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Cell-Context Specific Role of the E2F/Rb Pathway in Development and Disease

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Abstract

Development of the central nervous system (CNS) requires the generation of neuronal and glial cell subtypes in appropriate numbers, and this demands the careful coordination of cell-cycle exit, survival, and differentiation. The E2F/Rb pathway is critical for cell-cycle regulation and also modulates survival and differentiation of distinct cell types in the developing and adult CNS. In this review, we first present the specific temporal patterns of expression of the E2F and Rb family members during CNS development and then discuss the genetic ablation of single or multiple members of these two families. Overall, the available data suggest a time-dependent and cell-context specific role of E2F and Rb family members in the developing and adult CNS.

Keywords

proliferation; differentiation; glia; cell cycle; tumor; brain

INTRODUCTION

Proper development of the central nervous system (CNS) requires the coordinated generation of an appropriate number of neuronal and glial cells. This can only be achieved when proliferation, differentiation, and survival are tightly coordinated. The decision of a cell to enter into the replicative phase of DNA synthesis or stop proliferating is dependent on the presence of signals from the environment that activate signal transduction pathways and converge on the E2F/Rb molecular switch. Extracellular signals also affect cell fate, survival, and differentiation into neurons, oligodendrocytes, or astrocytes. The E2F/Rb pathway, originally characterized for its ability to modulate cell-cycle entry, has also been involved in regulating survival and differentiation of distinct cell types at precise developmental time points and this is the focus of this review.

The E2F family of transcription factors (see Fig. 1), originally named because of their ability to bind to the E2 gene promoter of adenovirus (Kovesdi et al., 1986; Reichel et al., 1987), positively and negatively regulate the expression of genes necessary for G1-S phase transition as well as many other genes necessary for cell cycle progression [reviewed in Dimova and

Dyson (2005)]. Classical E2F family members (E2F1-E2F6) form heterodimers with DP proteins and bind to DNA with high affinity (Bandara et al., 1993; Helin et al., 1993). The regulatory nature of the E2F complexes is contingent upon the ability of these transcription factors to interact with pocket proteins. Hypophosphorylated pocket proteins (Rb, p107, and p130) are able to bind E2Fs (E2F1-E2F5) (Ginsberg et al., 1994; Hijmans et al., 1995; Lees et al., 1993; Sardet et al., 1995), sequester their transactivation domain (Helin et al., 1993), and recruit chromatin modifiers that repress gene transcription [reviewed in Blais and Dynlacht (2007)]. In contrast, hyperphosphorylated pocket proteins are unable to interact (Qian et al., 1992; Suzuki-Takahashi et al., 1995; Zhu et al., 1995a) and the released E2Fs activate gene transcription by forming complexes with chromatin modifiers (DeGregori et al., 1995a; Rayman et al., 2002). Overall, phosphorylation of the pocket proteins provides a molecular switch to control the transcriptional nature of the E2F complexes, which the cell uses to control the expression of genes necessary for cell-cycle entry and progression.

During cell-cycle progression, the phosphorylation of the pocket proteins is mediated sequentially by different cyclin and cyclin-dependent kinase (CDKs) complexes (cyclinD-CDK4, CyclinD-CDK6, Cyclin E-CDK2, Cyclin A-CDK2, and CyclinB-CDK1) (Hannon et al., 1993; Hu et al., 1992; Kato et al., 1993; Li et al., 1993; Mayol et al., 1995; Xiao et al., 1996) in a temporal-specific manner (Lees et al., 1992). CDKs are the catalytic subunit of enzymes that are positively regulated by cyclins and negatively regulated by CDK inhibitors (CKIs). CKIs can be divided into the Cip/Kip (p21, p27, and p57) and Ink4 (p15, p16, p18, and p19) families, whose expression is tightly coordinated with the proliferative state of the cell (Sherr and Roberts, 1999, 2004). By sequentially modulating the levels of cyclins and CKIs, cells respond to environmental signals and either progress through checkpoints and proliferate or withdraw from the cell cycle. For example, in response to mitogens, cells upregulate the levels of cyclin D (Winston et al., 1996). Cyclin D forms complexes with CDK4 and CDK6 and phosphorylates pocket proteins (Kato et al., 1993; Mayol et al., 1995; Xiao et al., 1996), with consequent release of E2Fs. The free E2Fs are then able to form activating complexes that promote transcription of genes necessary for the G1/S transition and proliferation (DeGregori et al., 1995b; Mateyak et al., 1999; Xiao et al., 1996). Interestingly, the pocket proteins p107 and p130 can also act as CDK inhibitors by directly binding to cyclin-CDK complexes (Woo et al., 1997; Zhu et al., 1995a), whereas the CDKs can inhibit E2F DNA-binding activity by phosphorylating the E2Fs themselves (Dynlacht et al., 1997). Thus, cell-cycle entry or withdrawal is modulated by E2F transcriptional activity, which is dependent on the stoichiometry of negative and positive regulators. The intricacy of the system is further complicated by the existence of several E2Fs and pocket protein family members, with the ability to form repressive or activating complexes with chromatin modifiers. Additional complexity arises from the fact that each member of the E2F or pocket protein family is characterized by a specific cellular and temporal pattern of gene expression, and some E2Fs have the ability to modulate the levels of other members as well as p107, p21, and Cdk1 (Furukawa et al., 1999; Hurford et al., 1997; Iavarone and Massague, 1999; Johnson et al., 1993; Neuman et al., 1994; Smith et al., 1998; Takahashi et al., 2000; Tommasi and Pfeifer, 1995; Wu et al., 2001; Zhu et al., 1995b). In this review, we shall first introduce the cell-specific temporal pattern of expression and then analyze the phenotype in the CNS of knockout animals lacking single or multiple E2F (Table 1) and Rb family members to better understand their individual role in proliferation, survival, and differentiation in the nervous system.

THE E2F AND POCKET PROTEIN FAMILIES

E2Fs Bind to DNA

E2F1 was originally identified as a transcription factor, which activates and binds to the promoter of the adenovirus gene E2 (Kovesdi et al., 1986; Reichel et al., 1987). Later, it was discovered that it could also interact with Rb (Bagchi et al., 1991; Bandara et al., 1991;

Chellappan et al., 1991; Chittenden et al., 1991), and this encouraged an extensive search for homologues and the definition of E2F function. Nine members of the E2F family were identified, but only E2F1-5 were able to interact with the pocket proteins [reviewed in van den Heuvel and Dyson (2008)] (Fig. 1A). All the E2F proteins shared a highly homologous DNA-binding domain with affinity for the GC rich DNA sequence 'TTTCGCGC' (Bieda et al., 2006; Kovesdi et al., 1986; Yee et al., 1987). The nine members of the E2F family (van den Heuvel and Dyson, 2008) were classified into two broad categories: *activators*, which recruit activating complexes to chromatin and are released when Rb is hyperphosphorylated; and *repressors*, which bind to Rb family members (Fig. 1B) and recruit repressive complexes to chromatin [reviewed in (DeGregori and Johnson, 2006; Trimarchi and Lees, 2002)]. Thus, it was hypothesized that E2F complexes could either activate or repress cell-cycle genes (Muller, 1995). Indeed, several genes were identified as being regulated by only E2F activators or repressors [reviewed in Blais and Dynlacht (2004)]. However, ChIP-on-Chip studies revealed large groups of genes that were coregulated by both E2F activators and repressors (Ren et al., 2002; Xu et al., 2007) and suggested a reciprocal modulation of gene expression by distinct E2F family members. For instance, E2F activators and repressors were shown to be alternatively and sequentially recruited to S-phase genes during distinct stages of the cell cycle (Takahashi et al., 2000; Wells et al., 2000).

The E2F Family of Transcriptional Activators

The initial hint regarding the function of E2F1, E2F2, and E2F3 came from the ability to activate E2 gene transcription (Kovesdi et al., 1986; Reichel et al., 1987) and the observation that their levels increase upon cellular proliferation (Hsiao et al., 1994; Johnson et al., 1994; Leone et al., 1998; Neuman et al., 1994; Sears et al., 1997). The E2F1, E2F2, and E2F3 family members contain a transcriptional activating domain at the C-terminus (Helin et al., 1993; Lees et al., 1993) and promote cell-cycle entry by recruiting acetyl transferases to activate the expression of S-phase genes (Takahashi et al., 2000; Trouche et al., 1996). Overexpression of any members of this subgroup induced cell-cycle entry (DeGregori et al., 1995a,b; Johnson et al., 1993; Lukas et al., 1996; Mann and Jones, 1996; Schwarz et al., 1995), whereas deletion of all the E2F activators resulted in complete loss of cellular proliferation in mouse embryonic fibroblasts (MEFs) (Wu et al., 2001). Together, these findings supported the concept that the function of E2F1, E2F2, and E2F3 transcriptional activators is to induce cell proliferation (DeGregori et al., 1997; Johnson et al., 1993).

The discovery of two splice variants of the E2F3 gene, E2F3a, and E2F3b, however, added complexity to the model and raised the possibility that these two variants might play distinct regulatory roles (Leone et al., 2000). The differential expression of E2F3a only in proliferating cells in contrast to the ubiquitous expression of E2F3b (Leone et al., 2000) suggested that possibly only one of the two could be involved in cell proliferation (Tsai et al., 2008). Indeed, MEFs lacking E2F1, E2F2, and E2F3a, but still expressing E2F3b, exhibited severely defective proliferation (Tsai et al., 2008). Intriguingly, however, the expression of E2F3b protein or E2F1 protein from the E2F3a promoter rescued the phenotype of E2F1^{-/-}E2F3a^{-/-} mice (Tsai et al., 2008). These results were consistent with the biochemical data suggesting that both E2F3 splice variants function as transcriptional activators (Chong et al., 2009) and led to the model of proliferation resulting from the temporal and spatial integration of total E2F activator activity, rather than from the expression of single isoforms or spliced variants.

The E2F Family of Transcriptional Repressors

In contrast to the E2F activators, the expression of E2F4 or E2F5 was not linked to the proliferative state of the cell (Ikeda et al., 1996; Moberg et al., 1996), their overexpression was not sufficient to overcome cell-cycle arrest and induce proliferation (Mann and Jones, 1996) and MEFs lacking E2F4 and E2F5 were unable to exit the cell cycle in response to growth-

arrest signals (Bruce et al., 2000; Gaubatz et al., 2000). This cumulative evidence led to propose a model for E2F4 and E2F5 as negative regulators of cell-cycle entry.

Although the C-termini of E2F1-5 proteins share the pocket protein-binding domain, their individual interaction with distinct members of the pocket protein family is specific (see Fig. 1B). E2F1, E2F2, E2F3a, and E2F3b interact exclusively with Rb, which sequesters their activation domain (Bagchi et al., 1991; Chellappan et al., 1991; Lees et al., 1993; Leone et al., 2000). The repressor E2Fs (E2F4 and E2F5), in contrast, bind to p107 and p130, although E2F4 can also bind to Rb (Beijersbergen et al., 1994; Ginsberg et al., 1994; Hijmans et al., 1995; Vairo et al., 1995) and mediate transcriptional repression by direct recruitment of chromatin-modifying enzymes such as HDACs (Brehm et al., 1998; Ferreira et al., 1998; Luo et al., 1998; Magnaghi-Jaulin et al., 1998), Suv39H1 (Ait-Si-Ali et al., 2004; Nielsen et al., 2001), and HP-1 (Panteleeva et al., 2007). The E2F6, E2F7, and E2F8 proteins mediate transcriptional repression but do not bind to the pocket proteins (de Bruin et al., 2003; Di Stefano et al., 2003; Maiti et al., 2005; Trimarchi et al., 1998). E2F6, for instance, associates with polycomb group proteins to repress Hox genes (Courel et al., 2008; Storre et al., 2002; Trimarchi et al., 2001). E2F7 and E2F8 form homodimers and heterodimers with one another and repress genes involved in proliferation such as *E2F1* (Christensen et al., 2005; Di Stefano et al., 2003; Zalmas et al., 2008). Because the expression of E2F6, E2F7, and E2F8 in the adult brain is much lower than in any other tissue (de Bruin et al., 2003; Maiti et al., 2005; Trimarchi et al., 1998), they will not be further discussed.

The Rb Family of Pocket Proteins

The retinoblastoma gene (*Rb*) was initially discovered, because its mutation was associated with high frequency of familial retinoblastoma, a childhood tumor of the retina (Dunn et al., 1989; Friend et al., 1986; Knudson, 1971; Richter et al., 2003). Heterozygous individuals were highly predisposed to develop retinoblastoma. Since the discovery of the Rb protein (pRb), two homologous proteins were described (i.e., p107 and p130/Rb2), jointly, they were called “pocket proteins,” because they each contain a conserved pocket domain, which binds to viral proteins and E2Fs. Although the pocket proteins are highly homologous to one another, their functions appear to be distinct, with p130 protein being expressed in differentiating cells and p107 in proliferating precursors (Cobrinik, 2005; Cobrinik et al., 1993; Lees et al., 1992).

EXPRESSION PATTERNS OF E2F AND POCKET PROTEIN FAMILY MEMBERS IN THE NERVOUS SYSTEM

The biochemical analysis of E2F and pocket protein family members and the analysis of the properties of MEFs isolated from single or compound mice provided clues regarding the role of E2F/Rb pathway in the CNS, however, it was conceivable that individual members of these two families could uniquely contribute to distinct cell lineages. The concept of lineage-specific effects of E2F or pocket protein family members was nicely introduced by the characterization of the hematopoietic system in knockout mice for specific E2Fs or pocket proteins. Both E2F1 and E2F2, for instance, had been shown to be important for T-cell development, but the phenotype of single knockout mice suggested a role for E2F1 in regulating proliferation of mature T-lymphocytes and tumor formation (Field et al., 1996) and for E2F2 in regulating proliferation of immature T-lymphocytes and autoimmune diseases (Murga et al., 2001). Lineage specificity was also suggested by studies on p107 or its binding partner E2F4. Although they were both involved in the development of the hematopoietic lineage (Humbert et al., 2000; LeCouter et al., 1998a; Rempel et al., 2000), the loss of p107 resulted in myeloid hyperplasia (LeCouter et al., 1998a), whereas loss of E2F4 was associated with immature erythroid, myeloid, and B-cell lineages (Gaubatz et al., 2000; Humbert et al., 2000). To better understand the contribution of E2F and pocket protein family members in CNS development,

we shall first review their expression pattern and then analyze the phenotypes of the knockout mice in the CNS.

E2F Family Members in Neurons and Glia

All E2F proteins are expressed during the embryonic development of the CNS (Dagnino et al., 1997; Kusek et al., 2001; Li et al., 2008; Storre et al., 2002). *In situ* hybridization studies revealed that transcripts for E2F1, E2F2, and E2F5 were only highly expressed in regions enriched in proliferating progenitors, such as the neuroblastic layer of the retina and the ventricular zones of the neuroepithelium and spinal cord (Dagnino et al., 1997). The expression of E2F3 and E2F4 was more widespread, and their transcripts were detected in both proliferative and nonproliferative regions (Dagnino et al., 1997; Ruzhynsky et al., 2007). This differential pattern of expression suggested a role for E2F1, E2F2, and E2F5 in regulating cell-cycle entry and for E2F3 and E2F4 family members in lineage commitment or differentiation. Western-blot analysis using mouse brain lysates during development revealed the decrease of the major E2F and the existence of several isoforms whose precise role in the CNS remains undetermined (Kusek et al., 2001). In a pheochromocytoma line (PC-12 cells), an *in vitro* model for neuronal differentiation the levels of E2F1, E2F3, and E2F5 were also downregulated upon differentiation and E2F4 expression was detected only in differentiated cells (Persengiev et al., 1999). Downregulation of E2F1 transcripts and protein levels was also observed in differentiating cerebellar granule neurons (Panteleeva et al., 2007) and in oligodendrocyte progenitors induced to differentiate by thyroid hormone (Nygard et al., 2003). Altogether, these results suggest that downregulation of E2F1 is an event that is observed during differentiation of specific subpopulations of neurons and glial cells in the developing CNS.

Pocket Protein Family Members in Neurons and Glia

Interestingly, the expression of Rb and other members of the pocket protein family in the developing nervous system did not precisely map the distribution of the E2Fs, and distinct members of the family were expressed in different brain regions at distinct developmental times. *In situ* hybridization of mouse brains, detected Rb expression in the epithelium of the neural tube starting at day E8 (Jiang et al., 1997). At E10.5–E11.5, Rb was detected in the proliferative ventricular zone and in the postmitotic intermediate zone of various brain regions, including telencephalon, mesencephalon, hindbrain, and spinal cord (Jiang et al., 1997). *In vitro* Rb expression in differentiating P19 embryonic carcinoma cells correlated with the initial expression of neuronal traits (Slack et al., 1993), whereas P107 was exclusively detected in uncommitted proliferating cells and the levels of Rb and p130 protein levels progressively increased (Callaghan et al., 1999). In agreement with these findings, western blot analysis of mouse brain lysates at E10, E15, P1, and adulthood revealed a progressive decrease of p107 over time, a progressive increase of Rb and p130 during development in the adult brains (Kusek et al., 2001). Later studies reported p107 expression in proliferative areas of the adult brain, such as the subventricular zone (SVZ) (Vanderluit et al., 2004). A similar pattern of expression and temporal distribution was detected in the retina, where p107 was expressed in proliferating embryonic progenitors, Rb in postnatal progenitors, and p130 in postmitotic cells (Donovan et al., 2006; Lee et al., 2006). Because cell-cycle exit is often associated with the initiation of differentiation, it was inferred that Rb and p107 regulate the decision of progenitor cells to become postmitotic, whereas p130 might modulate differentiation. Indeed, the levels of p130 were elevated in PC12 cells differentiated in the presence of NGF (Persengiev et al., 1999) and in primary astrocytes, whereas they were decreased in transformed glial cells (Miyajima et al., 1996).

Interestingly, however, differentiated PC12 cells (Persengiev et al., 1999) and oligodendrocyte lineage cells during terminal differentiation (Huang et al., 2002) showed decreased, rather than

increased levels of Rb during differentiation, suggesting a cell-specific function of the pocket proteins in distinct lineages.

LESSONS FROM KNOCKOUT MICE

Knockout of the E2F Family Members

E2F knockout mice have been generated for each family member (i.e. single knockouts) and for multiple E2Fs (i.e. compound knockouts). The detailed phenotypic analysis of single-E2F knockouts (Table 1) revealed a unique and tissue-specific role of each E2F in development. The phenotypic analysis of compound mutants revealed the cell-specific ability of each family member to compensate for another. For instance, the detection of cardiac hypertrophy in *E2F3*^{-/-} and in double heterozygous animals *E2F1*^{+/-}*E2F3*^{+/-}, but not *E2F1*^{-/-} mice (Cloud et al., 2002; Field et al., 1996; Yamasaki et al., 1996), suggested that both E2F1 alleles were necessary to compensate for the loss of a single E2F3 allele for the development of the heart. The existence of compensatory mechanisms and the threshold amount of each E2F for proper development and proliferation was tissue specific, because all the other tissues developed normally in the double heterozygous *E2F1*^{+/-}*E2F3*^{+/-} mice (Cloud et al., 2002). The phenotypic analysis of compound E2F mutants is presented in Table 1 and supports the broad classification of the E2F family members into two groups: E2F1, E2F2, and E2F3 group of transcriptional activators and E2F4 and E2F5 of repressors.

Within the CNS, the loss of selected E2F family members also led to temporal and cell-type-dependent phenotypes. *E2F1*^{-/-} mice, for instance, displayed no aberrant brain anatomy and no change in the number of cortical neurons during the early postnatal period, although they were characterized by decreased adult neurogenesis in the olfactory bulb and dentate gyrus, and significant hypoplasia of the cerebellum (Cooper-Kuhn et al., 2002). The different requirement for E2F1 in regulating proliferation of embryonic versus neonatal neural progenitors suggested a dispensable role for E2F1 in cortical neurogenesis (occurring in mid-embryogenesis) and a critical role in adult neurogenesis (Cooper-Kuhn et al., 2002). Additional studies conducted in the postnatal brain of *E2F1*^{-/-} mice, however, revealed ectopic proliferation (Wang et al., 2007). Because no increase of neurons was reported in the adult brain, these results indicated an abortive attempt to re-enter the cell cycle and possibly accounting for decreased neurogenesis. Loss of *E2F3* also affected neurogenesis in the adult SVZ. Although no change in proliferation or apoptosis was detected at E13.5 in the single *E2F3* knockout (Ziebold et al., 2001), decreased BrdU1 cells were observed in the adult SVZ (McClellan et al., 2007). These studies suggested that E2F3 contribution to proliferation was dependent on the developmental stage. Therefore, both E2F1 and E2F3 have different roles in embryonic development and in the adult brain.

The importance of E2F repressors in the development of head structures was suggested by the phenotype of *E2F4*^{-/-} mice with craniofacial defects (Humbert et al., 2000; Rempel et al., 2000). However, in contrast to the phenotype reported for the loss of E2F activators, loss of E2F4 resulted in aberrant embryonic development of the CNS. *E2F4*^{-/-} mice at E11.5 showed reduced size of the ventral telencephalon accompanied by loss of homeodomain protein expression (such as Pax6, Nkx2.1, and Dlx) indicating a developmental delay in this region (Ruzhynsky et al., 2007). These changes were accompanied by normal neural specification (Ruzhynsky et al., 2007). Thus, E2F4 was considered as necessary for the differentiation but not for the specification of neurons or the proliferation of progenitor cells. It was defined that E2F4 modulated the expression of the morphogen sonic hedgehog (Shh) within the diencephalon and telencephalon but not in other ventral regions (Ruzhynsky et al., 2007). Shh is responsible for creating a gradient during development that confers positional information to ventricular progenitors and controls their fate (Dessaud et al., 2008). The ability of E2F4 to

regulate Shh expression suggests its role as a master regulator for the development of cells derived from the ventral telencephalon.

Despite the biochemical similarities of E2F4 and E2F5, the phenotypes of *E2F4*^{-/-} and *E2F5*^{-/-} mice are distinct. Loss of *E2F5* during embryonic development caused excessive cerebrospinal fluid (CSF) production from the choroid plexus resulting in hydrocephalus (Lindeman et al., 1998). The excessive CSF production was not due to increased proliferation of CSF-producing cells, but to improper development. These results suggested a role for E2F5 in the differentiation of the choroid plexus epithelium (Swetloff and Ferretti, 2005). Therefore, E2F4 regulates the expression of the morphogen Shh in the developing ventral telencephalon, whereas E2F5 is necessary for the proper development of the choroid plexus. In adult animals, E2F1 and E2F3 are needed for proper control of progenitor cell number. The temporally and spatially regulated function of individual E2F family members in the CNS is summarized in Table 2.

Knockout of the Pocket Proteins

Rb

Rb deficient mice were generated by three different groups and reported to be embryonic lethal between E12.5 and E14.5, depending on the genetic background (Clarke et al., 1992; Jacks et al., 1992; Lee et al., 1992). This embryonic lethality was originally attributed to aberrant erythropoiesis and neural defects in several regions of the CNS (i.e., hindbrain, diencephalons, and spinal cord) and PNS (i.e. spinal ganglia) (Clarke et al., 1992; Jacks et al., 1992; Lee et al., 1992). The major phenotype found in the CNS of *Rb*^{-/-} mice was increased apoptosis in hindbrain, spinal cord, and spinal ganglia (Clarke et al., 1992; Jacks et al., 1992; Lee et al., 1992) associated with the detection of ectopic mitosis (Lee et al., 1992). Interestingly, although *Rb* expression was found in both ventricular and intermediate zone of the developing neural tube (Jiang et al., 1997), the distribution of apoptotic cells in the *Rb*^{-/-} mice was confined to the intermediate zone, containing progenitors committed to the neuronal lineage. An important role for Rb in committed neuronal progenitors was also independently suggested by a study using transgenic mice expressing LacZ reporter from the α -tubulin promoter ($T\alpha 1^{LacZ}$) within a wild-type or *Rb*^{-/-} background (Slack et al., 1998). Because the alpha tubulin promoter is active in neuroblasts, which have completed terminal mitosis and started differentiation (Gloster et al., 1994), by following the distribution of LacZ expressing neurons in wild-type and *Rb*^{-/-} mice, the effect of Rb function in differentiating neuroblasts could be evaluated over time. A similar pattern of LacZ expression was detected in the brain and spinal cord of $T\alpha 1^{LacZ}$ *Rb*^{-/-} and $T\alpha 1^{LacZ}$ wild-type mice until E12.5, whereas fewer LacZ⁺ neurons were detected in $T\alpha 1^{LacZ}$ *Rb*^{-/-} mice by E14.5. These results indicated that Rb did not affect the early stages of specification but modulated survival of committed neuroblasts (Slack et al., 1998).

The embryonic lethality of the *Rb*^{-/-} mice was later attributed to a placental defect (Wu et al., 2003). The deletion of *Rb* in all embryonic tissues except for the placenta, using a Mox2-driven Cre-recombinase (Tallquist and Soriano, 2000), did not show the same neurological and erythropoietic abnormalities originally described for the *Rb*^{-/-} mice and questioned the actual role of Rb in the nervous system (Wu et al., 2003). This was addressed by several groups using a genetic approach, by crossbreeding Rb floxed mice (i.e. *Rb*^{lox/lox}) with mice expressing the Cre recombinase from cell-specific promoters (Table 3). Nestin-Cre lines (i.e. *Nes*^{+cre}*Rb*^{lox/lox}), were used to target *Rb* deletion to progenitors in the CNS, PNS, and in the retina (MacPherson et al., 2003, 2004). Foxg1-Cre lines (i.e. *Foxg1*^{+cre}*Rb*^{lox/lox}) were used to target telencephalic neurons and ablate *Rb* as early as E8.5 (Ferguson et al., 2002, 2005). Engrailed2-Cre lines (i.e. *En2*^{+cre}*Rb*^{lox/lox}) ablated *Rb* in the dorsal mid-hindbrain junction starting at E9.5 (Marino et al., 2003). In addition, Pax6alpha-Cre (i.e. *Pax6alpha*^{+cre}

Rb^{lox/lox} were used to target peripheral retinal progenitors at E10 (Chen et al., 2004), *Chx10-Cre* (i.e. *Chx10^{+/cre}Rb^{lox/lox}*) to target retinal progenitors from E11.5 to P3 (Ajioka et al., 2007), *IRBP-driven Cre* (i.e. *IRBP^{cre}Rb^{lox/lox}*) to target photoreceptor cells after cell-cycle exit (Vooijs et al., 2002).

Nes^{cre}Rb^{lox/lox} mice at E13.5 displayed inappropriate S-phase entry in the intermediate zone of the hindbrain in the absence of apoptosis and increased brain size by E18.5 (MacPherson et al., 2003). Deletion of *Rb* in the telencephalon at E8.5 in *Foxg1^{+/cre}Rb^{lox/lox}* mice yielded similar results, because ectopic proliferation of cells was detected in the intermediate zone in the absence of widespread apoptosis, and this resulted in increased neurogenesis at E16.5 (Ferguson et al., 2002). Further characterization of these mice revealed decreased survival of Cajal–Retzius neurons at E13.5 (Ferguson et al., 2005). Because these cells synthesize reelin, which is essential to cortical neuronal migration, it was not surprising that radial migration of cortical neurons was also disrupted in *Foxg1^{+/cre}Rb^{lox/lox}* mice (Ferguson et al., 2005). However, *in vitro* migration assays in wild-type cortical slice cultures, using wild-type or *Rb^{-/-}* neuroblast, revealed that only neurons from *Rb^{-/-}* mice failed to migrate (Ferguson et al., 2005). It was concluded that *Rb* is necessary to inhibit ectopic proliferation and favor radial migration in the telencephalon in a cell-autonomous manner. Impaired migration in the absence of *Rb* was also detected in granule cells of the cerebellum of *En2^{+/cre}Rb^{lox/lox}* mice (Marino et al., 2003).

The role of *Rb* in cell-cycle exit, differentiation, and survival of specific cell populations was also suggested by studies on conditional knockouts in the retina. Deletion of *Rb* in peripheral retinal progenitors at E9.5–E10.5, using *Pax6α-Cre* lines resulted in ectopic proliferation of specific populations of retinal cells (i.e., ganglion cells, photoreceptors, and bipolar cells) (Chen et al., 2004; MacPherson et al., 2004), whereas other cell types (i.e., horizontal, amacrine, and muller glia) were able to exit the cell cycle and differentiate (Chen et al., 2004). The incomplete deletion of *Rb* in progenitor cells at E9.5 in *Nes^{+/cre}Rb^{lox/lox}* mice (in a mosaic pattern) in all layers of the retina also revealed decreased thickness of the photoreceptor layer that was attributed to increased apoptosis (MacPherson et al., 2004), whereas complete loss of the rod photoreceptors was observed in *Pax6α^{+/cre}Rb^{lox/lox}* and *Chx10^{+/cre}Rb^{lox/lox}* mice (Chen et al., 2004; MacPherson et al., 2004; Zhang et al., 2004) and attributed to defective formation of the photoreceptors. However, the phenotype of *IRBP^{cre}Rb^{lox/lox}* mice, with ablation of *Rb* in precursors after cell-cycle exit had no effect on the differentiation of photoreceptors (Vooijs et al., 2002). Based on these studies, it was concluded that *Rb* was necessary for proliferation and survival, but not differentiation of photoreceptor cells (summarized in Table 3). Studies in compound mice for *Rb* and E2F family members revealed additional roles of *Rb* also in the differentiation of starburst amacrine interneurons (Chen et al., 2007) and suggested context-specific roles for *Rb* in regulating cell-cycle exit, survival, and differentiation of distinct cell populations.

p107

We have previously mentioned that p107 is expressed in proliferative areas of the developing CNS and that within the adult brain p107 continues to be expressed in highly proliferative regions, such as the SVZ. *In vitro*, p107 is expressed at high levels in embryonic neurospheres and at lower but detectable levels also in adult neurospheres (Vanderluit et al., 2004). These observations suggested a direct function of p107 in controlling proliferation of neural progenitor cells. Indeed, proliferation was increased in the embryonic ventricular zone and adult SVZ of adult *p107^{-/-}* mice, and it was associated with increased apoptosis (Vanderluit et al., 2004, 2007). The increased numbers of neurospheres obtained from cultures of *p107^{-/-}* embryonic telencephalon or adult SVZ and their ability to generate neuronal and glial cell types (Vanderluit et al., 2004) further supported a role for p107 in self-renewal. Overall,

the characterization of the knockout mice supported the concept of a direct involvement of p107 in the control of the proliferative and clonogenic ability of progenitor cells.

p130

Within postmitotic neurons, p130 is the major pocket protein in complex with E2F (Liu et al., 2005) to recruit chromatin modifiers into repressive complexes. Some of the repressed target genes are involved in apoptotic pathways, and therefore it was proposed that p130 function is required for the survival of postmitotic neurons (Liu et al., 2005). Indeed, animals deficient in *p130* were not viable, although they displayed a strain-dependent phenotype. Loss of *p130* in the BalbC genetic background (LeCouter et al., 1998b), but not in a C57Bl background (Cobrinik et al., 1996), resulted in increased proliferation and apoptosis in the central and peripheral nervous system at E10.5 (LeCouter et al., 1998b).

Compound Knockout of Pocket Proteins

Because of the biochemical similarities among members of the pocket protein family, it was hypothesized that they could compensate for one another. However, p107 was able to compensate for *p130* loss in MEFs (Cobrinik et al., 1996), but not the developing nervous system, where p107 and p130 are expressed in distinct cell populations at different developmental times (Cobrinik et al., 1996). In the retina, in contrast, loss of *Rb* and *p130* in progenitors (i.e. *Nestin^{+cre}Rb^{lox/lox} p130^{-/-}*) did not affect normal development but it promoted tumor formation at 11 weeks after birth (MacPherson et al., 2004). Because the phenotype was associated with a compensatory increase of p107 (MacPherson et al., 2004), it was suggested that p130 function was redundant with p107 during the early stages of development, but essential for the maintenance of the differentiated phenotype in the adult retina.

The similar pattern of p107 and Rb expression in the CNS also suggested that these two proteins could compensate for each other. Consistent with the concept of a functional overlap between these two members of the pocket protein family, the phenotype of the compound mutant (*Rb^{-/-}p107^{-/-}*) was worse than the single mutant and characterized by accelerated embryonic lethality at E12.5 and widespread apoptosis (Lee et al., 1996). This effect was dosage-dependent, because the *Rb^{+/-}p107^{-/-}* mice survived up to 3 weeks of age (Lee et al., 1996). The compensatory relationship between the pocket proteins in the CNS was also studied in the cerebellum and in the retina. The detection of increased proliferation and apoptosis in transgenic mice *En2^{cre} Rb^{lox/lox}* in a *p107^{-/-}* background (Marino et al., 2003) supported the functional overlap of these two molecules in the cerebellum. The detection of retinal dysplasia with loss of photoreceptors in chimeric *Rb^{+/-}p107^{-/-}* mice (Robanus-Maandag et al., 1998), and the development of retinoblastomas within the inner nuclear layer of chimeric *Rb^{-/-}p107^{-/-}* mice (Robanus-Maandag et al., 1998), further supported the functional overlap of these proteins in the retina. The loss of all pocket protein activity resulted in major CNS developmental phenotypes. Inactivation of all the members of the retinoblastoma family was achieved by overexpressing the N-terminal fragment of SV40 T-antigen (i.e. *T₁₂₁*) that binds and inactivates all pocket proteins. When *T₁₂₁* was overexpressed in neural progenitors during the neurogenic period (i.e. starting from E11.5), increased proliferation and apoptosis and decreased radial glia were detected in telencephalic regions surrounding the ventricles (McLear et al., 2006). Thus, pocket protein function was considered essential for brain development.

The major binding partners for the Rb family members are proteins of the E2F family; thus, a possible interpretation of the phenotype of mice with genetic ablation of genes encoding for pocket proteins is that they are functionally equivalent to the gain of function of E2F activators (Macleod et al., 1996). However, this predicted overlap between E2F overexpression and Rb loss of function is best validated by taking into consideration the phenotype of individual E2F

knockouts and determining whether they could rescue the phenotype of mice lacking specific pocket proteins.

Compound Knockout of E2F and Pocket Proteins

Further understanding of the role of distinct E2Fs in brain development was inferred by understanding their relative contribution to the CNS phenotype of *Rb*^{-/-} mice. The massive apoptosis and ectopic proliferation in the CNS of *Rb*^{-/-} mice, for instance, was rescued by the loss of *E2F1* (Tsai et al., 1998), *E2F3* (Ziebold et al., 2001), and *E2F3a* (Chong et al., 2009), but not *E2F2* (Saavedra et al., 2002) or *E2F3b* (Chong et al., 2009). This suggested that the ectopic proliferation and massive apoptosis in *Rb*^{-/-} mice were functionally related to increased activity of E2F1 and E2F3a. To validate this interpretation in a tissue and cell-specific manner, several conditional mutants for *Rb* were generated. Mice with conditional ablation of *Rb* and *E2F* in the telencephalon, obtained by crossing *Foxg1*^{Cre} lines with *E2F1*^{-/-}*Rb*^{lox/lox} or with *E2F3*^{-/-}*Rb*^{lox/lox} mice, confirmed that E2F1 and E2F3 were the mediator of ectopic proliferation and apoptosis in the embryonic CNS (McClellan et al., 2007). However, E2F1 and E2F3a did not equally affect *Rb* mediated neuronal migration. Specific deletion of *Rb* in the telencephalon resulted in decreased migration of interneurons into the dorsal cortex (Ferguson et al., 2005). Loss of *E2F3* in these *Rb* deficient mice rescued the number of migrating interneurons, whereas loss of E2F1 had no effects (McClellan et al., 2007). Therefore, E2F1 and E2F3 have overlapping roles in proliferation and apoptosis of the embryonic CNS, whereas E2F3 has a unique role in regulating neuronal migration (Table 2). Similar conclusions were reached by analyzing the retinal phenotype of mice-lacking *E2F1* or *E2F3a* and *Rb*. Loss of *E2F1* restored the thickness of the photoreceptor layer to wild-type levels (Chen et al., 2007) but unexpectedly revealed defective differentiation of a population of cholinergic interneurons called starburst amacrine cells (Chen et al., 2007). These results led to a deeper analysis of the phenotype of *Rb* null mice and to the discovery that impaired differentiation was observed in amacrine cells expressing E2F3 (while neurons lacking E2F3 differentiated normally even in the absence of *Rb*). Indeed, the differentiation of starburst amacrine cells was restored in compound mice-lacking *Rb* and *E2F3a* (Chen et al., 2007). Together, these data suggest a cell-type-specific role of the *Rb*/E2F pathway in distinct retinal cell types, with *Rb* controlling proliferation in the retina by sequestration of E2F1 mechanism and controlling starburst amacrine cell differentiation by inhibition of E2F3a but not E2F3b.

THE E2F/*Rb* PATHWAY IN NEURONAL AND GLIAL PATHOLOGY

Although the expression of cell-cycle regulators has been associated with physiological regulation of cell cycle during development and in proliferative areas of the adult brain, it is important to mention that their re-expression in neurons has been detected in pathological conditions characterized by neurodegeneration. Hyperphosphorylated *Rb* and altered distribution of E2F1 were detected in Alzheimer's brains (Ranganathan et al., 2001) and in cultured neurons exposed to toxic stimuli (Hou et al., 2000). Aberrant *Rb* expression (Jordan-Sciutto et al., 2003) and active E2F (Hoglinger et al., 2007) were also detected in Parkinson's disease and amyotrophic lateral sclerosis (Nguyen et al., 2003; Ranganathan et al., 2001). These changes were correlated with increased levels of E2F target genes including cyclin A, B, and E in Alzheimer's disease (Busser et al., 1998; Hoozemans et al., 2002; Vincent et al., 1996; Yang et al., 2001) and stroke (Burns et al., 2007; Love, 2003). It was proposed that neurodegeneration might be consequent to abortive cell-cycle re-entry associated with the activation of a suicide program (Herrup and Yang, 2007). Indeed, overexpression of E2F1 in cortical neurons was sufficient to induce apoptosis (Hou et al., 2000), whereas lack of E2F1 conferred protection to cerebellar granular neurons from dopamine-induced cell death (Hou et al., 2001) and to cortical neurons from death induced by β -amyloid (Giovanni et al., 2000).

Thus, neurons in the adult brain appear well versed at activating an apoptotic response as a consequence of increased expression of cell cycle genes, due to reactivation of Rb.

It is remarkable that glial cells appear to differentially handle aberrant expression of cell cycle genes. It is well accepted that inactivation of Rb in human astrocytes confers a proliferative advantage that can be maintained in the absence of growth factors, if Rasv12 is simultaneously expressed (Rich et al., 2001). Rb overexpression inhibits the growth of gliomas (Fueyo et al., 1998), and this is consistent with the fact that E2F1 promoter driven reporters are highly active in glioma cells (Parr et al., 1997). The detection of elevated E2F2 levels in cancer stem cells characterized by the expression of CD133 further supported this concept (Okamoto et al., 2007). An oversimplified interpretation of these results is a model proposing a cell-context function of reactivation of the Rb/E2F pathway in the adult brain. Neurons, traditionally considered as postmitotic cells, are unable to handle cell-cycle re-entry after differentiation, and this leads to apoptosis and possible neurodegeneration. Astrocytes, in contrast, are plastic cells with the ability to proliferate in response to injury. In these cells, the re-expression of cell-cycle genes leads to proliferation and successful cell-cycle re-entry.

A potential explanation for this cell-specific response to the re-expression of E2f/Rb is the differential regulation of target genes in distinct cell types, which is directly correlated with the pattern of expression of specific pocket proteins (see Fig. 2). It has been shown that p130 is the major pocket protein found in complex with repressive E2Fs in mature neurons (Liu et al., 2005). These p130/repressive E2F complexes contain chromatin modifying enzymes, which repress target genes involved in apoptosis and re-expression of Rb and activating E2F could alter the balance toward transcriptional activation of proapoptotic genes and negatively affect survival of postmitotic neurons (Liu et al., 2005). In astrocytes, in contrast, Rb in complex with activator E2Fs can regulate cell-cycle genes and result in proliferation. Indeed, the recent identification of compounds that are able to restore Rb function in gliomas have shown promising antitumor activity (Alonso et al., 2007), which is dependent on the other mutagenic mutations that are present in the cell (Alonso et al., 2008).

It is important to stress here that the “pathological” function of distinct members of the Rb/E2F family is also dependent on the temporal window of altered expression. Pathology could result from overexpressing molecules at a time when they should not be expressed or from inactivating them at a time when they should be active. For instance, inactivation of p107 might negatively affect differentiation and favor a dedifferentiated phenotype, due to lack of repression of Notch and its target genes (Vanderluit et al., 2004, 2007). During early development, specific populations of neuroblasts are dependent on Rb function not only for the control of proliferation, but also for the activation of a neurogenic program of gene expression. Several neurogenic transcription factors (i.e., Neurogenin or NeuroD) belong to the basic helix-loop-helix family of proteins [for full review, see Guillemot (2007), Kessaris et al. (2008), and Ross et al. (2003)], whose transcriptional activity is controlled by the Id family members, via a sequestration mechanism (Lasorella and Iavarone, 2006; Li et al., 2005). Because the Id proteins have also the ability to sequester Rb, it is conceivable that the levels of Rb during early development might be critical for the appropriate activation of a cell-specific transcriptional program [reviewed in Iavarone and Lasorella (2006) and Zebedee and Hara (2001)]. Aberrant modulation of this Id/Rb regulatory pathway may contribute to the formation of neuroblastomas during embryogenesis.

CONCLUDING REMARKS

In conclusion, we have presented multiple studies supporting a cell-type-specific and temporally defined role of distinct members of the E2F and Rb family. The coordinate expression of precise pocket proteins and E2F transcription factors differentially modulates

the development of neurons and glial cells, by shaping the chromatin landscape and modulating gene expression. Heterotopic and heterochronic patterns of expression are associated to pathological responses in the developing and mature brain, due to aberrant modulation of genes involved in cell-cycle regulation, apoptosis, and cell fate.

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Abbreviations

CDK	cyclin-dependent kinase
CDKi	cyclin-dependent kinase inhibitor
CNS	central nervous system
Cre	cre-recombinase
CSF	cerebrospinal fluid
Dp	dimerization protein
E2F	E2 promoter binding Factor
En2	Engrailed2
HDAC	histone deacetyltransferase
INL	inner nuclear layer of the retina
MEF	mouse embryonic fibroblasts
MGE	medial ganglionic eminence
NGF	nerve growth factor
ONL	outer nuclear layer of the retina
PNS	peripheral nervous system
Rb	retinoblastoma
SVZ	subventricular zone
Tα1	transgenic alpha tubulin promoter.

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Fig. 1.

Biochemical properties of the E2F family members. **A:** Schematic diagram of the conserved domains within E2F and Dp family members. E2F1-6 dimerize with Dp proteins to directly bind DNA via their conserved DNA-binding domains. E2F1-5 interact with pocket proteins via a conserved domain in their C-termini. E2F7 and E2F8 do not dimerize with DP proteins and contain two DNA-binding domains. E2F3 is alternatively spliced resulting in E2F3a and E2F3b isoforms, splice variants of E2F7 have also been described [not shown, Di Stefano et al. (2003)]. **B:** E2F1-5 dimerize with Dp proteins and these heterodimers interact with pocket proteins in distinctive ways, E2F1-4 proteins interact with Rb, E2F4, and E2F5 interact with p107 and p130.

Fig. 2.

Transcriptional regulation by E2F complexes modulates cell-cycle control, cell fate, and survival. Examples of known mechanisms where E2F complexes mediate the transcriptional regulation required for proper cell development. E2F1 recruits the acetyltransferase p300 to acetylate histones and promote the expression of genes involved in cell-cycle progression (Caretti et al., 2003; Rayman et al., 2002). An unknown E2F repressor mediates the necessary recruitment of p107 to the promoter of Notch1 during neuronal development (Vanderluit et al., 2004). E2F4 mediates neuronal survival in adult neurons by recruiting a complex containing p130, HDAC1, and the methyltransferase Suv39H1 to the promoter of the proapoptotic gene B-Myb (Liu et al., 2005).

TABLE 1

Summary of the E2F Mutant Phenotypes

Alleles					Genotype	Mouse Phenotype	Reference(s)
E2F1	E2F2	E2F3a	E2F3b	E2F4	E2F5		
X						Decrease of apoptosis in immature thymocytes, tumors of older adults tissues especially reproductive organs. Decreased progenitor proliferation in the CNS. Decreased apoptosis and adult neurogenesis in the olfactory bulb and dentate gyrus.	Cooper-Kuhn et al. 2002; Field et al. 1996; Wang et al. 2007; Yamaski et al. 1996
	X					Autoimmunity due to enhanced T lymphocyte proliferation	Murga et al. 2001
		X	X			Mixed strain dependent viability, congenitive heart failure causes premature death in a pure 129/Sv strain. Decreased proliferation of adult neural precursors.	Humbert et al. 2000b; Cloud et al. 2002; Wu et al. 2001; McClellan et al. 2007
		X				Viable, appear normal, aged mice have less white adipose tissue	Danielian et al. 2008; Chong et al. 2009;
			X			Viable, appear normal	Danielian et al. 2008; Chong et al. 2009;
				X		Decreased erythroid proliferation, fetal anemia, growth retardation, and craniofacial defects. Reduced size of the ventral telencephalon, reduced number of neural precursors, and reduced expression of sonic hedgehog.	Humbert et al. 2000a; Kinross et al. 2006; Rempel et al. 2000; Ruzhynsky et al. 2007
					X	Increased in cerebrospinal fluid causing hydrocephalus.	Lindeman et al. 1998
I		I	I			Viable, Congestive heart failure causes premature death.	Cloud et al. 2002
I		X	X			Viable, Congestive heart failure causes premature death.	Cloud et al. 2002
X		I	I			Viable, death from tumors - found to arise in E2F1 ^{-/-} .	Cloud et al. 2002
X	X					Viable	Wu et al. 2001
X	X		X			Viable	Tsai et al. 2008
X	X	X				Embryonic Lethal	Tsai et al. 2008
X		X				Reduced viability, premature death, increase in brain mass.	Danielian et al. 2008; Tsai et al. 2008
X		X	X			Embryonic lethal	Wu et al. 2001
X			X			Viable, no significant differences in tissue development.	Tsai et al. 2008
	X	X	X			Embryonic lethal	Wu et al. 2001
				I	I	Viable	Guabatz et al. 2000
				I	X	Viable	Guabatz et al. 2000

Alleles						Genotype	Mouse Phenotype	Reference(s)
E2F1	E2F2	E2F3a	E2F3b	E2F4	E2F5			
				X	1	E2F4 ^{-/-} E2F5 ^{+/-}	Reduced Viability	Guabatz et al. 2000
				X	X	E2F4 ^{-/-} E2F5 ^{-/-}	Late embryonic lethal	Guabatz et al. 2000

The left side of the chart graphically displays the E2F alleles which are present (+/+, green; +/-, yellow; -/-, red).

TABLE 2

Mice Lacking E2F and/or Rb Proteins in the developing CNS Display Abnormalities in Cellular Proliferation, Apoptosis, Migration, and Differentiation

Phenotypes of E2F and Rb mutant mice within the developing CNS				
Genotype	Proliferation	Apoptosis	Migration or differentiation	Reference(s)
Rb ^{-/-}	↑↑	↑↑	↓Embryonic neurogenesis	Clark et al. 1992; Jacks et al. 1992; Lee et al. 1992; Slack et al. 1998
E2F1 ^{-/-}	-(↑)	-(↓)	(↓Adult neurogenesis)	Cooper-Kuhn et al. 2002; Tsai et al. 1998; Wang et al. 2007
E2F2 ^{-/-}	ND	ND		
E2F3 ^{-/-}	-(↓)	-		McClellan et al. 2007; Ziebold et al. 2001;
E2F3a ^{-/-}	-	-		Chong et al. 2009
E2F3b ^{-/-}	-	-		Chong et al. 2009
E2F4 ^{-/-}	-	ND	Reduced size of the ventral telencephalon	Rudnicki et al. 2007
E2F5 ^{-/-}	ND	ND	Develop hydrocephalus	Lindeman et al. 1998
E2F1 ^{-/-} Rb ^{-/-}	-*	-*		Saavedra et al. 2002; Tsai et al. 1998;
E2F2 ^{-/-} Rb ^{-/-}	↑	↑↑		Saavedra et al. 2002
E2F3 ^{-/-} Rb ^{-/-}	-*	-*		Saavedra et al. 2002; Ziebold et al. 2001;
E2F3a ^{-/-} Rb ^{-/-}	-	-*		Chong et al. 2009
E2F3b ^{-/-} Rb ^{-/-}	↑	↑		Chong et al. 2009
Foxg1 ^{Cre} Rb ^{lox/lox}	↑↑	-*	↓migratory interneurons	Ferguson et al. 2002
Foxg1 ^{Cre} E2F1 ^{-/-} Rb ^{lox/lox}	-	-	↓migratory interneurons	McClellan et al. 2007
Foxg1 ^{Cre} E2F3 ^{-/-} Rb ^{lox/lox}	-	-	-migratory interneurons	McClellan et al. 2007

Quantitative differences in proliferation, apoptosis, migration, and differentiation are displayed within the graph as increases ↑, or decreases ↓ or no change (-) as compared with wild-type mice. Several phenotypes observed in the developing CNS are specific and not observed in the PNS (denoted in the chart by a *) or in the adult CNS (denoted by (↑) or (↓)). Phenotypes which have not been described are listed as ND.

TABLE 3

The Retinal Phenotype Caused by Deletion of Genes Encoding for Pocket Proteins is Dependent on Timing of Excision and Cell Type

Genotype	Effects of pocket protein loss in the Retina				Reference(s)
	Gene promoter	Timing	Cells targeted	Phenotype in Retina	
$Rb^{lox/lox}$	Nestin (maternally transmitted)	E9.5	Neuronal progenitors (mosaic)	Increased apoptosis leading to a thin photoreceptor layer. Ectopic proliferation outside of inner granular layer	MacPherson et al. 2003, 2004
$Rb^{lox/lox}$	Pax6 alpha enhancer	E10.5	Peripheral retinal Progenitors	Reduced ganglion, bipolar, and rod cells due to apoptosis. Disorganization of INL. Defect in amacrine cell differentiation.	Chen et al. 2004, 2007; MacPherson et al. 2004
$Rb^{lox/-}$	Chx10	E11.5	Retinal progenitors (mosaic)	Fewer rod cells but normal number of cone cells	Zhang et al. 2004
$Rb^{lox/lox}$	IRBP	E13.5	Rods and Cones	No retinal phenotype	Vooijs et al. 2002
$Rb^{1/-} p107^{-/-}$ chimeric	-	-	-	Retinodysplasia in neonates	Robanus-Maandag, 1998
$Rb^{-/-} p107^{-/-}$ chimeric	-	-	-	Retinoblastoma by 14 weeks	Robanus-Maandag, 1998
$Rb^{lox/lox} p107^{-/-}$	Nestin (paternally transmitted)	E9.5	All cell types in the optic nerve	Retinodysplasia at E18.5. Mice die prior to weaning age	MacPherson et al. 2004
$Rb^{lox/lox} p107^{-/-}$	Pax6 alpha enhancer	E10.5	Peripheral retinal progenitors	Decreased ganglion bipolar, rod and cone cells. Ectopic division of precursor cells, but normal progenitor proliferation. Retinoblastoma develops.	Chen et al. 2004
$Rb^{lox/lox} p107^{-/-}$	IRBP	E13.5	Rods and Cones	No retinal phenotype	Vooijs et al. 2002
$Rb^{lox/lox} p130^{-/-}$	Nestin (paternally transmitted)	E9.5	All cell types in the optic nerve	Retinoblastoma develops at ~11 weeks	MacPherson et al., 2004
$Rb^{lox/lox} p107^{+/+} p130^{-/-}$	Chx10	E11.5	Retinal progenitors (mosaic)	Retinoblastoma develops at ~11 weeks	Ajioka et al. 2007
$Rb^{lox/lox} E2F1^{-/-}$	Pax6 alpha enhancer	E10.5	Peripheral retinal progenitors	Rescue of ectopic cellular division and death seen in $Rb^{lox/lox}$	Chen et al. 2007

Effects of pocket protein loss in the Retina					
Genotype	Cre-recombinase Driver			Phenotype in Retina	Reference(s)
	Gene promoter	Timing	Cells targeted		
Rb ^{lox/lox} E2F3a ^{-/-}	Pax6 alpha enhancer	E10.5	Peripheral retinal progenitors	Rescue of amacrine cell differentiation defect seen in Rb ^{lox/lox}	Chen et.al. 2007