Review

Cyclin-dependent Kinase Inhibitors in the Development of the Central Nervous System

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Introduction

The development of the nervous system requires cell proliferation, cell cycle exit, differentiation, and migration of progenitor cells in a highly coordinated manner. These decisions are made after the integration of extrinsic cues with the cell cycle machinery. Results from expression analyses and deletion experiments of various positive cell cycle regulators and cdks have revealed their key role in neural development and in the maintenance of quiescence in adults.

In this review, we will attempt to provide an update of what is known about the cdks in specific regions of the CNS across the phyla, including Caenorhabditis elegans, Xenopus laevis, Drosophila melanogaster, Mus musculus, and Rattus rattus and discuss the relation of cdks with cyclin-cdk complexes and their substrate, the pRb. The first part of this review will highlight the current understanding of the mechanics of the cell cycle, provide a brief background of nervous system development, and outline the normal patterns of expression of the various cdks. In the second part, we will address several key points about the functional role of cdks in CNS development. Do cdks participate in early patterning of the CNS in parallel with their roles in proliferation? How do cdks regulate rates of proliferation and expansion of the neural progenitor pool? And finally, can cdks and other cell cycle regulators function cooperatively to trigger cell cycle withdrawal and direct differentiation? Although we tried to be inclusive, we would like to apologize to all our colleagues whose work may have been omitted and that we failed to acknowledge.

Cell Cycle Machinery

In neural cells as in other somatic cells, proliferation and growth arrest are regulated by a balance of extrinsic and intrinsic signals that direct either entry and progression into or exit from the cell cycle. The mitotic cell cycle is composed of four phases: one dedicated to synthesis of the genomic DNA (S), one to mitosis (M), and two gap (G1 and G2) phases (Fig. 1A). The first gap phase, G1, occurs between the end of mitosis and S phase. During G1, critical decisions are made to either commit to another round of cell division, exit the cell cycle permanently, or transiently exit to a G0 phase. Once committed to a new cell cycle, cells require sustained stimuli to reach the restriction point (R; Ref. 1) after which they replicate their genome and progress through the first cell cycle until the next G1 phase. The second gap phase, G2, between the end of S phase and the beginning of mitosis, allows cells to repair replication errors and strand breaks made during DNA synthesis and prepare for mitosis (2). Progression through each phase of the cell cycle is governed by cdks, which are activated by phosphorylation/dephosphorylation events and binding to regulatory subunits or cyclins to form heterodimers (Fig. 1A; Refs. 3, 4).

Mitogen stimulation induces entry and progression through G1 in part by ligand binding to receptors, activating multiple signaling pathways that converge on the transcription of immediate early genes, D-type cyclins, and cyclin assembly to cycl4/6 kinases (5, 6). Activated cyclin D-cdk4/6 complexes preferentially phosphorylate pRb and pRb-related proteins p107 and p130 (7, 8). This initial pRb phosphorylation is followed by additional phosphorylation by the cyclin E-cdk2 holoenzyme (Fig. 1B; Ref. 9). Once phosphorylated, pRb and related proteins release tethered E2F transcription factors that, in complex with DP subunits DP1 and DP2, either activate (E2F-1, 2, and 3) or repress (E2F-4 and 5) gene transcription essential for the G1 to S phase transition and commitment to mitosis (10, 11). Continuous cyclin A-cdk2 activity is required during S and G2 phase, whereas mitosis requires cyclin B-cdk1 activation by the dual-specific phosphatase cdck5C (Fig. 1A; Refs. 2, 12).

Cyclin-cdk complexes are negatively regulated by small polypeptides, cdks. In mammals, cdks are divided into two families. The Ink4 family (so named because they inhibit cdk4/6) includes p16^ink4a, p15^ink4b, p18^ink4c, and p19^ink4d (13, 14). The Cip/Kip family comprises p21^cip1, p27^Kip1, and p57^Kip2 (14). Whereas Cip/Kip proteins act as negative regulators of cyclin E-and A-cdk2 and cyclin B-cdk1 holoenzymes in a 1:1 stoichiometry (15), they also act as positive regulators of cyclin D-cdk4/6 complexes by mediating their assembly early in G1 (Refs. 16, 17; Fig. 1, A and C). This results in the titration of p27^Kip1 from cyclin E-cdk2, activating this complex (14). In turn, p27^Kip1 is phosphorylated by cyclin E-cdk2, targeting it for degradation (18, 19). Instead, Ink4 proteins compete with D-type cyclins to bind cdk4/6 (20). This frees cyclin D that becomes targeted for degradation and extinguishes cdk4/6 activity (21) and allows the cells...
to proceed through S phase. Enforced expression of Ink4 proteins can lead to G1 arrest by preventing the redistribution of Cip/Kip proteins and blocking cyclinE/cdk2 activity (Fig. 1C). Thus, in cycling cells, there is a reassortment of Cip/Kip proteins between cdk4/6 and cdk2 as cells progress through G1 alternately acting as positive and negative regulators of cdk activity.

Although cdk5, a cdk family member, is essential for neurogenesis and regulates neuronal migration and laminar configuration of the cerebral cortex (22), its binding to D-type cyclins is unable to induce its kinase activity. Rather cdk5 interacts with p35 and p39 proteins to form active complexes that phosphorylate substrates to regulate cell migration (23, 24). The cdk5/p35 or p39 complexes are also regulated, albeit indirectly, by Cip/Kip family members (25). Cip/Kip proteins can bind to inactive cyclin D-cdk5 complexes but cannot bind to cdk5 alone (25). However, binding to p35 or p39 allows cdk5 to escape inhibition by Cip/Kip proteins by displacing them from the cyclin D-cdk5 complexes, unleashing its kinase activity (25). In contrast, Ink4s are unable to bind cdk5 (20, 25). A formal possibility is that Ink4 binding to cdk4 displaces cyclin D, which can then complex with cdk5, preventing its activation by p35 or/and p39. Hypothetically, this would inactivate both cdk4 and cdk5, leaving cdk2 active. However, it is not known whether cdk2 activity is required for neuronal viability.

All eukaryotes possess homologues of the mammalian cdkis, evidence that negative regulation of the cell cycle was established with the evolution of mitotic cell division. To date we know there are equivalents of the mammalian Cip/Kip family of proteins in C. elegans, cki-1, and cki-2 (26); Drosophila, Dacapo (27, 28); and Xenopus, p27Xic1 (29). However, the Ink4 family is less well described, if at all, in lower vertebrates with the exception of Fugu, where two Ink4 family members have been found.3 A complete evolutionary study is warranted to establish the origins of each of the mammalian cdkis, to demonstrate points of similarity and divergence in their sequences, and to better understand their function.

CNS Development

The development of the CNS occurs in four major phases (Fig. 2). The first specifies the site of the nervous system and biases the ectoderm toward a neural fate known as neural induction. The second phase, neuralation occurs followed by an expansion of the stem cells to generate a pool of neuronal progenitors. Next, the period of symmetric division progres-

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3 Mike Fried, Cancer Center, University of California, San Francisco, personal communication.
Patterning of the vertebrate axis is a critical first step in CNS development. Anterior–posterior and dorso–ventral patterning is designated by homeodomain and zinc finger transcription factors. Neural induction begins in a specialized region, Speamann’s organizer or Hensen’s node, at the anterior end of the dorsal surface of the embryo (Fig. 2). This organizer establishes both the site and axis of the future CNS. The signals that first induce the site of the organizer are called neural inductive proteins and include molecules such as noggin, chordin, follistatin, and likely other genes (30). Once induction begins, signaling is mediated by inhibition of the BMPs (31). Inhibition of BMP activity transforms ectodermal cells to a neural fate. The specification of the neural phenotype or neuralization occurs along a midline stripe on the dorsal surface of the embryo, in a rostro–caudal direction. This specification is coordinated by other molecules including fibroblast growth factor, wingless (Wnt/Wg), Notch, Sox, Pou, Fox, and proneural genes encoding proteins such as the bHLH transcription family (32). The CNS takes shape from a layer of neuroectoderm that invaginates to form a furrow and fuses along its midline to make the early neural tube (Fig. 2). At this time the neural tube is a simple one cell thick layer. This layer expands through successive rounds of symmetric cell divisions to provide a pool of neural progenitors for the future CNS.

Each region of the CNS is generated from a founding pool of stem cells that reside in a replicative region, the VZ, or similar specialized area. For the cerebral cortex, cerebellum, and retina, around mid-gestation the cells within the VZ progressively switch from symmetric to asymmetric cellular division. Careful regulation of the proliferative versus quiescent output is required to ensure an appropriate cell number (33). Neurons emerge before the major glial types, suggesting that a single stem cell becomes progressively restricted in its fate potential.

Retinal development in the mouse begins around E10.5 from paired outgrowths of neuroepithelium from the forebrain that invaginate to form the optic cups. Soon after the first retinal cells leave the cell cycle from the retinal VZ, they differentiate into one of the three early generated neurons: ganglion, horizontal, or cone photoreceptor cells (Fig. 3A). Later in embryogenesis, amacrine and bipolar cells are born, and after birth, rod photoreceptors and Muller glia are generated (Fig. 3A). By about P8 in the mouse, the adult retinal structure appears (Fig. 3A) divided into three major cellular layers: ganglion cell, inner nuclear, and outer nuclear layers (Fig. 3B).

Mouse cortical development begins around E11 when the anterior portion of the neural tube dramatically expands and divides into the paired telencephalic vesicles, forming the future cerebral hemispheres. Concurrent with the formation of the hemispheres, the cell cycle shifts from symmetric to asymmetric division with cells leaving the VZ, differentiating, and migrating to the cortical plate (Fig. 4). Subsequent cells are generated and migrate through cells generated previously, contributing to new layers. In this way the brain is built from the “inside out.” Cortical neurogenesis is completed within the embryonic period, with the generation of glial types continuing for the lifetime of the organism. By birth, the cerebral cortex is divided into five cellular layers and one fiber layer consisting of many neuronal and glial cell types (Fig. 4).

The cerebellar cortex is phylogenetically one of the oldest parts of the brain, in the adult consisting of three cellular layers (molecular, Purkinje cell, and internal granular layers)
with an additional layer present during development, the external granular layer (Fig. 5). The cells of the cerebellar cortex derive from two proliferative regions, the rhombic lip and the VZ of the fourth ventricle. The former generates the granule cells, and the latter gives rise to the Purkinje cells. In mice, Purkinje cells are first born at E11, whereas granule cells are generated up until about P12. Granule cells exit the rhombic lip from about E13.5 and migrate over the existing Purkinje cells to form the external granular layer. Around P7 in mice, granule cells exit the external granular layer, descending through the Purkinje cells and settling in the internal granular layer. At the end of the second postnatal week,
the mature cerebellar cortex is discernible with its simple trilaminar structure.

Expression Patterns of Cdk Inhibitors in the CNS
Most of our information about the pattern of expression of the various cdkis in the nervous system is confined to three major regions: the retina, cerebral cortex, and cerebellum. Little or no information is available for the other areas of the brain including the diencephalon, midbrain, brainstem, and spinal cord (Fig. 2). For this reason, only these three areas will be discussed.

cdkis in Retinal Development. Expression of Kip1 and Ink4d mRNA is first detected in the retina around E11 concurrent with the birthdates of early born ganglion, horizontal, and cone cells4 (Ref. 34; Fig. 3A). Both p27Kip1 and Ink4d are present within the retina up until P5, after which their respective expression patterns dramatically change. This change occurs simultaneously with the cessation of retinal neurogenesis and the generation of the definitive inner and outer nuclear layers. At this time, expression of p27Kip1 is restricted to Muller glia, the last cell type to be born (34, 35), whereas Ink4d is restricted and maintained in regions consistent with horizontal, amacrine, and ganglion cells (Fig. 3B).4

In contrast, p57Kip2 has an unusual bimodal pattern of expression (36, 37) that does not overlap with p27Kip1 (38). It is first detected from E14.5 until just before birth in progenitor cells migrating away from the retinal VZ, after which it is turned off. This is the same period during which amacrine cells are born. At P3, p57Kip2 expression reappears and is restricted to a discrete sublineage of amacrine cells until adulthood (Ref. 36; Fig. 3B).

Of the remaining cdkis, Cip1 and Ink4b have not been detected in the retina and have only been reported in the lens (39) and cornea (40), respectively. To date, neither Ink4c or Ink4a have been examined in retinal development, although individual cdkis need not be ubiquitously expressed in all of the tissues.

cdkis in Cortical Development. Most of the cdkis are detected during development of the cerebral cortex (41, 42), although little is known about their changing expression patterns and the identity of the cells in which they are localized. Ink4c is the only cdki restricted to the dividing progenitor pool in the VZ, first appearing at E11 with the onset of asymmetric division, down-regulated at birth, and not detected in adulthood (Ref. 42; Fig. 4). Ink4d is expressed at a similar time as Ink4c, although only in cells exiting the cell cycle (42, 43). Once cells exit the cell cycle and migrate out of the VZ, p19Ink4d is down-regulated and is then re-expressed as cells approach their appropriate cortical layer, terminally differentiate, and become quiescent (Ref. 43; Fig. 4). Expression of Kip1 mRNA appears to overlap Ink4d. However, Kip1 expression is higher in cells exiting cycle in the VZ, whereas Ink4d is higher in differentiating populations in the expanding cortical plate (Refs. 25, 41, 44; Fig. 4). Despite their apparent overlapping patterns, careful immunohistochemical analysis has not been conducted to confirm that both cdkis are present in the same cells, limited by the availability of reagents.

Kip2 mRNA is present at lower levels in the VZ as cells exit the cell cycle but is very strongly expressed in the developing cortical plate up until birth and not at all in adulthood (Ref. 41; Fig. 4). In contrast, Cip1 mRNA is not detected in the cortex.

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(25) but is restricted to the olfactory bulb and ependymal cells that line the ventricles and the choroid plexus (41). Expression of Ink4a mRNA has also been reported in the developing cortex (41); however, immunohistochemical analysis is needed to definitively confirm p16Ink4a protein expression in the cortex.

**cdkis in Cerebellar Development.** From P5 to adulthood Ink4d, p16Ink4a, and p27Kip1 are present within granule cells exiting the cell cycle in the external granular layer and are sustained in these cells when they adopt their mature position in the internal granular layer (Refs. 45, 46; Fig. 5). These proteins are also expressed in Purkinje cells, although there is some dispute as to whether this is true for p27Kip1, with one study unable to corroborate this finding (46). The reported differences in p27Kip1 expression may be attributed to discrepancies in the antibodies used. Purkinje cells also express p21Cip1 during the postnatal period (Fig. 5), suggesting that p27Kip1 and p21Cip1 may cooperate to keep this major cell type in a state of quiescence (47).

Both p15Ink4b and p18Ink4c are also detected in the cerebellum, but the identity of the cells in which they are expressed is uncertain (Ref. 45; Fig. 5). However, p57Kip2 has not been examined in the cerebellum, but this does not preclude its involvement in cerebellar development.

**Functions of cdkis in the Developing CNS**

**Role of Cell Cycle Regulators in Patterning, Induction, and Expansion of the Neuroectoderm.** Although cdkis have not been detected during patterning and neural induction, their activity is required for release of tethered E2Fs that have been implicated in these early developmental events. Recently, elegant studies by Suzuki and Hemmati-Brivanlou (48) in Xenopus and by Myster et al. (49) in Drosophila show that E2F acts upstream of early ventro–posterior patterning genes. Enforced expression of Xenopus E2F, XE2F, induces expression of homologues of an homeodomain, Hox3, and a zinc finger transcription factor, Krox20, (48). In contrast dominant negative XE2F results in the ectopic expression of dorsal markers including the general neural marker, neural cell adhesion molecule (48). These results suggest that XE2F regulates patterning of the ventral mesoderm and hindbrain by actively repressing the expression of dorsal-specifying genes.

In Xenopus, specification of the neuroectoderm and proliferation of neural progenitors occurs simultaneously (50). The same is likely to be true in higher vertebrates. Whereas neural induction is not dependent on cellular division (51), coordination of the two events facilitates the proper development of the CNS. It is uncertain how these events are linked, because few molecules have been shown to regulate both neural induction and cell cycle progression. Recently Hardcastle and Papalopulu (52) provided the first evidence that p27Xic1, a Xenopus Cip/Kip, acts as a downstream target of neural inductive signaling by XBF1, a forkhead transcription factor. In these experiments, high doses of XBF1 suppressed p27Xic1 expression and promoted neuroectodermal proliferation, whereas low doses induced p27Xic1 expression, exit from the cell cycle, and differentiation of ectopic neurons (52). It is important to note that misexpression of p27Xic1 did not induce the production of ectopic neurons, clearly indicating that cdki expression alone is not sufficient to induce neuron differentiation. It is uncertain how XBF1 regulates p27Xic1 expression, but some clues might come from the Drosophila system, where patterning genes like Cut regulate the transcription of p21Cip1.

The Cut transcription factor is conserved from flies to humans and, like the homeotic patterning genes Hom and Hox, contains a DNA-binding homeodomain (53). In flies, Cut demarcates the peripheral wing axis specifying chemosensory and mechanosensory organs along the wing margin and is necessary for the development of sensory organs in the peripheral nervous system (53). Little is known of the function of Cut in vertebrate and particularly mammalian development. However, a recent functional study in NIH 3T3 cells suggests that Cut acts downstream of Cdc25A to initiate S phase entry by repressing transcription of p21Cip1 (54). Cut does this by binding to the p21Cip1 proximal promoter or TATA box, blocking transcription of p21Cip1, and promoting cellular proliferation (54). In this way, cdkis like p21Cip1 are not deterministic of the neuronal phenotype but participate in the expansion of the neuronal population.

bHLH transcription factors and a variety of other genes including Notch play key regulatory roles in specifying the neuronal phenotype. Normally Notch activation suppresses neuronal determination favoring glial fates, but there are cases where this is not strictly true. For example, in Drosophila wing bud, Wingless and Notch induce the pan-neural bHLH gene Deadpan to down-regulate expression of DE2F and DCDc25, arresting cells in G1 and G2 (55). Similarly, in Drosophila eye development, Notch signals proneural bHLH genes achaete and scute to synchronize G2 and M phases by down-regulation of the cdc25 phosphatase (56). This synchronization is necessary for the normal posterior to anterior progression of the eye morphogenetic furrow. Disruption in this synchronization results in defects in neural patterning in the eye (56). Similarly, in the Drosophila optic lobes, changes in the expression of the bHLH transcription factors, Deadpan and Asense, play opposing roles to alter proliferation rates (57). Deadpan and Asense mediate proliferation in part by changing the expression of Dacapo, the Drosophila Cip/Kip homologue (57). In these examples, genes inducing a neural phenotype alter rates of proliferation by mediating the expression of cell cycle genes, including DE2F, DCDc25, and Dacapo, respectively.

Like Notch, BMPs normally antagonize neurogenesis. However, in the rat rostral migratory stream, retroviral-mediated expression of wild type BMP receptor 1a triggers SVZa cells to exit cell cycle without inhibiting their neuronal commitment. Consequently, expression of BMP levels up-regulate p16Ink4a protein levels (58). This is the first mammalian example of a neural repressing molecule (BMP), inducing cell cycle exit via the up-regulation of a cdki.

Thus, in several model organisms, inhibitors and activators of cell cycle checkpoints are regulated by upstream effectors specifying patterning and neuroectodermal proliferation. Given the high degree of conservation of cell cycle regulators during evolution, it is likely that such regulation will also be the rule rather than the exception in mammals.
Requirement for cdkis in Asymmetric Division and Lengthening of the Cell Cycle. The onset of asymmetric division requires cdki function to force cells to exit the cell cycle and differentiate. In the mouse neocortex, asymmetric division begins on E11 and continues until shortly before birth. A concomitant lengthening of the cell cycle occurs with times increasing from 8.1 h (E11) to 18.4 h (E17; Ref. 59). This increased cell cycle time is primarily attributed to an increase in the length of G1 (59). The cues that signal the transition from symmetric to asymmetric division and the lengthening of G1 are likely to be controlled by G1 regulators. Ink4c and Ink4d are expressed during neurogenesis and, thus, are well positioned to regulate this cellular transition. Whereas Ink4d is expressed preferentially in postmitotic neurons, Ink4c is exclusively expressed in proliferating cells of the VZ during asymmetric division (42). These expression patterns suggest that Ink4c participates in the switch from symmetric to asymmetric division, although the mechanistic basis for this transition is uncertain. Despite the fact that neither Ink4c-null nor Ink4c/Ink4d-double-null adult mice have no obvious neural phenotype,5 neural cells may have altered cell cycle length, an hypothesis that will require closer examination of these animals. More recently, enforced expression of p27Kip1 was shown to lengthen G1 phase in the developing mouse neocortex, although it was unable to increase the length of G1 beyond a physiological maximal time suggesting possible collaboration between p27Kip1 and other cell cycle regulators (60).

cdkis and Control of Neural Terminal Cell Cycle Exit, Differentiation, and Maintenance of Quiescence. Neuronal, like somatic cells, must exit the cell cycle, differentiate, and maintain a state of quiescence. However, unlike somatic cells, neuronal cells permanently exit the cell cycle. How do cdkis participate in the pathways leading to these decisions?

Several lines of evidence suggest that cdkis function only to regulate cell cycle arrest and withdrawal. In the eye of invertebrates like Drosophila, overexpression of either Dacapo or human Cip1 triggers cells to prematurely withdraw from cell cycle, whereas deletion of Dacapo promotes additional cellular divisions (61). Regardless, altered expression of Dacapo still permits cellular differentiation although the proportion of cell types is disrupted. Dacapo might be part of a mechanism that monitors daughter cell production, regulating final numbers. Similarly, in the mouse retina, cerebellum, and inner ear, targeted deletion of Kip1 causes hyperproliferation of specific cell lineages without affecting differentiation patterns (34, 45, 46, 62–64). Likewise, oligodendrocyte progenitors deleted for p27Kip1 continue to proliferate (65). Ectopic expression of Kip1 arrests cultured progenitors despite elevated mitogen levels but fails to trigger differentiation into mature oligodendrocytes unless mitogens are withdrawn (65–67).

p27Kip1 cooperates with p19ink4d to maintain neuronal quiescence independent of differentiation, and both are required to regulate exit from the cell cycle. It is only after both cdkis are deleted that proliferation continues inappropriately in the cortex (68), retina,4 and the inner ear.6 This mouse model provides the most compelling evidence that blocking activity of both G1 and S phase kinases is critical to ensure permanent cell cycle exit and maintenance of long term quiescence.

Maintenance of quiescence in neurons is crucial for the stability of the complexly wired brain. In the cortex and retina, maintenance of cellular quiescence is in part ensured by the sustained expression of Ink4d-(42). Retina from mice deficient for Ink4d exhibit prolonged cellular division that is compensated by apoptosis, suggesting that continued expression of Ink4d might function to guard against inappropriate cell cycle entry.4 Similarly, maintenance of Ink4d expression is required for inner ear hair cell viability and quiescence, because Ink4d-null mice are severely hearing impaired because of progressive loss of hair cells.9 Mature Ink4d-null animals show no apparent change in the architecture of the brain or retina compared with wild-type mice, suggesting that deletion of p19ink4d does not affect retinal differentiation patterns.

Expression analyses clearly demonstrate the presence of cdkis during neuronal development. However, it is uncertain whether these cdkis instruct cell fate or set the proper phase during which cells respond to signals to specify differentiation. An elegant study by McConnell and Kaznowski (69) provided the first example that the fate of cortical neural cells was specified some time before terminal cell cycle exit. They extracted early embryonic cortical cells from ferrets, established these cells in culture and synchronized their cycles, and transplanted them into the brains of older ferret embryos. Cells transplanted shortly after having completed their final round of mitosis migrated to layer six, consistent with the fate of cortical progenitors normally exiting the cell cycle in late embryogenesis. In contrast, cells transplanted in S phase migrated to ectopic positions in cortical layers two and three. Because of this work, other studies have suggested that cell cycle proteins actively participate in cellular differentiation.

Recently, Ohnuma et al. (29) proposed that p27Xic1, the Xenopus homologue of p27Kip1, could serve a novel function in directing retinal Muller glial cell fate. Overexpression of p27Xic1 favors Muller glial fate at the expense of the formation of bipolar cells. Deletion constructs made from the NH2-terminal domain of p27Xic1 showed that one region between residues 31 and 91 had both Muller glia-inducing and cdk2-inhibitory activity, suggesting there were separate regions to regulate cell cycle exit or differentiation (29). Cell specification involving p27Xic1 might operate via Notch/Delta signaling, a pathway that is normally associated with the inhibition of neural differentiation (29). Despite these results, it is still uncertain whether p27Xic1 or any other cdkis regulate the expression of Notch or Delta.

Like p27Xic1, the mammalian p57Kip2 is thought to play dual roles in retinal development, arresting cells to exit the


cell cycle during embryogenesis and specifying a novel subset of amacrine cells postnatally (36, 37). Targeted deletion of p57^Kip2 leads to the loss of this lineage, suggesting that p57^Kip2 might affect fate determination of a particular subgroup of amacrine cells (36, 37). However, given that Kip2-null mice exhibit increased apoptosis, an alternative interpretation might be that p57^Kip2 induces cell cycle arrest of a subset of amacrine progenitor cells. Its deletion would result in progressive death of these progenitors before the differentiation of the subsequent cell lineage.

The signals that trigger a cell to leave the cell cycle are dependent on an interplay between cell autonomous pathways and extracellular cues. Recent evidence from the developing cortex suggests that p57^Kip2 is part of a signaling cascade inhibiting progenitor proliferation. In this tissue, pituitary adenylate cyclase-activating polypeptide is a potent antimitogenic signal (70, 71) that regulates cortical cell cycle exit by promoting expression of p57^Kip2. p57^Kip2 then forms complexes with cyclin-E-cdk2 and inhibits S phase entry. 7

Secreted growth factors like fibroblast growth factor (72), TGFβ (73, 74), and neurotrophic factors (75–77) can modulate the expression and activity of cdkis. The same exogenous proteins are also linked to roles in cellular differentiation. It is possible that by gating the time for cell cycle exit, cdkis might passively direct cellular fate by deciding the time of action of neural- or glial-specifying genes (78, 79). To date, experiments in many animals models favor a role for cdkis in triggering cell cycle exit and maintenance of the quiescent state rather than being deterministic for differentiation. It is likely that, as the expression pattern and the upstream regulators of cdkis is evaluated in different cellular contexts, this prediction will or will not be confirmed.

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