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# Playing with the cell cycle to build the spinal cord

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ABSTRACT

A fundamental issue in nervous system development and homeostasis is to understand the mechanisms governing the balance between the maintenance of proliferating progenitors versus their differentiation into post-mitotic neurons. Accumulating data suggest that the cell cycle and core regulators of the cell cycle machinery play a major role in regulating this fine balance. Here, we focus on the interplay between the cell cycle and cellular and molecular events governing spinal cord development. We describe the existing links between the cell cycle and interkinetic nuclear migration (INM). We show how the different morphogens patterning the neural tube also regulate the cell cycle machinery to coordinate proliferation and patterning. We give examples of how cell cycle core regulators regulate transcriptionally, or post-transcriptionally, genes involved in controlling the maintenance versus the differentiation of neural progenitors. Finally, we describe the changes in cell cycle kinetics occurring during neural tube patterning and at the time of neuronal differentiation, and we discuss future research directions to better understand the role of the cell cycle in cell fate decisions.

## 1. Introduction

Spatial and temporal regulation of cell proliferation and tissue growth is associated with specific physiological conditions and is important for the proper development of multicellular organisms. Thus, cell proliferation and cell cycle progression must be tightly coordinated with pattern formation, cell differentiation and tissue morphogenesis, in different contexts including the central nervous system development.

The aim of this review is to illustrate the major links occurring between the cell cycle and the main cellular and molecular events governing specification and differentiation in spinal cord development. From this perspective, we will give an overview of the cell cycle and the key players in this process; we will describe the relationship between the cell cycle and interkinetic nuclear migration; we will highlight the interplay between morphogens, cell cycle regulators and transcription factors governing neural progenitor specification and neuronal differentiation; finally, we will summarize our current knowledge of cell cycle dynamics during spinal cord development and how it may influence cell fate.

## 2. The cell cycle: the key players and regulators

The prototypical eukaryotic cell cycle is composed of four phases. In the first gap phase or G1 phase, cells decide whether to enter the replication phase, or to exit the cell cycle and go into a G0 or quiescent state. If the decision is to continue, then the synthesis phase or S phase comprises a DNA replication step. A second gap phase (G2), which prepares the cell to enter mitosis, is followed by the M phase or mitosis, in which two new daughter cells are generated (Fig. 1). The cell cycle is finely controlled at restriction and checkpoints to ensure integrity of the genomic material and its proper repartition for correct cell division (Fig. 1) (Harashima et al., 2013).

The transition between cell cycle phases occurs in a coordinate way and is regulated by the activity of serine/threonine kinases, the CDKs (Cyclin-Dependent Kinases) that bind a cyclin subunit for activation. Cyclin binding induces conformational changes, allowing activation of the CDK complex by phosphorylation on conserved threonine residues and also the release of the blockade at the entrance of the CDK catalytic cleft. Activation of CDKs occurs at specific points in the cell cycle, i.e., CDK4 and CDK6 are activated during G1, CDK2 at the G1/S transition and during S phase, and CDK1 during G2 and M phases (Fig. 1), thereby promoting cell cycle progression through the phosphorylation of selected substrates (Vermeulen et al., 2003).

D-type cyclins (cyclin D1, D2, and D3; formally CCND1, CCND2, CCND3) couple the cell cycle machinery to extracellular signals. When cells are exposed to mitogens, cyclin Ds are upregulated and bind to CDK4 and CDK6 to form active complexes that promote progression through the G1 phase. Cyclin D-CDK4/6 complex initiates phosphorylation of members of the retinoblastoma protein (pRB) family. Partially phosphorylated RB proteins lead to the release of E2f transcription factors, which promote the expression of genes

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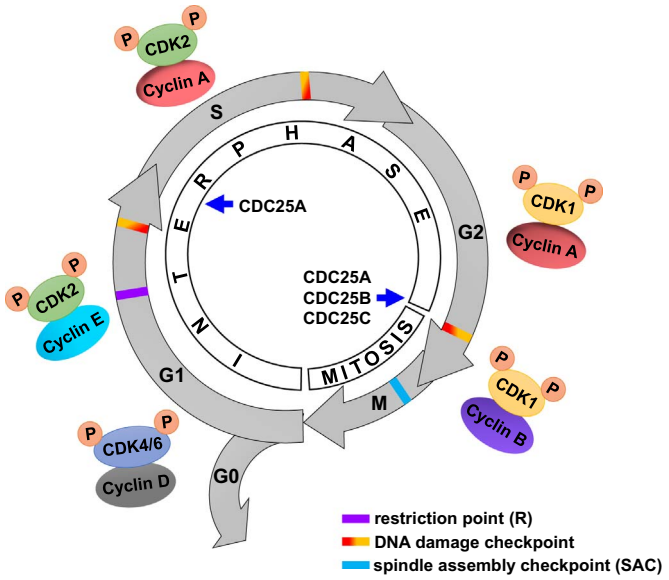


**Fig. 1.** Schematic overview of the cell cycle. The cell cycle is composed of 4 phases: G1, S and G2 corresponding to cell interphase, and the M phase or mitosis. Cell cycle progression is controlled by phase-specific cyclin-CDK complexes. Inhibitory phosphorylations at highly conserved Thr and Tyr residues (represented in light orange) are removed by CDC25 phosphatases, allowing the activation of these complexes (see text for details). The cell cycle is controlled at the restriction point and checkpoints. The restriction point (R) in late G1 phase is defined as a point of no return in G1, at which time the cell is committed to enter the cell cycle. DNA damage checkpoints arrest the cell cycle to allow DNA repair prior to replication (G1/S checkpoint), during S phase (S checkpoint), or prior to mitosis (G2/M checkpoint). The spindle assembly checkpoint (SAC) detects improper alignment of the chromosomes and stops the cell cycle in metaphase.

required for G1 to S phase transition and S phase progression including cyclin E (CCNE) and cyclin A (CCNA). Cyclin E binds to CDK2 in late G1, reinforces RB phosphorylation and inactivation. The point in late G1 at which RB becomes fully phosphorylated is called the “restriction point”, where cells are committed to enter S phase and to progress irreversibly to cell division. In complex with cyclin A, CDK2 controls S phase completion. Continuation through the G2 phase results from activation of cyclin A-CDK1, and progression through mitosis requires activation of the cyclin B (CCNB)-CDK1 complex (Fig. 1) (Sherr et al., 2016; Vermeulen et al., 2003). Unlike CDKs which are present during the cell cycle, cyclins are unstable proteins (Murray, 2004). Cyclin D and cyclin E display a PEST sequence (segment rich in proline (P), glutamic acid (E), serine (S) and threonine (T)) and cyclin A and cyclin B contain a destruction box, both required for efficient cyclin proteolysis. Thus, cyclin Ds will rapidly disappear after mitogen withdrawal. The periodic oscillations of cyclin E, cyclin A and cyclin B coordinate DNA replication with mitosis. Cyclin E appears in late G1, peaks at the G1/S transition and disappears in S phase. Cyclin A accumulates during S and G2 phases and decreases during mitosis. Cyclin B peaks at the G2/M transition and persists in mitosis. Cyclin B proteolysis by the anaphase-promoting complex or cyclosome (APC-C), is required for complete division into two daughter cells.

In addition to cyclin binding, CDKs are regulated by phosphorylation-dephosphorylation at highly conserved threonine and tyrosine residues (e.g., Thr14 and Tyr15 in human CDK1) (Harashima et al., 2013). Inhibitory phosphorylations are caused by Myt1 and Wee1 kinases, which inactivate CDKs. The CDC25 (Cell Division Cycle) phosphatases play an important role in cyclin-CDK activation by removing these inhibitory phosphate residues, thereby ensuring timely progression through the different cell-cycle phases (Fig. 1) (Boutros et al., 2007; Sur and Agrawal, 2016). There are three CDC25 phosphatases in mammals: CDC25A, CDC25B, and CDC25C whose expression and activity are highly regulated by different mechanisms, including cell cycle controlled expression and degradation, phosphorylation-dephosphorylation cycles and intracellular localization. CDC25A is mainly involved in the G1/S transition, whereas CDC25A, CDC25B and CDC25C play a role in the G2/M transition (Fig. 1). Thus, when CDK1 becomes necessary to enter mitosis, dephosphorylation by CDC25B phosphatase, activates CDK1 and turns on the cyclin-CDK complex.

CDK activity can be counteracted by CDK inhibitors or CKIs, which bind CDKs or CDK-cyclin complexes to regulate their activity. Two families of CKIs have been described so far: INK4 (formally CDKN2) and Cip/Kip (CDKN1) (Sherr and Roberts, 1995). The INK4 family comprises 4 members (INK4 a-d), which specifically inactivate G1-CDKs (CDK4/6) by competing with cyclin D (Carnero and Hannon, 1998). The Cip/Kip family includes $p21^{Cip1}$, $p27^{Kip1}$ and $p57^{Kip2}$ that inactivate CDK2-cyclin E, CDK2-cyclin A complexes and to a lesser extent the CDK1-cyclin B complex (Hengst and Reed, 1998).

All these components of the cell cycle machinery work in a coordinate fashion to promote cell cycle progression. Their functions and regulation are thoroughly documented in numerous cell lines under physiological and pathological conditions, but how cell cycle control is integrated into building a functional organ remains poorly understood. Here we present early and recent findings exploring this question, mainly focusing on spinal cord development.

## 3. Cell cycle occurs in phase with nuclear movement in the neural tube

The neuroepithelium is a pseudostratified epithelium. A common feature of such epithelia is an oscillatory nuclear movement synchronized with cell cycle progression, known as the Interkinetic Nuclear Migration (INM) (Lee and Norden, 2013).

In neuroepithelia, progenitor cells located in the ventricular zone display an elongated shape, due to the cytoplasmic extensions toward both surfaces, apical and basal (Fig. 2A). Nuclei of progenitor cells migrate and occupy a specific position inside the neuroepithelium according to the cell cycle phase: nuclei migrate basally in the G1 phase, so that the S phase occurs on the basal side, and apically in the G2 phase, allowing mitosis to happen at the apical surface (Fig. 2A left inset) (Langman et al., 1966; Sauer, 1935). This pseudostratified organization was proposed to allow more cells to pack into the neural tube, since the nuclei require more space than the end feet and that cell cycle of progenitor cells are asynchronous (Murciano et al., 2002). Accordingly, INM would also be a way to clear nuclei from the apical side after mitosis, and therefore maximize cell density in the neural tube (Kosodo et al., 2011).

Over more than a century, INM and the cell cycle have appeared as important elements in the regulation of neural tube shape and growth, and more recently in the balance between proliferation and differentiation.

The spinal cord develops from the caudal neural plate, a thickened pseudostratified epithelium containing immature neural progenitors. Neural tube closure starts with the bending of the flat neural plate, in order to approach neural folds in the dorsal midline, where the folds fuse to form the closed neural tube (Fig. 2B a-d) (Cearns et al., 2016).

In 1902, apical constriction was proposed as a mechanism of cell shape change that could facilitate neural plate bending, known as neurulation. In the neural plate, apical constriction consists of narrowing the apical side of the hingepoint cells (asterisks in Fig. 2B b-c), resulting in wedged-shaped cells that allow neural tube closure (Fig. 2B a-d) (Sawyer et al., 2010). Subsequently in 1987, Smith and Schoenwolf proposed that creation of the ventral hingepoint (the prospective floor plate) occurred by basal expansion of the median hingepoint cells (Fig. 2B c′) (Smith and Schoenwolf, 1987). Thus, while apical constriction was dependent on myosin, Rho GTPases and the actin cytoskeleton (for review (Sawyer et al., 2011; Sawyer et al., 2010)), basal expansion depended on INM (McShane et al., 2015;

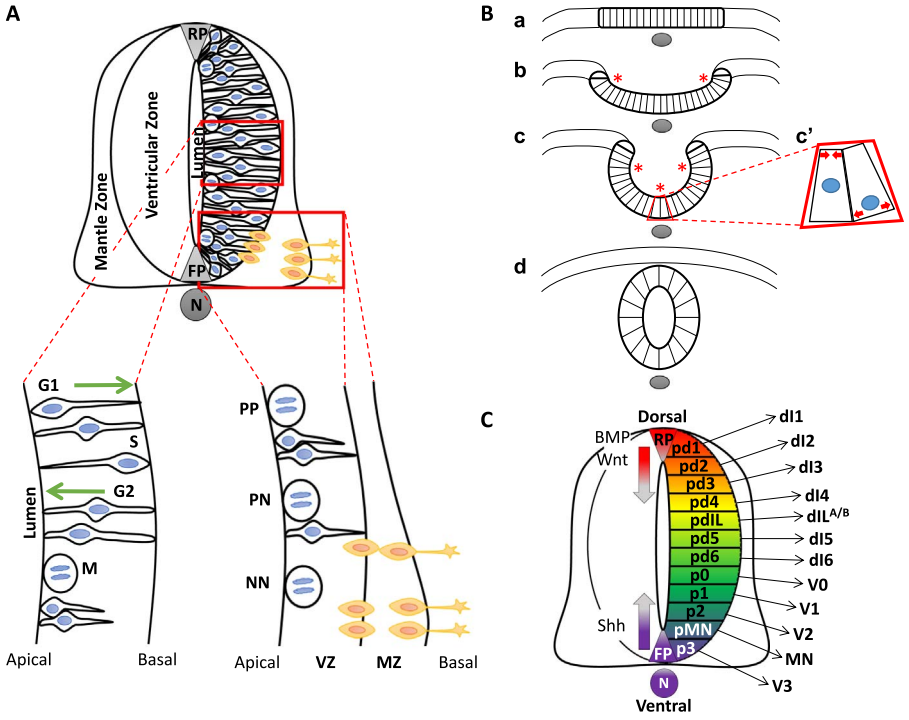


**Fig. 2.** The vertebrate developing spinal cord. A) Schematic representation of a neural tube showing proliferative progenitors (white cells) located in the ventricular zone and differentiating neurons (yellow cells) in the mantle zone. Within the ventricular zone, progenitor cell nuclei migrate (green arrows) between the apical and basal sides, as they progress through the cell cycle in a process known as interkinetic nuclear migration (INM) (left inset). Three modes of cell division have been described for neural progenitors: PP, PN or NN divisions (right inset). RP: roof plate, FP: floor plate, N: notochord, VZ: ventricular zone, MZ: mantle zone. B) Primary neurulation in vertebrates (a – d). A flat neural plate (a) bends in the ventral midline in order to approach neural folds (b, c) until closure forms the neural tube (d). Bending occurs at the hingepoints, where hingepoint cells (asterisks in b, c) change their morphology to facilitate the closure. Shape changes occur through apical constriction (left cell in c') and/or by basal expansion (right cell in c'). C) Cell specification in the spinal cord. Cellular identities are defined by morphogen gradients: Shh, from the notochord and the floor plate, to establish the identity of the ventral progenitor cell domains; BMP and Wnt, from the roof plate, to produce dorsalizing signals. Different neuronal subtypes are generated from the specific progenitor domains. RP: roof plate, FP: floor plate, N: notochord.

Smith and Schoenwolf, 1987, 1988). Accordingly, median hingepoint cells presented different cell cycle kinetics as compared to other neural progenitors: nuclei of these cells spent more time in the basal position of the neural tube (longer S phases) than in the apex (shorter M phases), suggesting that cell cycle length modifications could be the mechanism underlying cell shape changes during neural plate bending (McShane et al., 2015; Smith and Schoenwolf, 1987, 1988).

Since its discovery in 1935, the mechanisms involved in INM have been largely studied using different models, such as the developing mouse brain and the zebrafish retina (Kosodo et al., 2011; Laguesse et al., 2015). Apical migration of nuclei in G2 has been shown to be microtubule-, microtubule associated proteins- and actomyosin-dependent (Hu et al., 2013; Norden et al., 2009; Spear and Erickson, 2012). A more precise comprehension of these movements was obtained using live imaging. In chicken neural-tube-slice cultures, it was shown that nuclei migrate apically during G2 using the dynein/microtubule motor system (Spear and Erickson, 2012). During late G2-phase, the centrosome is released from the apical surface and moves basally to meet the apically migrating nucleus. Mitosis is initiated away from the apical surface as a result of cyclin B1-CDK activation. Actin contraction then induces cell rounding and pushes the cell the rest of the way towards the apical surface (Spear and Erickson, 2012). Dynein nuclear pore recruitment, essential for apical migration in G2, was analyzed in more detail during brain development (Hu et al., 2013). This study showed that in early G2, dynein is recruited via the RanBP2-BicD2 pathway, while in late G2, the Nup133-CENP-F pathway is involved. Moreover, it showed that apical nuclear migration is a required event for progression from G2 to mitosis, indicating that INM is required for cell cycle progression. The mechanisms underlying basal migration of nuclei in G1 are more controversial. While some studies evoke a passive and stochastic process, possibly driven by a crowding effect (Kosodo et al., 2011), others suggest that it involves microtubule/kinesin3 or actomyosin cytoskeleton (for a review see (Laguesse et al., 2015)).

In the spinal cord mitosis occurs exclusively along the lumen (Fig. 2A left inset); that is the main difference with the developing neocortex. In the latter, two main populations of progenitors have been described, apical progenitors (APs) located in the ventricular zone and undergoing mitosis at the apical surface and, basal progenitors (BPs) dividing at an abventricular location in the subventricular zone (Florio and Huttner, 2014; Pilz et al., 2013; Wilsch-Brauninger et al., 2016). These two progenitor populations are essential for neocortex expansion in development and evolution, with large brain size being correlated with a higher ratio of highly proliferative BPs compared to APs. The developing spinal cord is less complex, since it contains only progenitors dividing at the apical surface, making it a suitable model to better understand the role of the cell cycle in cell fate choice.

## 4. Cell cycle, INM and cell fate choice

A key question is to determine whether INM has an instrumental role in determining the cell fate of neural progenitors. Numerous examples of INM perturbations correlating with neurogenesis modifications are present in the literature (Baye and Link, 2007; Del Bene, 2011; Del Bene et al., 2008; Hu et al., 2013; Murciano et al., 2002) (reviewed by Formosa-Jordan et al. (2013)). In the chicken spinal cord and Zebrafish retina, it was proposed that nuclei movements along the apico basal axis could lead to varying exposure of progenitor nuclei to Notch signaling that in turn could regulate neurogenesis (Del Bene, 2011; Del Bene et al., 2008; Murciano et al., 2002). The Notch intercellular signaling pathway results from the interaction between the transmembrane receptor Notch and its ligands Delta or Serrate/Jagged in neighboring cells. The Notch signaling pathway plays an important role in neurogenesis, controlling the balance between progenitor maintenance and differentiation, by a process called lateral

inhibition (Collier et al., 1996). Accordingly, a neurogenic precursor expressing the Delta ligand inhibits its expression in the neighboring cells to prevent their differentiation. Binding of the ligand to the Notch receptor leads to the proteolytic cleavage of the Notch intracellular domain (NICD), which enters the nucleus and activates its target genes, including the transcriptional repressor Hes (Hairy-Enhancer of Split) and related genes. It thereby prevents expression of proneural transcription factors of the neural basic helix-loop-helix (bHLH) family, such as NeuroG2 (Neurogenin 2; Ngn2), thus maintaining the cell in an undifferentiated state (Collier et al., 1996). Conversely, in the neurogenic precursor expressing Delta, proneural transcription factors are induced at a high level by positive feedback mechanisms, leading to the induction of bHLH differentiation genes of the NeuroD family and to neuronal differentiation (Formosa-Jordan et al., 2013).

In the chicken developing spinal cord, the Notch ligand cDelta-1 is expressed transiently by newborn neurons located on the basal side of the neural tube. These cells are on their way towards the mantle zone. They are mixed with progenitors in S and the onset of G2 (Myat et al., 1996). In the zebrafish retina, the ligand Delta is expressed in a baso-apical decreasing gradient, and altering INM dynamics leads to alterations of the duration and level of exposure of nuclei to Notch signaling (Del Bene et al., 2008), resulting in differentiation defects. Based on the same model, the time the nucleus will spend on the basal side of the neural tube, can be critical for its level of Notch activity. Accordingly, Notch expression has been shown to be upregulated in progenitors undergoing G2/M/early G1 (Del Bene et al., 2008; Murciano et al., 2002). However, real time measurement of Notch signaling in the chicken neural tube reveals that the onset of Notch activity can be a random event, occurring in different phases of the cell cycle (Vilas-Boas et al., 2011). Thus, elucidating the role of Notch signaling and INM in spinal neurogenesis will require more in-depth investigation.

## 5. Mitosis and cell fate

Mitosis has also been proposed as being critical to cell fate determination. Neural progenitors can divide symmetrically to enrich a cell population, or asymmetrically to generate a differentiated progeny (Fig. 2A right inset). Accordingly, three modes of cell division have been described for neural progenitors: self-expanding or proliferative division that generates two progenitors (PP), self-replacing or asymmetric neurogenic division in which a progenitor and a neuron are generated (PN), and self-consuming or terminal neurogenic division that gives rise to two neurons (NN). The mechanisms that control the choice between proliferative and neurogenic divisions remain poorly understood. The most widespread but still debated hypothesis is that the orientation of the mitotic spindle controls cell fate choice (Dewey et al., 2015; di Pietro et al., 2016; Fededa et al., 2016; Paridaen and Huttner, 2014; Peyre and Morin, 2012). Specifically, the orientation of the mitotic spindle with respect to the apical surface of the neuroepithelium is proposed to determine the axis of cell division. 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 (reviewed in (Paridaen and Huttner, 2014)). In the developing spinal cord, 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 (Morin et al., 2007). Indeed, neural progenitors detached prematurely and migrated to the mantle zone, staying as proliferating neural progenitors. The planar divisions were thus essential for the maintenance of neural progenitors in the ventricular zone. However, it was shown that misexpressing the adaptor protein Inscuteable in the chicken neural tube, shifts the spindle towards an oblique orientation, at the expense of planar divisions, and simultaneously causes accelerated neurogenesis (Das and Storey, 2014). Recently, a cell and axon guidance molecule, Semaphorin3B (Sema3B), has been shown to orient the divisions of neuroepithelial cells in the mouse developing spinal cord (Arbeille et al., 2015). Sema3B, expressed in floor plate cells, is released in the cerebrospinal fluid and binds to the apical pole of spinal progenitors. Adding Sema3B to neural tube cultures promotes planar orientation of cell divisions whereas Sema3B deletion leads to a shift of planar to oblique divisions. However, Sema3B -/- progenitors neither detach prematurely, nor display a modified ratio of neurogenic/proliferative divisions. Thus, understanding the role of mitotic spindle orientation in asymmetric fate acquisition in spinal progenitors requires further investigations.

In mouse corticogenesis, it was recently reported that asymmetry in the size of the mitotic spindle correlated with asymmetric cell division and neuron generation (Delaunay et al., 2014). The daughter cell issued from the larger spindle preferentially became a neuron, whereas the daughter cell arising from the smaller-spindle stayed an apical progenitor. It was proposed that spindle-size asymmetry is controlled by the Wnt planar cell polarity (PCP) signaling pathway (Delaunay et al., 2014). Whether the spindle-size asymmetry exists in the spinal cord, remains to be explored.

Once committed to neuronal differentiation, neural progenitors sever their apical foot to detach from the ventricular surface and then migrate to the differentiation or mantle zone (Fig. 2A right inset) (Das and Storey, 2014). Different types of neurons are generated in precise temporal and spatial patterns under the influence of extrinsic signals and downstream transcriptional networks (Fig. 2C) (Kicheva et al., 2014; Kicheva and Briscoe, 2015). Numerous examples illustrate the interplay between morphogens, cell cycle regulators and the transcriptional program governing neural progenitor specification and differentiation.

## 6. Morphogens, cell cycle machinery and controlling the balance between proliferation and neuronal differentiation

The spinal cord grows mainly along the antero-posterior axis, accompanying the posterior body elongation. This growth is not only due to progenitor proliferation, but also to cell rearrangements that may be distinct from the surrounding tissues (Benazeraf and Pourquie, 2013; Loganathan et al., 2016; Neijts et al., 2014; Steventon et al., 2016).

Spinal progenitors originate from a population of neuromesodermal progenitor cells located in the caudal epiblasts, near the node-streak region of the embryo (for reviews see (Gouti et al., 2015; Henrique et al., 2015)). Cells contributing to the spinal cord remain in the epiblast to form the caudal neural plate, where they are maintained in an undifferentiated state under the control of FGF signaling (Agius et al., 2015; Akai et al., 2005; Bertrand et al., 2000; Diez del Corral et al., 2002; Wilson et al., 2009). FGF signaling promotes Wnt8c expression, which in turn prevents neuronal differentiation (Olivera-Martinez and Storey, 2007). As the body axis elongates, FGF activity decreases while Wnt8c persists in the preneural tube. Importantly, Wnt but not FGF signaling is permissive for retinoid signaling. Thus, retinoic acid coming from the somite will inhibit FGF and Wnt signaling, switching the cell from a proliferative undifferentiated state to a more mature state (Olivera-Martinez and Storey, 2007). This is achieved through the upregulation of major determinants of neuronal fate, such as the homeodomain transcription factors Pax6 and Irx3, or the bHLH protein Olig2, and the pan neuronal genes such as NeuroG2 (Bertrand et al., 2000; Diez del Corral et al., 2003; Novitch et al., 2003).

Cells in the caudal neural plate and in the neural tube are actively proliferating. Although they express some cell cycle regulators in common, these two groups of cells basically have two distinct expression patterns (Fig. 3). Thus, cyclin D2 is strongly expressed in the chicken caudal neural plate under the control of FGF signaling, whereas cyclin D1 appears in the closing neural tube and is associated

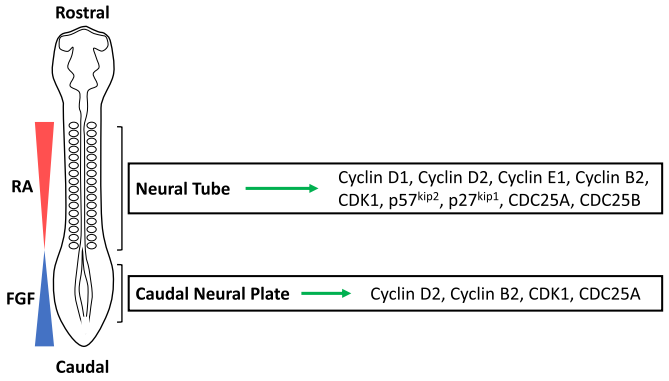


**Fig. 3.** Discrete expression of cell cycle regulators is associated with neuroepithelium maturation. Schematic representation of the dorsal view of an early (E1.5) chick embryo. The mature neural tube and the caudal neural plate are represented. In the caudal neural plate, neural progenitors are maintained in an undifferentiated state under the control of FGF. Neuronal differentiation occurs when FGF is repressed by RA (retinoic acid) coming from the somitic mesoderm (Gouti et al., 2015). Distinct cell cycle regulators are differentially expressed between the neural tube and the caudal neural plate as reported previously (Benazeraf et al., 2006; Lobjois et al., 2004; Olivera-Martinez et al., 2014).

with neuroepithelium maturation (Lobjois et al., 2004). More unexpectedly, transcripts encoding the CDC25B phosphatase regulating entry into mitosis, are absent from the caudal region of the neuroepithelium, where proliferation is highly active (Benazeraf et al., 2006). In contrast, CDC25A is expressed both in the caudal neural plate and neural tube (Fig. 3) (Benazeraf et al., 2006). More recently, transcriptomic analysis performed by the group of Kate Storey identified numerous cell cycle regulators, including cyclins and CKIs, as being differentially expressed between chicken caudal neural plate and neural tube (Fig. 3) (Olivera-Martinez et al., 2014).

Within the more mature neural tube, spinal progenitors are subjected to morphogens that pattern it along the dorso-ventral axis: Wnts and BMPs originating from the roof plate, and Shh diffusing from the notochord and floor plate (Fig. 2C) (Kicheva and Briscoe, 2015). Accumulating data link these three signaling pathways to the cell cycle machinery and/or the mode of division of neural progenitors.

Wnt signaling has been implicated with neural progenitor proliferation, and also with the inhibition of cell cycle exit. Although multiple Wnt proteins are expressed in and around the neural tube, only Wnt1 and Wnt3a promote neural progenitor proliferation by positively regulating cell cycle progression (Megason and McMahon, 2002). Consistent with this, overexpression of Wnt1 in mouse embryos, or a constitutively active form of β-catenin in mouse or chick embryos, increases progenitor proliferation and decreases neuronal differentiation (Dickinson et al., 1994; Ille et al., 2007; Megason and McMahon, 2002; Zechner et al., 2003). Conversely, β-catenin deficiency in mouse embryos, or expression of a dominant negative Tcf-4 in chick embryos, reduces cell proliferation and increases differentiation within the neural tube (Megason and McMahon, 2002; Zechner et al., 2003). Wnts control proliferation by regulating cyclin D1 and cyclin D2 transcription, thereby G1 to S progression and thus cell cycle reentry (Alvarez-Medina et al., 2009; Megason and McMahon, 2002). In the neural tube, the transcription factor Sox5 counteracts Wnt-β-catenin activity resulting in cyclin D1 downregulation and cell cycle exit (Martinez-Morales et al., 2010).

Sonic Hedgehog (Shh) is a morphogen secreted from the notochord and the floor plate to establish the identity of the five ventral progenitor cell domains (p0, p1, p2, pMN and p3) (Fig. 2C) (Kicheva and Briscoe, 2015). Shh controls proliferation and survival in the neural tube. It acts upstream of Wnt signaling by regulating Tcf3/4 expression and thus cyclin D1 expression and G1 progression in ventral neural progenitors, (Alvarez-Medina et al., 2009). In addition, the Shh/Gli pathway regulates G2 length, by controlling late cyclin expression including cyclin A and cyclin B and the regulator of the G2/M transition CDC25B (Alvarez-Medina et al., 2009; Benazeraf et al., 2006). Whether these links are direct, remains to be elucidated. A link between Shh and cyclin A2, cyclin B1 and CDC25C, has also been observed in the Xenopus retina (Locker et al., 2006). In that context, it is associated with the conversion of slowly-dividing stem cells into fast cycling transient amplifying progenitors, closer to exiting the cell cycle and to differentiating.

Cyclin D1 has also been shown to be a target gene of the Hippo signaling pathway, influencing cell proliferation and survival during neural tube development (Cao et al., 2008). Overexpression of YAP transcriptional activator and of its DNA binding partner TEA domain transcription factor (TEAD), in chick neural tube, induces cyclin D1 expression. Furthermore, the repression of genes downstream of YAP and TEAD, leads to downregulation of cyclin D1, resulting in cell cycle exit and neuronal differentiation (Cao et al., 2008).

Shh and BMP have been shown to affect the division mode of neural progenitors. To determine the three modes of division with single cell resolution, two reporters were designed: Sox2p-GFP(green) that is expressed in progenitor-generating divisions (PP and PN) and Tis21p-RFP(red), whose expression is restricted to neurogenic progenitors (PN and NN). Thus, progenitors performing PP, PN or NN divisions appear respectively in green, in yellow (green plus red) or red (Le Dreau et al., 2014; Saade et al., 2013). Electroporation of these reporters show that high Shh activity coincides with PP divisions and the expansion of motor neuron progenitors, when Shh signaling decreases PP divisions are replaced by PN and NN divisions (Saade et al., 2013). In addition, a dominant active form of Smoothened receptor, which maintains high Shh activity, promotes self-expanding (PP) divisions and prevents neurogenic divisions (PN+NN). Conversely, when a dominant active form of Patched1 receptor that reduces Shh activity is electroporated, an increase in NN divisions is observed at the expense of PP divisions, indicating that Shh signaling is important to maintain stem cell identity in the spinal cord (Saade et al., 2013).

Transforming growth factor β (TGFβ) family member bone morphogenic protein (BMP), diffuses from the roof plate to specify the most dorsal progenitor domains (pd1, pd2 and pd3) (Fig. 2C). Additionally, BMP activity is required for the generation of three subpopulations of interneurons (dI1, dI3 and dI5) (Le Dreau et al., 2012). Elisa Marti's group reported that BMP signaling promotes self-expanding (PP) divisions of dorsal interneuron progenitors (Le Dreau et al., 2014). More precisely, they show a direct correlation between the different modes of division and endogenous canonical BMP activity. By measuring SMAD 1/5 (BMP effector) activity within the neural tube, they observe that strong SMAD activity is related to PP divisions, intermediate activity correspond to PN divisions, and weak activity is associated to NN divisions. Their results suggest that high BMP activity is required to maintain PP divisions, while a progressive decrease in BMP activity allows the switch to PN and NN divisions (Le Dreau et al., 2014).

Taken together, these observations show that even if Shh and Wnt/BMP appear to have antagonistic activities in dorso-ventral patterning, enough evidence demonstrates that these signaling pathways have multiples roles and that they collaborate in controlling neural tube patterning, neural progenitor proliferation and mode of division, in order to ensure correct spinal cord morphogenesis.

## 7. Cell cycle regulators can act on transcriptional networks related to neurogenesis and neuronal differentiation

Different studies have addressed the question regarding involvement of the cell cycle machinery in the transcriptional initiation of developmental genes. Such functions have been described for cyclin Ds, and research has focused on cyclin D1, which is able to interact with different transcription factors to regulate their activity (Coqueret, 2002). High-throughput mass spectrometry has shown that during

mouse development, cyclin D1 is able to bind the promoters of a large number of expressed genes (Bienvenu et al., 2010). Of note, this group shows that in the mouse retina, cyclin D1 binds the upstream regulatory region of the Notch1 gene. The recruitment of CBP (CREB binding protein) histone acetyltransferase by cyclin D1, promotes the histone acetylation of the Notch1 promoter region to regulate Notch transcript and protein levels (Bienvenu et al., 2010).

In the spinal cord, Lukaszewicz and Anderson proposed that cyclin D1 regulates not only cell proliferation but also promotes neurogenesis independently of its interaction with the cell cycle machinery (Lukaszewicz and Anderson, 2011). They found that down-regulation of cyclin D1 in the chick spinal cord reduces the number of progenitor cells committed to differentiation, as well as the number of newborn motor neurons. Even if the phenotype was rescued by the expression of cyclin D1, it was not with cyclin D2, indicating that this activity cannot be explained by regulation of cell cycle progression. Furthermore, overexpression of a mutant form of cyclin D1 that lacks the ability to bind CDK, and which cannot promote cell proliferation, increased significantly the percentage of neurogenic progenitors as compared to the GFP control. Finally, they also found that this neurogenic function is mediated by the interaction between cyclin D1 with Hes6, a noncanonical Notch effector (Lukaszewicz and Anderson, 2011).

More recently, the group of Ludovic Vallier showed that in human embryonic stem cells, cyclin D1 acts as a cell cycle dependent transcriptional regulator, able to control lineage specification of stem cells. By means of ChIP-seq they identify cyclin D1 target genes, including transcription factors and components of the Wnt, BMP4 and Activin/Nodal/TGFβ signaling pathways (Pauklin et al., 2016). Interestingly, they show that cyclin D1 control of transcription is independent of its interaction with CDK6 and consequently of its phosphorylation activity as well. They also find that cyclin D1 recruits transcriptional coactivators (such as SP1) to neuroectoderm genes (*Pax2*, *Sox3*, *PBX1*, *DACH1* and *Otx1*) and corepressors (such as E2fs) to endoderm genes (*Sox18*, *Wnt3* and *Smad2*), thereby promoting or blocking the induction of germ layers. Finally, using the FUCCI reporter tool (Sakaue-Sawano et al., 2008), it was shown that the binding of cyclin D1 to its target genes occurs during late G1 phase and G1/S transition, suggesting that the regulatory function of cyclin D1 is cell cycle-dependent (Pauklin et al., 2016).

As mentioned before, the transcription factor Sox2, is a key regulator of neural progenitor maintenance. In mouse corticogenesis, the cell cycle regulators E2f3a and E2f3b fine-tune the Sox2 level in opposite ways, thereby controlling the balance between proliferation and differentiation (Julian et al., 2013).

Cell cycle regulators can control the activity of proneural proteins, as illustrated with NeuroG2. NeuroG2 is progressively phosphorylated on multiple Serine/Proline (SP) sites by CDK1/2 (Ali et al., 2011). This phosphorylation acts quantitatively to control Ebox binding. Phospho-NeuroG2 forms cannot activate the NeuroD promoter, whose activation is needed for cell autonomous neuronal differentiation. However, phospho-NeuroG2 forms are still able to activate the Delta promoter, whereby driving non-autonomous Notch mediated progenitor maintenance through lateral inhibition. Phospho-NeuroG2 forms predominate in rapidly cycling cells, while cell cycle lenghthening results in underphosphorylated forms. This differential activation of NeuroD and Delta allows the preferential coupling of cell cycle lenghthening to differentiation, over progenitor maintenance (Ali et al., 2011; Hindley et al., 2012; Hindley and Philpott, 2012). Whether the NeuroG2 phosphorylation state also regulates in turn the repression of progenitors genes and cell cycle positive regulators, remains to be determined.

Cip/Kip family members can also interact with NeuroG2. In the mouse cerebral cortex, by stabilizing NeuroG2, $p27^{Kip1}$ triggers the differentiation and migration of cortical neurons, independently from its cell cycle regulatory activity (Nguyen et al., 2006).

Together, these examples illustrate that cell cycle regulators may act at the transcriptional and post-transcriptional levels to regulate cell fate acquisition in different contexts.

## 8. 50 years of cell cycle measurements in spinal progenitors and still a fuzzy picture

A large number of groups, mostly studying mouse retinogenesis and corticogenesis, have proposed cell cycle kinetics as a mechanism controlling cell fate decisions, highlighting the importance of analyzing the links between cell cycle dynamics and cell fate (Arai et al., 2011; Calegari and Huttner, 2003; Gruber et al., 2011; Lange et al., 2009; Locker et al., 2006; Pilaz et al., 2009).

Measurements of cell cycle and phase lengths have been performed since the 1960s. Nevertheless, different groups are still interested in studying and measuring cell cycle kinetics in order to understand its role in organogenesis, including spinal cord morphogenesis (Table 1). In 1962, the first cell cycle length data were obtained using thymidine incorporation. In experiments geared to determine the turnover rate of proliferating cells in the chick neural tube, S Fujita reported a cell generation time of 5 h and a mitotic length of 24 min in 1 d embryos (Fujita, 1962). Similarly, two more groups aiming to characterize the normal behavior of neural progenitors during closure of the neural tube, measured a total cell cycle length of 8 h in 0.75 to 1.3 d embryos (Langman et al., 1966), and a total cell cycle time of 6.3 h and 9.9 h (for dorsal and ventral neural progenitors, respectively) in 1.2 to 1.6 d embryos (HH stage 9 - HH stage 10 embryos (Hamburger and Hamilton, 1992)) (Smith and Schoenwolf, 1987) (Table 1). These data suggest that cell cycle length may vary according to position within the neural tube and age of the neuroepithelium.

As reported before, transcriptomic analysis identified numerous cell cycle regulators as being differentially expressed between chicken caudal neural plate and neural tube (Fig. 3) (Olivera-Martinez et al., 2014). The group of Kate Storey, aiming to validate transcriptome changes, analyzed the cell cycle parameters in the caudal neural plate versus the neural tube. They show that total cell cycle length doubles once neuronal differentiation is ongoing: from 8 h in cells from the caudal neural plate (CNP), where progenitors are mostly performing PP divisions, to 16 h in cells from the neural tube (NT), where neurogenesis has already started (Fig. 3) (Olivera-Martinez et al., 2014). In addition, they showed that S phase lengthening from 3 h in cells from the CNP to 5 h in the NT contributes to lengthening of the cell cycle (Table 1). Since epigenetic changes may happen during S phase, they propose that it is this S phase elongation that drives the epigenetic events that together with chromatin rearrangements lead to neural differentiation (Olivera-Martinez et al., 2014).

In the spinal cord, Elisa Marti's group, aiming to quantify the dynamics of cell division in the motor neuron progenitor (pMN) domain, found a correlation between cell cycle kinetics and MN differentiation (Saade et al., 2013). Using the chick developing spinal cord at two different developmental stages (HH stage 14 versus HH stage 24 (Hamburger and Hamilton, 1992)), they showed that cells in the neurogenic phase (HH stage 24) have a shorter total cell cycle length due to a decrease in the S and G2 phases (Table 1). Shortening of S phase length and its correlation with neurogenesis, was previously reported in corticogenesis: S phase length decrease correlated to neurogenic division and was thus concomittant to neuronal differentiation (Arai et al., 2011). Results from Saade's work also showed that even if the length of the G1 phase did not change between the developmental stages analyzed, the proportion occupied by the G1 phase was bigger at HH stage 24 and represented 52% of the whole cell cycle (Saade et al., 2013), a result similar to that already described for mouse corticogenesis (Arai et al., 2011; Lange et al., 2009; Pilaz et al., 2009). In the spinal cord, the PP, PN and NN populations were sorted using the Sox2p-GFP and Tis21p-RFP reporters and their DNA content was analyzed by flow cytometry (Saade et al., 2013). The DNA content profiles for PP and PN populations were comparable, suggesting that the switch from PP to PN divisions is not associated with major

**Table 1**
Cell cycle total and phase lengths measured in the chick developing spinal cord.

| Developmental Stage | Domain | Cell cycle phases length (h) | | | | Cell cycle length (h) | Method | Ref |
|---|---|---|---|---|---|---|---|---|
| | | G1 | S | G2 | M | | | |
| HH6 | Nd | | Nd | | 0.4 | 5 | Thymidine | Fujita, 1962 |
| HH29 | Ventral | 9 | 4 | 2 | 1 | 16 | | |
| HH4 - HH9 | Nd | 0 | 5 | 2.5 | 0.5 | 8 | Thymidine | Langman et al., 1966 |
| HH9 - HH10 | Ventral | | Nd | | | 9.9 | Thymidine | Smith and |
| | Dorsal | | Nd | | | 6.3 | | Schoenwolf, 1987 |
| HH14 | pMN | 5 | 8 ± 1 | 1.4 ± 0.05 | 1.5 ± 0.4 | 16 ± 2 | EdU | Saade et al., 2013 |
| HH24 | | 5 | 3 ± 0.5 | 0.8 ± 0.1 | 1.2 ± 0.3 | 10 ± 1 | pH3 | |
| HH17 – HH20 | Dorsal | Nd | Nd | 2 | Nd | Nd | EdU/BrdUpH3 | Peco et al., 2012 |
| | pMN | 6.7 | 3.7 ± 0.4 | 1.4 | 0.5 | 12.3 ± 1.5 | | |
| HH17 – HH20(CDC25B-RNAi) | | 5.4 | 3.1 ± 0.5 | 2 | 0.5 | 11.1 ± 1.3 | | |
| E9-E9.5 (mouse)HH18 | p3 | 4.5 | | 4 | 0.5 | 9 | IdU/BrdUpH3FUCCI S/ | Kicheva et al., 2014 |
| (chick) | pMN | 4 | | 4.5 | 0.5 | | G2/M | |
| | pI | 3 | | 5.5 | 0.5 | | | |
| | pD | 3 | | 5.5 | 0.5 | | | |
| E9.5 (mouse)HH19 | p3 | 7 | | 4.5 | 0.5 | 12 | | |
| (chick) | pMN | 6 | | 5.5 | 0.5 | | | |
| | pI | 5 | | 6.5 | 0.5 | | | |
| | pD | 5 | | 6.5 | 0.5 | | | |
| E10 (mouse)HH22 (chick) | p3 | 9.5 | | 3 | 0.5 | 13 | | |
| | pMN | 7 | | 5.5 | 0.5 | | | |
| | pI | 5.5 | | 7 | 0.5 | | | |
| | pD | 5 | | 7.5 | 0.5 | | | |
| HH10 | CNP* | Nd | 3 | Nd | | 8 | EdU | Olivera-Martinez |
| | NT* | Nd | 5 | Nd | | 16 | | et al., 2014 |
| HH25 | Dorsal | 5 ± 1.7 | 5.9 ± 0.6 | 1.3 ± 0.1 | 0.64 ± 0.1 | 12.9 ± 0.9 | BrdUpH3 | Le Dreau et al., 2014 |
| HH25 (SMAD1/5-RNAi) | | 5.4 ± 1.3 | 3.7 ± 0.4 | 1.4 ± 0.1 | 0.8 ± 0.1 | 11.3 ± 0.7 | | |
| HH10 | Dorsal/ | | Nd | | 0.5 ± 0.1 | 16 | BrdU | Wilcock et al., 2007 |
| HH10/11 – HH16 | Intermediate | | Nd | | 0.5 ± 0.2 | 9 – 28 | | |
| HH10/11 – HH16(PP) | | | | Nd | | 15 | Timelapse Imaging | |
| HH10/11 – HH16 (PN) | | | | Nd | | 20 | | |

Nd: not determined; pH3: phospho-histone H3;
* CNP: caudal neural plate, NT: neural tube.

changes in cell cycle kinetics. The NN DNA content profile, however, was atypical and suggested a high level of aneuploidy.

By measuring G2 phase length in proliferating dorsal progenitors (Pax7+ cells) and ventral progenitors (Olig2+ cells, pMN) at HH stage 17, we also observed a shorter G2 phase in the pMNs (Peco et al., 2012), due to upregulation of the G2/M regulator CDC25B in ventral neural progenitors. CDC25B downregulation resulted in a lengthening of G2-phase without significantly modifying the S-phase or total cell cycle duration, suggesting that it was compensated by a shortening of the G1 phase. Such a compensatory mechanism has already been described in Drosophila wing disc, where a delay in G2/M progression is compensated by an acceleration of the G1/S progression and vice-versa (Reis and Edgar, 2004). This mechanism, intrinsic to the core cell cycle machinery, involves String, the Drosophila ortholog of CDC25B. In the neural tube, a similar compensatory mechanism would contribute to maintaining the proliferation rate even along the dorso-ventral neural tube, while cell cycle kinetic modifications would be required for the onset of differentiation (Kicheva et al., 2014). CDC25B down-regulation maintains neural cells in the progenitor state at the cost of neuronal differentiation, indicating that the function of the phosphatase is also to promote differentiation (Peco et al., 2012). Whether CDC25B's action on neuronal differentiation results from the modification of the G2 phase length or is uncoupled from the cell cycle remains to be elucidated.

Briscoe and coworkers (Kicheva et al., 2014) investigate how tissue growth and specification are coordinated to generate the relative proportions of different cell types in the mouse and chick spinal cord. They measured cell proliferation of progenitors from different domains within the dorso-ventral axis (Fig. 2C): p3 (ventral interneuron progenitors), pMN (motor neuron progenitors), pI (intermediate interneuron progenitors) and pD (dorsal interneuron progenitors) (Table 1). By means of IdU/BrdU incorporation and using mouse neural tube, they measure a total cell cycle length of 9 h in mouse embryos between E9 and E9.5 (corresponding to chick stage HH18). Before E9, the proliferation rate is constant; once differentiation starts it decreases and the cell cycle length increases. Even if cell cycle length is similar for all progenitor domains along the dorso-ventral axis at each given stage, cell cycle phase distribution is different (Table 1). Using S/G2/M FUCCI transgenic mice, they show that the G1 phase increases during development relative to the cell cycle length, but that this increase is not the same within the different domains. Differentiation progress from ventral to dorsal in the developing spinal cord and at each given stage, ventral progenitors display a longer G1 and shorter S/G2 than dorsal progenitors. At E10, therefore, when the maximum differentiation rate is reached for MNs, pMNs exhibit a long G1 phase (7 h) and a short S/G2 phase (5.5 h). In contrast, pD at E10 show a short G1 phase (5 h) accompanied by a long S/G2 phase (7.5 h). Thus, the increase in total cell cycle length, and more precisely G1 length, was correlated with neuronal differentiation. Finally, when analyzing chick neural tube behavior, they found that pattern formation proceeds in a similar way as in the mouse (Kicheva et al., 2014).

In all these studies, total cell cycle and phase lengths were calculated from fixed tissues, thereby treating neural progenitors as a homogeneous population. All these results, therefore, correspond to estimated average length values. To analyze cell behavior underlying neuron production, Wilcox and colleagues addressed the question by measuring cell cycle length directly in the living neuroepithelium, using a chicken neural-tube-slice culture system (Wilcock et al., 2007). Calculation of cell cycle duration, using time-lapse imaging, showed that the neural progenitors are a dynamic cell population, with cell cycles ranging from 9 h to 28 h. Importantly, using their single cell analysis, they showed that neurogenic asymmetric division (PN) takes

longer than progenitor-generating division (PP) (Table 1). Finally, they suggest that symmetric terminal divisions (NN) must be even longer since a full cell cycle was not recordable for such divisions (Wilcock et al., 2007).

Altogether, these studies illustrate the correlations existing between cell cycle kinetics and neurogenesis, and suggest that cell cycle kinetics may be linked to cell fate decisions. Nevertheless, there is no consensus of how exactly, and not surprisingly, the molecular mechanisms involved are not yet understood.

## 9. Cell cycle exit and neuronal differentiation

Besides the differences observed in total cell cycle length and cell cycle phase duration, cell cycle exit has been described as a process that may determine cell fate. As neurons are no longer able to re-enter the cell cycle, a large number of studies have addressed the question of whether cell cycle withdrawal is a prerequisite for neuronal differentiation.

In the early developing spinal cord, we have shown that over-expression of cyclin Ds (D1 and D2) initially promotes neural progenitor proliferation and reduces neuronal differentiation (Lobjois et al., 2004). However, this phenotype is not maintained, and 24 h later, cyclin D electroporated cells migrate towards the mantle zone and differentiate into abnormal neurons, capable of migrating and extending axons while still being in the cell cycle (Lobjois et al., 2008). CKI silencing experiments show similar results, which indicate that forcing cells to proliferate is not sufficient to keep them undifferentiated (Gui et al., 2007). By means of $p57^{kip2}$ loss-of-function, it was found that young neurons are able to re-enter the cell cycle (Gui et al., 2007).

Data also indicate that cell cycle arrest is not sufficient to promote neuronal differentiation. Overexpression of the transcription factor Gata2, involved in the production of V2 interneurons, reduces proliferation of spinal progenitors but does not increase neuronal differentiation (El Wakil et al., 2006). We also showed that downregulation of cyclin D1 reduces cell proliferation without promoting neuronal differentiation (Lacomme et al., 2012). Taken together, these results indicate that cell cycle withdrawal is not necessary for neuronal differentiation, but rather that it is an event coordinated together with the activation of the transcriptional program of neuronal differentiation genes.

As mentioned previously, cell cycle arrest involves CDK inhibitors (CKIs). A detailed analysis of CKIs expression performed during spinal cord development (Gui et al., 2007), shows that while $p21^{Cip1}$ expression is restricted to ventral interneurons (Fig. 2C), $p57^{Kip2}$ is broadly expressed along the dorso-ventral axis, except for V2 and motor neuron (MN) domains. Finally, $p27^{Kip1}$ is expressed in the young motor neurons and V3 interneurons. Moreover, in contrast to other CKIs, $p27^{Kip1}$ is also present in most of the cells located in the mantle zone, where it persists during differentiation and therefore is used as a generic marker for cell cycle exit (Gui et al., 2007). Together these observations suggest that different CKIs alone or in combination, will participate in cell cycle arrest in different neuronal subtypes.

The question is then how are cell cycle arrest and differentiation coordinated? For several years now, it has been proposed that proneural genes may induce cell cycle arrest by upregulating CKIs (Bertrand et al., 2002). Overexpressing NeuroG2 in the neural tube results in differentiating neurons accumulating $p57^{Kip2}$ and $p27^{Kip1}$ (Gui et al., 2007; Novitch et al., 2001). More recently, we show that cell cycle exit driven by NeuroG2 is indeed a multistep process. In a first step, NeuroG2 induces cell cycle arrest by rapidly shutting down cyclin D1 and cyclin E, thus preventing cell cycle reentry. At this step, CKIs expression is still not modified. In a second step, upregulation of CKIs such as $p27^{Kip1}$ leads to irreversible cell cycle withdrawal in differentiating neurons (Lacomme et al., 2012).

The timing of cell cycle arrest and neuronal differentiation has been proposed to be under the control of crosstalk between patterning genes and proneural genes. NeuroG2 represses the pan neural genes Sox1-3 and those involved in subtype specification, such as Olig2 or Pax6, a prerequisite for neuronal differentiation (Bel-Vialar et al., 2007; Lee et al., 2005; Novitch et al., 2001; Sandberg et al., 2005; Sugimori et al., 2007). In the chicken neural tube, NeuroG2 upregulates the expression of the repressor Sox21, which promotes cell cycle arrest and neuronal differentiation by counteracting the function of Sox1-3 (Sandberg et al., 2005). The balance between Sox1-3 and Sox 21 activities, defines whether neural cells maintain the progenitor state or commit to neuronal differentiation. Using Pax6 as a paradigm, we show that low levels of Pax6 are permissive to neural progenitors' proliferation, whereas increasing Pax6 levels leads to NeuroG2 upregulation and cell cycle arrest. This is associated with the upregulation of Delta ligand and NeuroM/NeuroD4, indicating that cells are engaged in the neuronal program. However, these high-level Pax6 cells arrested in the cell cycle are blocked in an intermediate state and cannot differentiate, unless they extinguish Pax6 via a negative feedback mechanism involving NeuroG2 (Bel-Vialar et al., 2007). Thus in the spinal cord, the proneural genes play a key role in coordinating cell cycle withdrawal and neuronal differentiation.

The Down's syndrome candidate gene (*Mnb/Dyrk1a*) has also been proposed to coordinate cell cycle exit and neuronal differentiation (Hammerle et al., 2011). Gain-of-function of MNB/DYRK1A in chicken neural tube and mouse telencephalon, upregulates $p27^{Kip1}$ at the transcriptional level, promoting cell cycle exit; it also suppresses Notch signaling, by acting downstream of the Notch intracellular domain (NICD) and by stimulating *Delta1*, thereby promoting neuronal differentiation.

## 10. Regenerative neurogenesis in the adult spinal cord

The adult spinal cord contains a population of latent progenitor cells, the ependymoglial cells lining the central canal. Under normal conditions, these cells display little if any proliferation. After a lesion, these latent progenitors could potentially give rise to new neurons. Across species, latent progenitors located in the spinal cord behave differently after injury; for example, while in mammals they generate astrocytes and oligodendrocytes but not neurons, in fish regenerative neurogenesis is observed together with the production of mature motor neurons. Neuron production is associated with Sox2 activation that allows cell cycle re-initiation. Other important players have been associated with spinal cord regeneration in zebrafish, including an upregulation of the Notch signaling pathway and Dopamine, which displays a proliferation-promoting and/or neurogenic function on ependymoglial cells partly through Shh pathway activation (Alunni and Bally-Cuif, 2016). Even if a large number of studies aiming to understand neuronal repair have been published as illustrated in several recent reviews, (Alunni and Bally-Cuif, 2016; Barbosa and Ninkovic, 2016; Jessberger, 2016), we are still far away from understanding how regenerative neurogenesis can be achieved following spinal cord injury or in neurodegenerative diseases.

## 11. Conclusion

Several questions remain open. The mechanisms controlling a neural progenitor cell's decision to continue to proliferate or to differentiate are far from being well understood. As we presented in this review, increasing evidence links cell cycle kinetics and cell cycle machinery with neural cell fate. Nevertheless, it is still unclear how changes in either one of these systems, or both, in the mother cell, modify the fate of the daughter cells. This caveat is partly related to the fact that cell cycle analyses were mainly performed on fixed tissue and show the mean length for each cell cycle phase in a cell population, but does not necessarily reflect the heterogeneity existing within this population. Moreover, the impact of a cell cycle change on cell fate decision was most often not directly addressed. For this reason, single

cell analysis to characterize accurately cell cycle kinetics of mother cells performing different modes of division, will be of great interest. The introduction of cell cycle biosensors (Sakaue-Sawano et al., 2008) and real time imaging of neural tube slice cultures (Das et al., 2012), or transgenic quail models (Huss et al., 2015) should facilitate such experiments. It will then be possible to identify the cell cycle kinetics of self-renewing progenitors versus neuron-generating progenitors with single-cell resolution and, combined with manipulation of the cell cycle, to determine how changes in cell cycle dynamics impact cell fate decision. This could reveal novel mechanisms controlling cell fate in the developing spinal cord, which most likely will be applicable to other stem cells, including human neural stem cells.

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