Neurogenesis and the Cell Cycle

Review

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For a long time, it has been understood that neurogenesis is linked to proliferation and thus to the cell cycle. Recently, the gears that mediate this linkage have become accessible to molecular investigation. This review describes some of the progress that has been made in understanding how the molecular machinery of the cell cycle is used in the processes of size regulation in the brain, histogenesis, neuronal differentiation, and the maintenance of stem cells.

Introduction

Neurogenesis involves proliferation and differentiation. In cell cycle terms, this means re-entering and exiting the cell cycle. The number of times neuroblasts re-enter the cell cycle largely determines the size of the brain. But it isn't simply the number of cell cycles that is important; it is also the fraction of progenitors that exit at each cycle. This fraction increases as neurogenesis proceeds (Caviness et al., 2000). As cells exit the cell cycle in the developing brain, they take up particular neuronal or glial fates. This process of neural differentiation is linked to the cell cycle in two important ways. The first is histogenesis: cells that exit the cell cycle early take on early fates, while cells that exit the cell cycle later take on later fates. The second is the molecular coordination between cell cycle exit and neuronal cell fate determination, in which determination factors influence the cell cycle, and cell cycle factors influence determination. Finally, some cells in the nervous system remain undifferentiated throughout the life of the animal. These are neural stem cells. Recent work on adult neural stem cells suggests that part of the mechanism by which these cells remain undifferentiated has to do with keeping these cells in the cell cycle. In this review, we hope to update readers on some of the work concerning the links between the cell cycle and these various aspects of neurogenesis.

Cell Cycle and Size Regulation in the Nervous System

The overall size of the brain is governed by cell cycle machinery. This is clearly demonstrated by the enlarged

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brains of mice lacking the rather ubiquitous cell cycle inhibitor p27Kip1 (Fero et al., 1996; Nakayama et al., 1996). But as some parts of the brain tend to be larger than others, many cell cycle components are expressed in specific areas and control regional growth. For example, cyclin D1 has a highly restricted expression pattern in the retina and cerebellum (Fantl et al., 1995; Huard et al., 1999), while in the hindbrain, cyclin D1 and cyclin D2 are expressed in distinct rhombomeres (Wianny et al., 1998). Mouse knockouts for these genes show specific proliferative defects of these respective regions (Fantl et al., 1995; Huard et al., 1999; Sicinski et al., 1995).

How are these cell cycle components expressed and activated appropriately so that each part of the CNS ends up being the correct size? Studies in the retina provide some insight. The retina is specified by a number of transcription factors, some of which have a role in controlling proliferation. Thus, overexpression of the eye-specific transcription factors Optix2/Six6, Six3, and Rx1 in *Xenopus* and zebrafish embryos all result in giant eyes (Figure 1; Andreazzoli et al., 1999; Kobayashi et al., 2001; Zuber et al., 1999). That this is due to extra cell divisions is shown by the fact that clones overexpressing these genes have more cells than control clones. Both Optix2/Six6 and Six3 bind to groucho, a generic transcriptional corepressor, and overexpression of groucho also leads to increased eye size (Lopez-Rios et al., 2003; Zhu et al., 2002). This suggests that the eye-specific factors may work by repressing cell cycle inhibitors locally. Indeed, knockouts of Optix2/Six6 show upregulation of two cdk inhibitors, p27Kip1 and p19Ink4d (Li et al., 2002).

Switching from a pattern of symmetric divisions, in which both cells stay in the cycle, to asymmetric divisions, in which one daughter leaves the cell cycle, can also be used to regulate neuronal number (Takahashi et al., 1996). This kind of control mechanism may explain why transgenic mice expressing constitutively active β-catenin under a neuron-specific enhancer develop enlarged brains. β-catenin is a downstream component of the wnt pathway, which has been implicated in the control polarity of asymmetrical cell divisions (Bellaiche et al., 2001). Consistent with this, in the β-catenin transgenic mice there is a 2-fold increase in the proportion of precursors that re-enter the cell cycle during cortical neurogenesis, implicating a substantial increase in symmetrical divisions (Chenn and McConnell, 1995; Chenn and Walsh, 2002).

How big cells are is another a factor that determines brain size. Highly related invertebrates, for example, may have similar numbers of neurons to each other but great differences in the size of these neurons. Even within a single animal, neurons may vary greatly in size. One way that this is accomplished is through the process of endoreplication, moving through the cell cycle without cytokinesis. Cyclin Es seem to be critically involved in endoreplication in *Drosophila* (Edgar and Orr-Weaver, 2001) and mouse (Geng et al., 2003). Thus, natural giant neurons are often polyploid, and it is possible to make unnatural giant neurons experimentally by
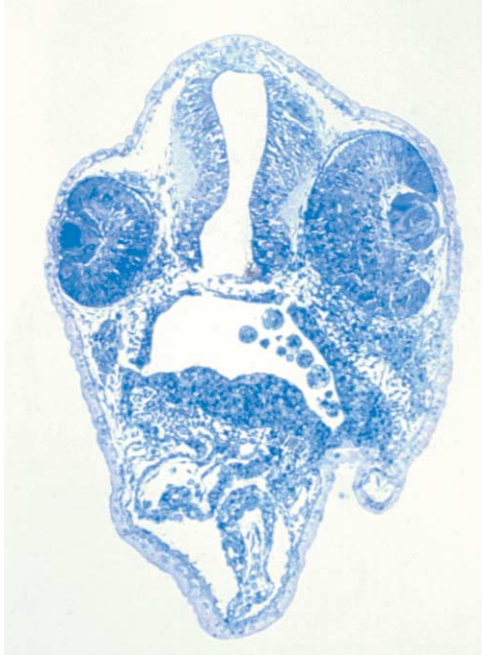
Figure 1. Giant Eye Induced by Optix2

Injection of a low dose of Optix2 mRNA into right side of *Xenopus* embryo (dorsal blastomere) at the 8-cell stage produced a significant enlargement of the eye on that side.

interfering with cytokinesis (Wu et al., 1990). Cell size is also controlled by cell growth during the G phases of the cell cycle. The insulin receptor pathway has been implicated in this. In mice, a conditional brain-specific knockout of PTEN, a component of the insulin receptor-PI3K pathway, shows increased neuron size (Backman et al., 2001). In *Drosophila*, mutations in almost all components of this pathway produce cells of abnormal size (Bohni et al., 1999; Leevers et al., 1996; Potter et al., 2001; Saucedo et al., 2003; Stocker et al., 2003; Tapon et al., 2001; Verdu et al., 1999; Zhang et al., 2003). The size of the brain is thus determined by the number of its constituent cells times the average size of each cell, and both of these features are linked by developmental pathways of neurogenesis to components of the cell cycle.

Cell Cycle and Neural Histogenesis

The time at which the cell exits the cell cycle is its "birth date." In most systems studied, there is a correlation between birth date and fate, giving rise to the process known as histogenesis (Caviness and Sidman, 1973; Holt et al., 1988). One of the clearest examples of histogenesis in the vertebrate brain is the generation of neurons before glial cells. Even in clonal culture experiments, glial cells are formed after neurons, suggesting that this may be an intrinsic property of mammalian neuroblasts (Qian et al., 2000). Components of the cell cycle, it turns out, may be involved in coordinating this aspect of histogenesis. Suggestions that this is so come from experiments using the cell cycle inhibitors. One of these, p27Kip (a cdk inhibitor), gradually increases in cultures of glial progenitors, and when it accumulates

to a high enough level, it causes oligodendrocyte precursors to exit the cell cycle and differentiate (Durand and Raff, 2000). In experiments where the increase of p27Kip1 is accelerated, oligodendrocytes differentiate after a smaller number of cell divisions (Gao et al., 1997), suggesting that cells may be measuring proliferative time by monitoring the accumulation of cell cycle inhibitors. Similarly, in the *Xenopus* retina, the partially homologous cell cycle inhibitor p27Xic1 builds up gradually until it eventually reaches a level high enough to drive cells out of the cell cycle. As elsewhere in the brain, the last-born cells in the retina are glial cells, in this case Müller glial cells. In this system, p27Xic1 appears to be directly involved in the differentiation of Müller glial cells, as overexpression of p27Xic1 not only drives the progenitors out of the cycle early, but also turns most of them into Müller glial cells. Analysis of the functional domains of p27Xic1 show that it is, in fact, a bifunctional molecule, with overlapping yet separable domains for cyclin kinase inhibition and Müller glial determination (Ohnuma et al., 1999).

The evidence of cell cycle inhibitor influences on cell fate contends in a way with a much larger body of evidence suggesting that transcription factors are the key determinants of neural and glial cell fate. Clearly, the proneural genes, especially the bHLH type, have a major role in cell determination. For example, the proneural bHLH transcription factor ath5 is the determination factor for retinal ganglion cells, the first-born neuronal type in the retina (Brown et al., 1998; Kanekar et al., 1997). But such findings raise the question of how transcription factors like ath5 influence cells to exit the cell cycle at the appropriate "histogenetic" moment. Let us first look at ath5 mutants, in mice and zebrafish. In these mutants, retinal ganglion cells are not made (Brown et al., 2001; Kay et al., 2001), and what is particularly interesting for this review is the fact that in such mutants no retinal progenitors exit the cell cycle at the time when RGCs are normally born. Rather, they all stay in the cycle and produce later-type neurons. This is also consistent with the observation that the overexpression of Xath5 in *Xenopus* produces extra RGCs, which are all born at the appropriate time for RGC genesis (Ohnuma et al., 2002). These studies suggest that ath5 helps retinal progenitors leave the cell cycle. How they do this is not clear, but some evidence suggests that bHLH genes downregulate the cell cycle by activating expression of cdk inhibitors (Farah et al., 2000).

In the *Xenopus* retina, the idea that Xath5 might activate the expression of p27Xic1 so that the cell exits the cell cycle early leads to an interesting question, since Xath5 induces ganglion cells and p27Xic1 induces Müller glial cells. To address this question, p27Xic1 was transfected together with Xath5 into retinal progenitors; the result was that these cells exit the cell cycle early but that they are almost all retinal ganglion cells, not Müller glial cells. Thus, the determinative power of the proneural genes appears to be dominant to that of the cell cycle inhibitor. The simple answer seems to be that p27Xic1 makes glial cells only in the absence of co-expressed bHLH proteins.

Observations concerning the role of the cell cycle in histogenesis have been also been reported in the *Drosophila* CNS (Figure 2). The cell cycle protein *string*
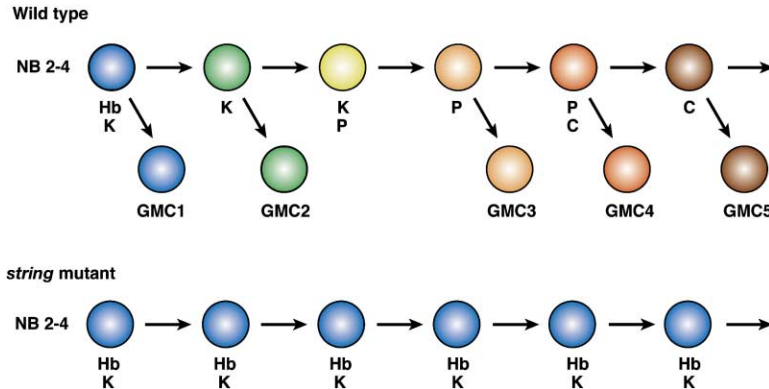
Wild type

codes for the phosphatase *cdc25* and, in *string* mutants, cells are arrested at the G2/M transition. Interestingly, *string* mutant neuroblasts fail to undergo the temporal transitions in transcription factors that lead to the succession of different fates in their sequential progeny (Isshiki et al., 2001). Indeed, a delay in the cell cycle results in a delay in the expression of the determinant *even-skipped* in the GMC 1-1 lineage (Weigmann and Lehner, 1995), and complete cell cycle arrest in S phase by injection of the DNA synthesis inhibitor aphidicolin results in complete inhibition of *even-skipped* expression. These observations indicate that progression of the cell cycle is required to progress a clock that progenitors use to drive histogenesis.

Could these findings be revealing an entangled network in which the molecular machinery regulating the cell cycle influences the molecular machinery of determination and vice versa? This may be quite a reasonable suggestion. One mechanism by which such cross-coordination of determinative transcription factors and cell cycle components might be accomplished involves what are known as heterochronic genes. Heterochronic genes are defined by mutations that cause changes of timings of developmental events. Such genes were identified first in *C. elegans*, although homologs have now been found in flies and mammals (Dostie et al., 2003; Frantz et al., 1994; Honma et al., 1999; Lagos-Quintana et al., 2002, 2003; Lim et al., 2003; Mourelatos et al., 2002). The main members of these heterochronic genes encode microRNAs (miRNAs) and RNA binding proteins (Ambros, 2000; Rougvie, 2001). In nematodes, for example, they turn off the expression of genes like *hbl-1* and *lin-29*, homologs of the sequentially expressed neuroblast determinants in *Drosophila, hunchback* and *Krüppel* (Isshiki et al., 2001). Recently, it has been reported that the mammalian heterochronic gene miRNA-23 regulates the timing of expression of the basic-helix-loop-helix transcription repressor Hes1 at the posttranscriptional level (Kawasaki and Taira, 2003). Mutants in heterochronic genes generally cause precocious development. Importantly, the functions of heterochronic genes may be sensitive to phases of the cell cycle and thus may couple developmental decisions to cell cycle transitions and thereby provide a mechanism for ordering cell fate choices (Ambros, 1999; Euling and Ambros, 1996).

In *C. elegans*, the heterochronic gene *lin12* is a homolog of *Notch*. The Notch pathway leads us back to the issue of why neurons are born before glia. The overexpression of an activated form of Notch increases gliogenesis at the expense of neurogenesis in several systems (Gaiano and Fishell, 2002; Lundkvist and Lendahl, 2001). This is almost certainly mediated, at least in part, by the ability of the Notch pathway to inhibit the transcription of proneural genes (Chitnis, 1995). As precursor neurons decide their fate in the retina, they may thus use the Notch pathway to inhibit neighboring cells from acquiring the same fate (Dorsky et al., 1995). Interestingly, in several systems such as the *Xenopus* retina, the Notch pathway is active in cells that already express bHLH proneural genes (Perron et al., 1998). Experimentally, in these systems, when a constitutive activator of the Notch signaling pathway is co-expressed with proneural genes, this increases the number of neurons induced by the proneural genes, not glia (Ohnuma et al., 2002). As in the case of the cdk inhibitor p27Xic1, the proneural action of the bHLH genes seems dominant to the gliogenic activity of Notch activation in this system. It turns out, however, that these two findings are linked by the fact that Notch activation pulls the cells out of the cell cycle in this system just as effectively as p27Xic1, though the mechanism by which it does so is not yet clear (Bao and Cepko, 1997; Dorsky et al., 1997; Ohnuma et al., 2002; Scheer et al., 2001; Sriuranpong et al., 2001). As a result, if a precursor cell already expresses a proneural gene like Xath5, Notch activation may cause it to exit the cell cycle. Thus, the Notch pathway regulates neurogenesis early by helping precursors that express bHLH out of the cell cycle and regulates gliogenesis later by inhibiting proneural gene expression in cells that are starting to express high levels of p27Xic1.

Cell Cycle Phases and Neuronal Differentiation
We know that neuronal fates are often determined around their final cell cycle (Cremisi et al., 2003; Edlund and Jessell, 1999; Ohnuma et al., 2001), but what is less clear is that these determination events are often linked to specific phases of the cell cycle. This is illustrated in experiments where young cortical progenitor cells are transplanted into older animals. Young cells change their fate in accordance with the older environment, but only if they are transplanted at G1 or S phase. Cells transplanted at M phase retain their early fates (McCon-
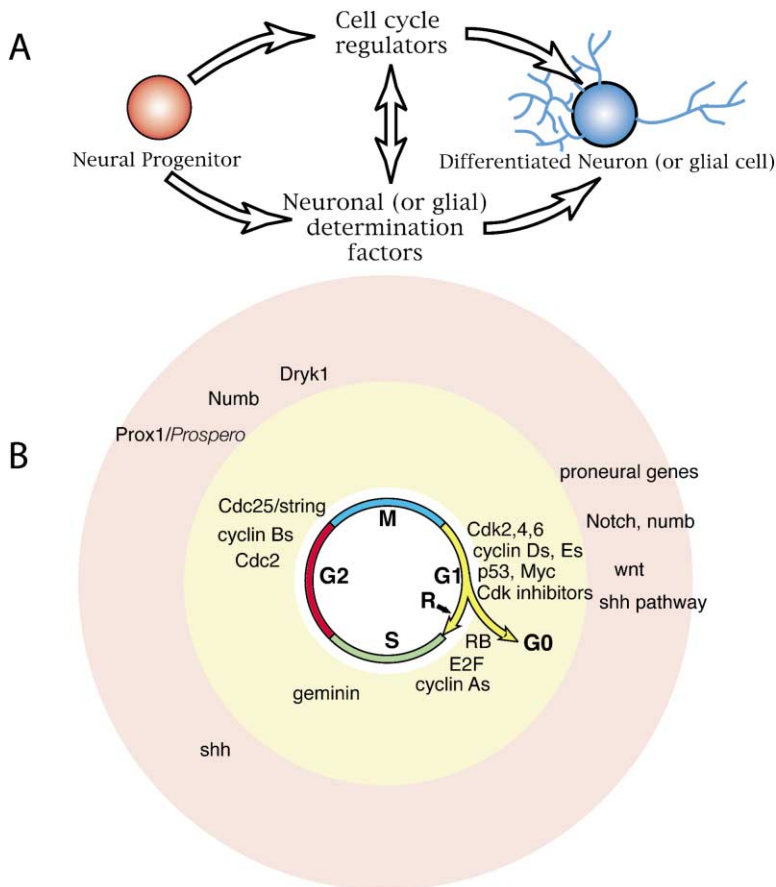
Figure 3. Interaction between Cell Cycle Regulation and Neural Cell Fate Determination

(A) The basic model that is most consistent with our present understanding is shown in this simple diagram in which cell cycle regulators affect the expression and function of neural determination factors and vice versa. (B) The cell cycle components that are mentioned in this review and which influence neurogenesis are shown at their approximate working positions (in yellow background) around a schematic drawing of cell cycle. The neural determination factors mentioned in this review that affect the cell cycle are indicated at their possible working points (in orange background). R means "cell cycle restriction point."

nell and Kaznowski, 1991). Retinal cells also lose their responsiveness to some extrinsic determinants as they enter M phase (Belliveau and Cepko, 1999). Intrinsic determinants may also be linked to specific phases of the cell cycle. For example, Prox1, a homeobox protein that is required for determination of horizontal cells in the vertebrate retina, is initiated and has its greatest expression at G2 (Dyer et al., 2003). Phase dependency may provide clues about the mechanisms that coordinate cell cycle exit with neural determination. For example, in S phase, chromosomal DNA is must be exposed by the removal of chromatin so that replication can take place. Thus, if chromatin remodeling is involved in neural determination, this phase might be more susceptible to transcriptional determinants than to other phases such as M phase, in which the chromatin is highly condensed. However, asymmetric divisions occur in M phase. Thus, where determination is regulated by asymmetric inheritance of determinants, M phase might be critical. Therefore, in the following paragraphs and Figure 3, we will review how some determination events may be linked to particular phases of the cell cycle during neurogenesis.

M Phase

At M phase, inherited determinants become localized to one pole of a mother cell undergoing asymmetric cell division. Asymmetric divisions of neural precursor cells have been extensively studied in fly neurogenesis (Chia and Yang, 2002; Doe and Bowerman, 2001; Jan and Jan, 2001; Lu et al., 2000; Matsuzaki, 2000). There are two basic points to be made here. The first is that the

basic cell cycle machinery is involved in setting up asymmetric divisions. For example, in *Drosophila string* mutants, the proteins Partner of Numb, Numb, and Prospero do not localize properly (Lu et al., 1999; Spana and Doe, 1995). This brings us to the second basic point, which is that the segregated determinants may affect both cell fate and further cell proliferation. Numb, a well-known antagonist of the Notch pathway (Guo et al., 1996; Spana and Doe, 1996), appears to be asymmetrically inherited in both *Drosophila* (Rhyu et al., 1994) and vertebrate (Cayouette et al., 2001; Silva et al., 2002; Wakamatsu et al., 1999; Zhong et al., 1996) neurogenesis. In humans, alternative splicing of Numb generates four different transcripts (Verdi et al., 1999). Two of these forms mediate neuronal cell fate choice, while the other two forms activate proliferation. Another molecule inherited asymmetrically is known as Dryk1, an ortholog of *Drosophila minibrain*, which is essential for the normal neuroblast proliferation (Tejedor et al., 1995). In chicks, Dryk1 is transiently expressed in neuroepithelial progenitor cells from M phase to G1 phase, and interestingly, the mRNA of Dryk1 is asymmetrically localized during mitosis to the differentiating rather than the proliferating lineage, leading to the proposal that Dryk1 defines a transition step between proliferation and differentiation in neuroepithelial cells (Hammerle et al., 2002).

G1 Phase

A key cell cycle restriction point is located at the end of G1 phase. If cells pass this point, they will almost invariably complete the cell cycle. This restriction point

is regulated by many G1 phase components, including cdk4, cdk6, cdk2, cyclin Ds, cyclin Es, cyclin As, RB, E2F, p53, and cdk inhibitors. In order to differentiate, cells need to leave the cell cycle in G1 and enter G0 without passing the cell cycle restriction point. This suggests that blocking the cell cycle in G1 by overexpression of certain cdk inhibitors should promote differentiation, while driving cells through G1 should inhibit differentiation. This prediction is borne out in experiments in the *Xenopus* retina. Here, blocking the cells in G1 by overexpressing the cdk inhibitor p27Xic1 potentiates the activity of proneural genes, while driving cells through G1 by overexpressing cyclin E1 reduces the activities of proneural genes (Ohnuma et al., 2002). Similarly in mammalian cells in culture, Cyclin D1 downregulates the transcriptional activity of the bHLH gene NeuroD (Ratineau et al., 2002), while Cyclin D3 directly regulates the transcriptional activity of the retinoic acid receptor α, which promotes neurogenesis (Despouy et al., 2003; Sharpe and Goldstone, 1997). In fact, nearly all the components of G1 cell cycle regulation have been reported to influence neural determination, and most studies agree that the factors that direct cell cycle arrest in G1 phase somehow also activate determination pathways (Carruthers et al., 2003; Dyer and Cepko, 2000b; Vernon et al., 2003; Zezula et al., 2001).

While many G1 phase cell cycle components affect determination, the reverse is also true, i.e., that many determination factors also affect the cell cycle at G1, allowing cells to take the G0 branch (Carey et al., 2002; Farah et al., 2000; Gallo et al., 2002; Geling et al., 2003; Hardcastle and Papalopulu, 2000; Insua et al., 2003; Li and Vaessin, 2000; Lin et al., 1998; Lyden et al., 1999; Wu et al., 2003). For example, overexpression of the proneural gene NeuroD2, Mash1, or neurogenin-1 can convert mouse P19 embryonic carcinoma cells into differentiated neurons. In this process, they induce the expression of p27Kip1 and cause cell cycle arrest in G1 (Farah et al., 2000). External neural determination and proliferation signals also appear to work through G1 cell cycle components. Wnt, for example, regulates the cell cycle by modulating the expression of cyclin D1, cyclin D2, and c-Myc (Baek et al., 2003; Kioussi et al., 2002), while Shh modulates the transcription of cyclin D1 and N-myc (Kenney et al., 2003; Oliver et al., 2003).

S Phase
The degree of condensation of chromatin changes during the cell cycle (Hakimi et al., 2002; Machida et al., 2001; Olave et al., 2002). In S phase, the chromosomes lose their histones and decondense to replicate their DNA, and then the chromosomes recondense, sometimes incorporating remodeling of the chromatin. Thus, neural development might be particularly susceptible to factors that affect chromatin remodeling. Indeed, several studies show that the chromatin-remodeling complex known as SWI/SNF plays a critical role in neural development. A dramatic example comes from studies of the Srg3 protein, a core subunit of SWI/SNF. Mice that are heterozygous for a mutant form of the *Srg3* gene show severe defects in neural proliferation and neural differentiation in association with exencephaly (Kim et al., 2001). Not only does SWI/SNF affect replication, but it may also interact directly with particular neural determination factors, as recent studies have shown that the remodeling protein CBF-1 recruits the SWI/SNF

complex to the promoters of Notch-regulated genes such as Hes1 and Hes5 (Kadam and Emerson, 2003). Interestingly, the SWI/SNF remodeling complex may also regulate cell division, as the tumor suppressors prohibitin and retinoblastoma appear to recruit SWI/SNF to E2F-dependent promoters and thus promote the action of these proteins in hanging cells up in G1 and leading to their differentiation (Martens and Winston, 2003).

Once assembled on the chromatin, histones may become acetylated, which encourages transcription of nearby genes. This epigenetic process is also critical for normal neural development, as shown, for example, by the knockout of Querkopf, a histone acetyltransferase. These mutant mice show abnormal cerebral cortex development including a reduction of Otx1-positive neurons (Thomas et al., 2000). These observations that chromatin structure is critical for normal neural determination are not necessarily very surprising. The idea that neural determination factors might affect chromatin structure is perhaps more so. The protein called Geminin, because of its dual function in neural determination and cell cycle progression, is a good example of this. In *Xenopus* and fly embryos, overexpression of geminin both (1) neuralizes ectoderm and induces expression of neural determination factors such as neurogenin-1 and (2) takes cells out of the cell cycle (Kroll et al., 1998; Quinn et al., 2001). Like p27Xic1, Geminin is a bifunctional molecule with separable domains for neuronal determination and cell cycle control (McGarry and Kirschner, 1998). Geminin, as it turns out, is a component of a prereplicative complex affecting chromatin condensation by modifying the binding of topoisomerase II with chromatin (Cuvier and Hirano, 2003; McGarry and Kirschner, 1998; Yanagi et al., 2002).

Staying Undifferentiated and in the Cycle—Neural Stem Cells
Mature neurons cannot re-enter the cell cycle, though no one quite knows why. Therefore, it is generally believed that most regions of the adult mammalian brain are unable to generate new neurons and that as a consequence, we are basically stuck with the neurons we are born with. These neurons have to last us for the rest of our lives, which is one of the key reasons why brain damage can cause such devastatingly long-term negative outcomes. However, it has been shown recently that undifferentiated neural stem cells exist in the adult mammalian nervous system (Gage, 2000) and that these cells can be activated to produce new neurons even in the adult. The majority of these stem cells are localized in specialized areas or niches such as the subventricular zone, the olfactory epithelium, the dentate gyrus of the hippocampus, and the ciliary marginal zone of the retina. Stem cells may divide very slowly in a symmetric manner (Sommer and Rao, 2002). Recent observations indicate that this slow cycling may be due to the low expression of cell cycle activators in stem cells. For example, in the CMZ of *Xenopus* retina, expression of the cell cycle activators cyclin A2, cyclin E1, cyclin D1, cdc2, and cdk2 is much lower in the stem cell population than in the quickly dividing early retinoblasts (Ohnuma et al., 2002). More intriguing still is the possibility that some mature glia may have stem cell-like properties. For example, in

the retina, Müller glial cells can re-enter the cell cycle and produce progeny that transdifferentiate into neurons (Reh and Levine, 1998). In a process called reactive gliosis, Müller glial cells re-enter the cell cycle after retinal injury. A key question is why these cells do not divide under normal circumstances. In this case, it appears to be high levels of cell cycle inhibitors. Adult Müller cells express high levels of p27Kip1, but upon reactivation, there is a downregulation of p27Kip1 and the cells move on to S phase. Consistent with this observation, mice lacking p27Kip1 showed a constitutive state of reactive gliosis (Dyer and Cepko, 2000a).

The localized distribution of stem cells suggests that the environment may be responsible in part for keeping stem cells in the cell cycle (though perhaps dividing only slowly) and maintaining their multipotency. Clues to what these environmental cues might be come from attempts to culture adult neural stem cells (Weiss et al., 1996). In high-density culture, FGF2 alone is sufficient to maintain neural stem cells (Gage et al., 1995; Gritti et al., 1996), although EGF also appears to promote neural stem cell proliferation (Martens et al., 2000). Interestingly, FGF2 and EGF also affect the cellular commitment, as FGF2 induces neurogenesis from gliogenic precursors and EGF converts neurogenic precursors to multipotent stem cells (Doetsch et al., 2002; Palmer et al., 1999). Notch signaling may also be involved in maintaining stem cells. Mice lacking RBP-Jk, an essential component of the Notch pathway, or presenilin1, a regulator of the Notch pathway, produce reduced numbers of multipotential neural stem cells (Hitoshi et al., 2002). This effect in presenilin1 knockout mice is rescued by overexpression of an active form of Notch1. Exactly how these factors and others, like Wnts and hedgehogs that have been implicated in this process (Ahlgren and Bronner-Fraser, 1999; Machold et al., 2003; McMahon and Bradley, 1990; Perron et al., 2003), maintain the neural stem cell fate is not understood, but it may have to do with their ability to regulate the regional expression of transcriptions factors such as *Emx2* (Ligon et al., 2003; Theil et al., 1999, 2002; Tole et al., 2000b). *Emx2* is expressed in adult stem cells in the telencephalon and the dentate gyrus (Galli et al., 2002; Pellegrini et al., 1996; Tole et al., 2000a). Inhibition of *Emx2* activity increases the population of symmetrically dividing stem cells, while activation of *Emx2* decreases this type of division (Galli et al., 2002). Sox2 is another such transcription factor that is expressed in neural stem cells. Constitutive expression of Sox2 results in the maintenance of the progenitor state, while inhibition of Sox2 causes neural progenitors to exit the cell cycle and differentiate (Graham et al., 2003).

Conclusions

In this review, we have highlighted some of the ways that the cell cycle is fundamentally linked with neural development and some of the molecular mechanisms that form the links. Understanding these links better may have relevance to human developmental diseases of the brain, many of which are associated with abnormal proliferation (Walsh, 1999). For example, Dyrk1A may be a causal factor in Downs' syndrome (Altafaj et al., 2001; Hammerle et al., 2003). Mutations in Six3 result

in holoprosencephaly (Roessler et al., 1996; Wallis et al., 1999), while defects of Emx2 and TSC2 cause schizencephaly and focal dysplasias (thickened and disordered structure) in the cortex, respectively (Brunelli et al., 1996; Consortium, 1993). Moreover, almost all signaling pathways involved in neural development are also implicated in tumorigenesis (Allenspach et al., 2002; Lustig and Behrens, 2003; Wetmore, 2003). It is therefore important to understand the links these pathways have to the cell cycle.

One of the important lessons we have learned from explorations into the cell cycle during neural development is that many cell cycle genes show restricted expression and affect cellular fate. Conversely, many neural differentiation factors regulate the cell cycle, both directly and indirectly. Indeed, there seem to be many complex relationships between cell cycle components and developmental factors, as one might imagine there would be in building an organ as complex and refined as the nervous system. We might be just scratching the surface of this issue now, but in the next few years we can hope that the underlying logic of the network that links the cell cycle to various aspects of neurogenesis will be elucidated.

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