown that the lengthening G2 phase, following inhibition of CDC25B phosphatase levels in the neural tube, is correlated with an increase in the number of proliferative neural cells and a reduction of differentiated neurons, suggesting that controlling G2 phase duration is of importance for cell fate decision (Peco et al. 2012). Altogether, these studies show that cell fate choices are tightly linked to the duration of cell cycle phase length. The precise molecular mechanisms involved and the timing of their actions still remain to be characterized.

Phosphatase CDC25, cell cycle kinetics and cell fate

Recent data indicate that the CDC25 phosphatases known to regulate cell cycle progression are also involved in cell fate

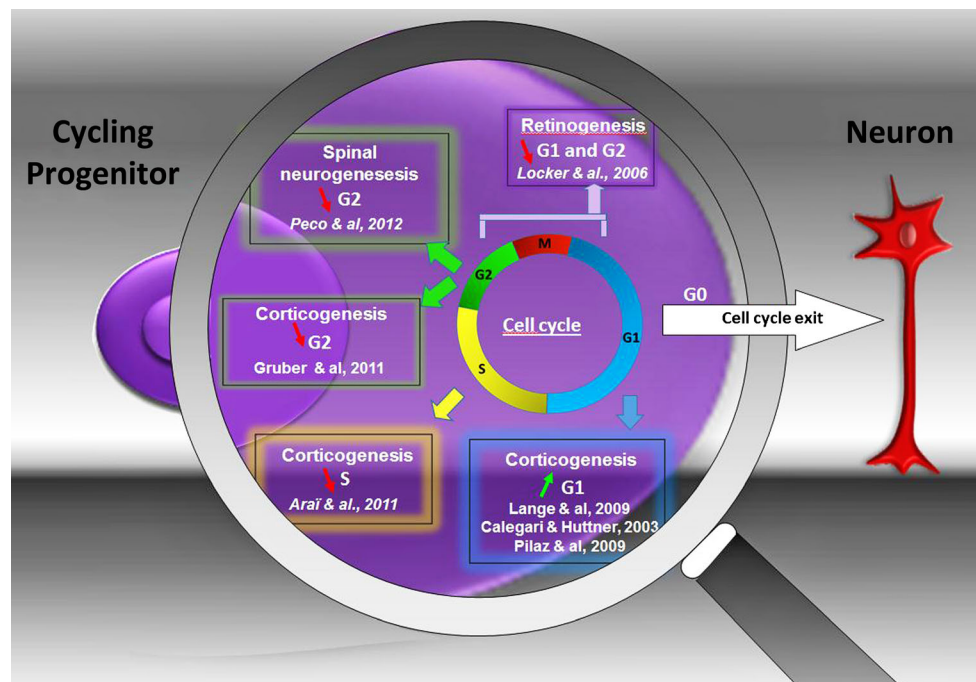


Fig. 4 Evidence for a role of cell cycle kinetics in neural fate choice. Schematic drawing of the relationship between the acquisition of a neuronal fate (neuron, red) and the cell cycle kinetics of a proliferative neural progenitor (cells, purple). The S phase is in yellow, Mitosis in red, G1 in blue and G2 in green. Concomitantly with differentiation, neuronal precursors stop cycling and reach a quiescent state (G0 phase). During neurogenesis, cell cycle kinetics is tightly linked to cell fate decisions.

During spinal neurogenesis, a short G2 is associated with an increase of neuronal production. In the retina, both G1 and G2 duration are reduced prior to differentiation. In the cortex, G1 lengthening promotes neuronal differentiation and proliferating neural progenitor cells have a longer S phase than those committed to the neuronal lineage; G2 phase shortening has also been correlated with premature neuronal differentiation in the microcephaly mouse model

decision. We thus choose to describe in more detail this family of cell cycle regulators, highlighting the links reported between CDC25B and neurogenesis.

Genomic, molecular organisation and biochemical activity of CDC25 phosphatases

The cell division cycle 25 family (CDC25) is highly conserved throughout evolution (Boutros et al. 2007a). In mammalian cells, three genes have been identified: CDC25A, CDC25B and CDC25C, which range from 470 to 566 amino acids in length. Orthologues of these genes have been found in chicken (only two in *Gallus gallus*; CDC25A and CDC25B, (Benazeraf et al. 2006)), in *Xenopus laevis* (CDC25A B, C, D, (Nakajo et al. 2011)), in zebrafish (CDC25A B, C, D, (Nogare et al. 2007)), in *Drosophila*, (string and twine (Edgar and O'Farrell 1989; Jimenez et al. 1990) and in *C. elegans* (cdc-25 1–4, (Ashcroft et al. 1998)). Among the different species, the C-terminal domains enclose the catalytic pocket of CDC25 proteins and are highly evolutionarily conserved (Fig. 1b). The N-terminal domains of the proteins encode the regulatory regions that are globally more divergent. The non-catalytic domain contains many phosphorylation sites involved in the regulation of enzymatic activity, as well as protein stability. This amino-terminal moiety also contains

signal peptides (NLS and NES sequences) that control the intracellular localization of CDC25 phosphatases (Boutros et al. 2007a).

As mentioned above, these phosphatases regulate positively transitions between cell cycle phases (Fig. 1a), since they activate cyclin-dependent kinase (CDK) complexes by removing inhibitory Threonine and Tyrosine phosphorylations (Boutros et al. 2007a). CDC25A is implicated in the control of G1-S and G2-M transitions by regulating the activities of CDK1 and CDK2, whereas CDC25B seems to be mainly involved in activating CDK1-cyclin B at the G2-M transition (Boutros et al. 2007a; Timofeev et al. 2010). CDC25B has also been shown to be recruited to the mother centrosome and to be involved in the centrosome duplication cycle and in microtubule nucleation (Boutros and Ducommun 2008; Boutros et al. 2007b, 2011). Invalidation of the three CDC25 genes has been performed in mouse. CDC25B-deficient mice are viable but females are sterile, because CDC25B^{-/-} oocytes are unable to resume meiosis (Lincoln et al. 2002). Mice lacking CDC25C individually or in combination with CDC25B develop normally and are viable and embryonic fibroblasts derived from these mice exhibit normal cell cycle parameters in culture (Chen et al. 2001; Ferguson et al. 2005). So far, no defect in nervous system development has been reported. Only CDC25A provides an essential

function during early embryogenesis, since *CDC25A*-deficient embryos exhibit growth retardation and die before E7.5 (Lee et al. 2009), suggesting that *CDC25A* is required for very early steps of development. This family of phosphatases has also been particularly well studied in tumorigenesis due to their abnormal expression in various human cancers, often correlated with more aggressive disease and poor clinical prognosis (Boutros et al. 2007a).

Regulation of *CDC25* expression by morphogens

CDC25B expression is known to be tightly regulated in a cell cycle-dependent manner (Kakizuka et al. 1992; Korner et al. 2001). The transcript begins to be detected in early S phase, it peaks in G2 and M phases before dropping abruptly in early G1 phase (Kakizuka et al. 1992; Korner et al. 2001). Surprisingly for a core cell cycle regulator, *CDC25B* is expressed in only a subset of proliferating progenitors in the developing nervous system of chicken and mouse embryos (Fig. 5; Benazeraf et al. 2006; Kakizuka et al. 1992; Peco et al. 2012). *CDC25B* is absent from the caudal stem zone containing progenitors performing only PP divisions (arrows in Fig. 5a, b), its transcription being initiated in the ventral neural tube correlating with the onset of neuronal differentiation (Hammerle and Tejedor 2007). The spatial and temporal dynamics of *CDC25B* expression will then remarkably accompany neurogenesis rather than proliferation all along spinal cord development (Fig. 5c–e) (Peco et al. 2012).

We showed that, in the closing neural tube, *CDC25B* expression is turned on by the Shh signaling pathway (Benazeraf et al. 2006). Misexpressing Shh in the caudal stem zone is sufficient to induce precocious transcription of *CDC25B*, whereas blocking the pathway with cyclopamine totally abolishes *CDC25B* expression in the chicken neural tube (Benazeraf et al. 2006). *CDC25B* upregulation is not a consequence of Shh action on proliferation, as arresting the cell cycle for a short time does not reduce *CDC25B* expression in the neural tube, whereas blocking Shh signaling drastically diminishes *CDC25B* expression without arresting cell cycle progression (Benazeraf et al. 2006). Thus, the onset of *CDC25B* transcription depends upon Shh signaling activity in the neural tube and is associated with that of neuronal differentiation. Whether there is a direct link between the *CDC25B* phosphatase and the Shh/Gli pathway remains to be elucidated.

As mentioned above, we only found one other *CDC25* phosphatase in the chicken genome, *CDC25A*. *CDC25A* transcripts display a different expression pattern than *CDC25B* along the cell cycle, being highly expressed in late G1 and S phase and decreasing during G2 and mitosis (Jinno et al. 1994). As expected for a positive cell cycle regulator, *CDC25A* is present in the caudal stem zone and in all high proliferation domains (Benazeraf et al. 2006; Peco et al.

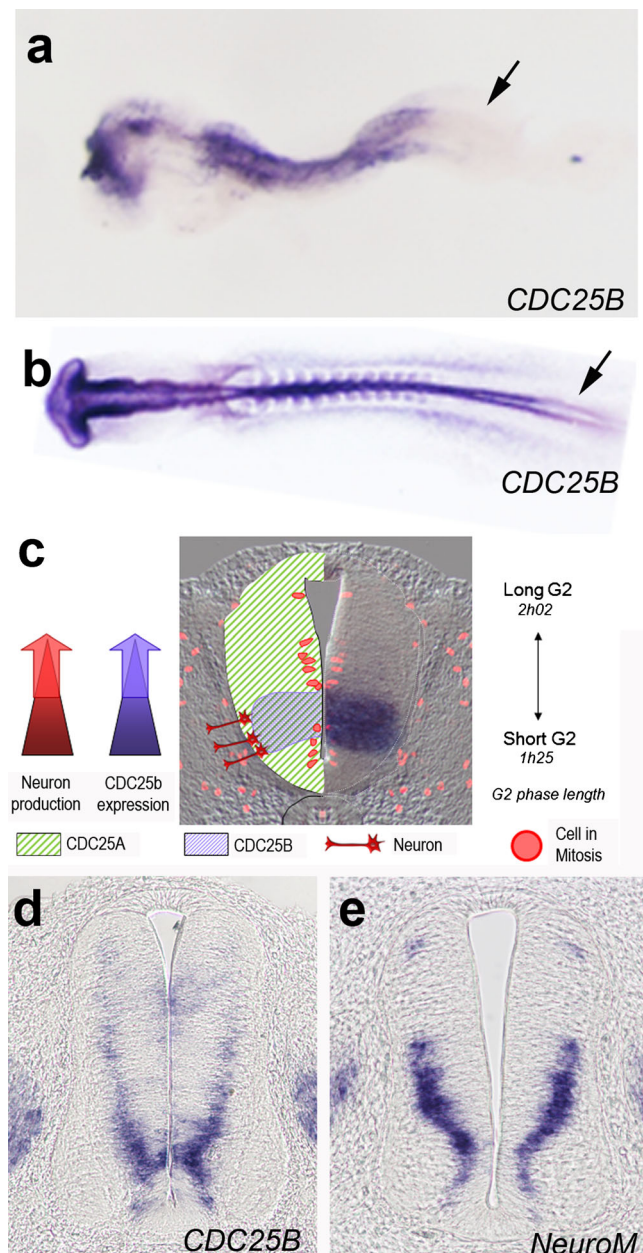


Fig. 5 *CDC25B* expression correlates with neurogenic domains in chick and mouse neural tubes. **a, b** In situ hybridization of *CDC25B* on a 8.5-dpc mouse embryo (**a**) and a 1.5-day-old chick embryo (**b**). The arrow points to the caudal neural stem zone containing proliferative progenitors devoid of *CDC25B* expression. **c** Cross-section in the chicken neural tube. At E2.5, *CDC25A* is expressed throughout the neural tube (green hatching) whereas a high level of *CDC25B* transcripts is detected in the ventral neural tube. Mitosis can be observed in the dorsal region where *CDC25B* is not expressed, meaning that this phosphatase is dispensable for entry into mitosis. Neural progenitors expressing both *CDC25A* and *CDC25B* display a shorter G2 phase than those expressing *CDC25A* alone and this is associated with a more efficient neuronal production. The ventral domain of high *CDC25B* expression corresponds with that containing the progenitor of motor neurons at the time of motor neuron production. **d, e** Cross-section in the neural tube at E4.5 showing an in situ hybridization of *CDC25B* (**d**) and of a marker of young neurons, *NeuroM* (**e**). Note that the dorsal progression of *CDC25B* is concomitant with the dorsal extension of *NeuroM*

2012). Whether CDC25A expression is modulated by signaling pathways during neurogenesis remains to be explored.

A direct functional link between the Wnt signaling pathway and CDC25A has been demonstrated recently in human sarcomas (Vijayakumar et al. 2011). The authors reported a high frequency of increased canonical Wnt activity in sarcomas of multiple histological subtypes and showed that CDC25A is indeed a direct TCF/b-Catenin transcriptional target, acting as a major mediator of that signaling pathway, which controls cell proliferation both in vivo and in vitro. As Wnt also acts as a major morphogen in nervous system development, targeting CDC25A by such signaling pathways could also be an important way to control neural progenitor proliferation. Thus, these phosphatases may be targeted by the main signaling pathways to regulate cell fate choice during neurogenesis.

CDC25A's role in pluripotency maintenance

In mouse embryonic stem cells (ESCs), high levels of CDC25A are observed in G1 (van der Laan et al. 2013). CDC25A abundance depends upon high expression of Dub3, a deubiquitylase that fine-tunes CDC25A steady-state levels. Interestingly, Dub3 is a target of estrogen-related receptor- β , a key transcription factor of the self-renewal machinery. Knockdown of Dub3 or CDC25A induces spontaneous differentiation of the ESCs, showing that the Dub3-CDC25A pathway is important for maintaining pluripotency (van der Laan et al. 2013). Similarly, a study in zebrafish linked the CDC25A level to the differentiation status of the cells (Bouldin et al. 2014). In this work, heat shock gain of the function of CDC25A in the posterior region of the tail bud in zebrafish embryos induced the maintenance of spadetail expression (T-box transcription factor) and the inhibition of muscle differentiation genes. Interestingly, this study also established a link between S/G2 phase duration and the ability of cells to differentiate. This shows that fine-tuning the cell cycle by regulating CDC25 expression is essential for normal muscle differentiation and establishing proper embryo length (Bouldin et al. 2014). Both studies implicate CDC25A in the control of maintaining the balance between proliferating progenitors versus differentiation but the mechanisms of action involved have not yet been characterized.

CDC25B, a FoxM1 target involved in neural development

A role for CDC25B phosphatase in several aspects of neural development was initially suggested in studies of the Forkhead transcription factor FoxM1. FoxM1, known as a transcriptional regulator of G1/S progression, also acts at the G2/M transition through CDC25B and Cyclin B. In *Xenopus* embryos, FoxM1 is expressed in the neuroectoderm and disruption of its function using morpholinos leads to decreased

expression of neuronal markers NCAM and N-tubulin and to the slight expansion of a proliferating neural progenitors marker, Sox2 (Ueno et al. 2008). This phenotype is accompanied by an altered G2/M transition and a significant reduction in CDC25B expression. Moreover, downregulating CDC25B using morpholinos phenocopies FoxM1 loss of function suggesting that altering the G2/M transition is sufficient to hinder neurogenesis. In mouse, conditional disruption of FoxM1 in the developing telencephalon slows down cell cycle progression, lengthening G1, S and G2/M phase duration. This leads to a precocious transition from AP to BP probably as a consequence of the G1 phase lengthening (Arai et al. 2011) and to a reduction of neurogenesis in the adult telencephalon (Wu et al. 2014). In that study, the G2 phase lengthening is associated with a downregulation of CDC25B expression, in addition to a reduction in the level of CyclinB1 and an impaired basal to apical interkinetic nuclear migration (INM) (Wu et al. 2014). The function of FoxM1 was also explored in Cerebellar Granule Neuron Precursors (CGNP), using transgenic mice with either complete or conditional loss of function (Schuller et al. 2007). In the cerebellum, FoxM1 is upregulated in response to Shh signaling and its function is restricted to the G2/M transition. Accordingly, in FoxM1 loss of function, this transition is delayed in correlation with decreased levels of Cyclin B1 and CDC25B. Here, the postponed mitotic entry is associated with spindle abnormalities and centrosome amplification, suggesting that FoxM1 is crucial for the spindle apparatus and centrosome duplication in CGNP (Schuller et al. 2007). Altogether, these data suggest a function of the FoxM1 target CDC25B phosphatase in key cellular events associated with neurogenesis including INM and proper mitotic spindle assembly.

CDC25B activity and neurogenesis

Recently, we addressed directly CDC25B's function in neurogenesis using the chicken developing spinal cord as a model (Peco et al. 2012). As mentioned above, in the chicken neural tube, CDC25B expression correlates remarkably well with areas where neurogenesis occurs, whereas CDC25A is broadly expressed in proliferating domains (Fig. 5c). Interestingly, neural progenitors expressing CDC25A alone have a longer G2 phase (2 h02) than those expressing both CDC25B and CDC25A (1 h25). Moreover, reducing CDC25B expression in these progenitors results in a specific lengthening of the G2 phase (2 h06), whereas S-phase length and total cell cycle duration are not significantly modified. Reduction of CDC25B levels also leads to an increase in the number of proliferating neural progenitors and a concomitant reduction in neuron production. The downregulation of CDC25B affects both motor neurons and interneurons production (Peco et al. 2012), indicating that the phosphatase is part of the generic molecular network involved in neurogenesis. Hence, in the

developing spinal cord, decreasing CDC25B levels leads to G2 phase lengthening and neuronal differentiation defects, indicating that the function of the phosphatase is to promote neurogenesis.

How might CDC25B promote neurogenesis?

In mouse, loss of function of genes whose human counterparts are associated with microcephaly, such as ASPM (Fish et al. 2006) and microcephalin1 (MCPH1; Gruber et al. 2011), results in both defective spindle orientation and accelerated neurogenesis. Similar phenotypes are also observed upon loss of Huntingtin (Godin et al. 2010) or loss of Treacle, a centrosome and kinetochore protein (Sakai et al. 2012). In particular, in mice mutants, for the centrosomal protein MCPH1, the level of checkpoint Kinase 1 (CHK1), a negative regulator of CDC25B, is reduced. This triggers increased CDC25B activity, premature activation of CDK1 and early entry into mitosis, associated with a delay in centrosome maturation. The authors describe significant defects in planar spindle orientation, suggesting that the asynchrony between mitotic entry and centrosome cycle disturbs mitotic spindle alignment. Interestingly, silencing CDC25B rescues premature neurogenic production and spindle misalignment. Thus, downstream of MCPH1, the CHK1-CDC25B-CDK1 pathway may control neurogenesis through a modification of spindle orientation. Whether it is the principal way CDC25B affects the balance between proliferation and differentiation remains to be explored. Indeed, all these molecules (ASPM, treacle, Huntingtin, MCPH1) also play a role in centrosome maturation and spindle formation. In light of increasing evidence involving asymmetric maturation of centrosomes in the regulation of asymmetric fate choices (Wang et al. 2009), an interesting alternative is that CDC25B might affect fate choices via an effect on mitotic spindle formation and centrosome maturation.

Other hypotheses can be proposed to explain how CDC25B loss of function and the induced G2 phase lengthening can lead to neuronal differentiation defects. In *Drosophila* S2 cells or in *Xenopus* embryos, G2/M arrest induced by knocking down CDC25B is associated with increased Wnt signaling (Davidson and Niehrs 2010; Davidson et al. 2009). This is due to the accumulation of the CDK14/CyclinY complex that promotes Wnt signaling through phosphorylation of the LRP6 co-receptor and increases the receptiveness of cells for incoming Wnt signals. Interestingly, disrupting the 3 CDC25 genes by homologous recombination in the mouse small intestine results in enhanced Wnt/ β -catenin signaling (Lee et al. 2009). Since this pathway is involved in keeping neural progenitors proliferating in the developing spinal cord (Megason and McMahon 2002), one tempting hypothesis is that CDC25B phosphatase activity, by shortening G2 phase duration in spinal progenitors, diminishes their sensitivity to the Wnt pathway, thereby favoring

neuronal differentiation at the expense of neural progenitor proliferation.

Another possibility could be that CDC25B's action on neuronal differentiation is uncoupled from the cell cycle. Such an independent function of cell cycle regulators has been described for several members of the cyclin families and has been best illustrated for cyclinD1 (Coqueret 2002). In the small intestine, CyclinD1 is able to repress the transcriptional activity of Beta2/NEUROD by indirect interaction through p300 and in the muscle it represses MYOD transcriptional activity independently of CDK kinase activity (Ratineau et al. 2002; Skapek et al. 1996, 1995). In both cases, cyclinD1 acts independently of its interaction with CDK4/6 or pRB phosphorylation. These data among others describe a new function for cyclinD1 that represses the activity of transcription factors triggering differentiation. More recently, genome-wide analysis shows that cyclinD1 binds to DNA in the promoters of many genes and that in the developing retina, cyclinD1 binds to regulatory regions of the Notch gene where it recruits CBP histone deacetylase, thereby increasing Notch signaling (Bienvenu et al. 2010). A similar action of cyclinD1 on Notch signaling, independent of its interaction with CDK4/6, has also been revealed in the developing spinal cord (Lukaszewicz and Anderson 2011). In view of these examples, one can postulate that CDC25B might also regulate neuronal differentiation by acting on specific substrates, independently of its action via CDK1 dephosphorylation, even though, up to now, no other CDC25 phosphatase substrates other than CDK/Cyclin have ever been identified.

Further work is required to explore these non-mutually exclusive hypotheses and to unravel the molecular mechanism involved downstream of CDC25B to regulate the transition between a proliferating neural progenitor and a differentiated post-mitotic neuron.

Conclusion

As exemplified in this review, an increasing amount of data indicate that cell cycle kinetics and actors of the cell cycle machinery play a major role in cell fate decision. This involves cross-interactions between morphogens and core cell cycle regulators such as the CDC25 phosphatases. It is thus tempting to propose that these phosphatases that modulate CDK-Cyclin activities will affect cell cycle kinetics thereby changing cell destiny. A key question that remains to be clarified is how a change in the mother cell cycle determines the destiny of the daughter cells. To solve this point, we need to begin with an accurate characterization of the cell cycle features of mother cells performing self-renewal versus differentiating divisions. So far, it has not been possible to link cell cycle kinetics to cell fate choice in the developing nervous system

because neural progenitors are heterogeneous, those performing proliferative or differentiating divisions being spatially intermingled and indistinguishable. The landscape has recently changed, however, with the introduction of fluorescent cell cycle indicators allowing the visualizing of the different phases of the cell cycle in living cells, offering a promising strategy to measure in integrated organs the cell cycle kinetics of single cells and to track their progeny (Sakaue-Sawano et al. 2008). Another challenge will be to organize a comprehensive scheme of all the connections between cell cycle regulators, such as the CDC25 phosphatases and the molecular networks orchestrating cell fate decisions. Finally, dissecting the interplay between cell cycle dynamics and cell fate decisions is important not only in a normal context but also in pathological settings such as tumorigenesis where it may help devise new curative strategies.

Acknowledgments We thank Pr. B. Ducommun and Dr. X. Morin for critical reading of the manuscript and C. Monod-Wissler for improving the English writing. Work in F.P.'s laboratory is funded by the Centre National de la Recherche Scientifique, L'Université P. Sabatier, le Ministère de l'Éducation Nationale et de la recherche, the Fondation pour la Recherche sur le Cancer (ARC) and the Fédération pour la Recherche sur le Cerveau (FRC).

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