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Control of Retinal Development by Tumor Suppressor Genes

Robert Cantrup, Gaurav Kaushik and Carol Schuurmans
Department of Biochemistry & Molecular Biology,
Hotchkiss Brain Institute,
Alberta Children's Hospital Research Institute,
University of Calgary
Canada

1. Introduction

Tumor suppressor genes are so named because of their actions in preventing cancer – but it is often overlooked that they also have normal functions in non-pathological cellular contexts. Here we review the evidence that tumor suppressor genes are key regulators of developmental events, focusing on the neural retina as a model system. At no other time during an organism's lifespan are the biological processes that tumor suppressor genes control - including cell division, differentiation, migration and programmed cell death, more pronounced than during the embryonic and early postnatal period. The developing retina serves as an excellent model of tissue development based on its experimental accessibility, well-characterized cell types, and sophisticated laminar organization. The retina also has an important physiological function - processing light signals so that vision is possible. Visual processing requires functional neural circuits, the formation of which requires that appropriate numbers of the correct types of neuronal and glial cells differentiate during development. Here we introduce our current knowledge of how the retina develops and then review the evidence that tumor suppressor genes control several aspects of this process, including: 1) cell division/proliferation, 2) appropriate cell fate specification/differentiation, 3) cell migration, and 4) cellular apoptosis. By better understanding the normal functions that tumor suppressor genes play in the developing embryo, we are better positioned to understand why their deregulated expression leads to tumor growth and cancer.

1.1 Retinal structure and morphogenesis

The retina is the neural layer of the eye and is responsible for converting light photons into electrical impulses that are transmitted to the brain. It is comprised of one glial and six neuronal cell types that are organized into three nuclear layers: 1) an outer nuclear layer (ONL) of rod and cone photoreceptors, which receive light signals; 2) an inner nuclear layer (INL) of supporting Müller glial cells and bipolar, horizontal and amacrine cell interneurons, which refine and transmit signals from photoreceptors; and 3) a ganglion cell layer (GCL) of retinal ganglion cells (RGCs) – the output neurons of the retina (Figure 1).
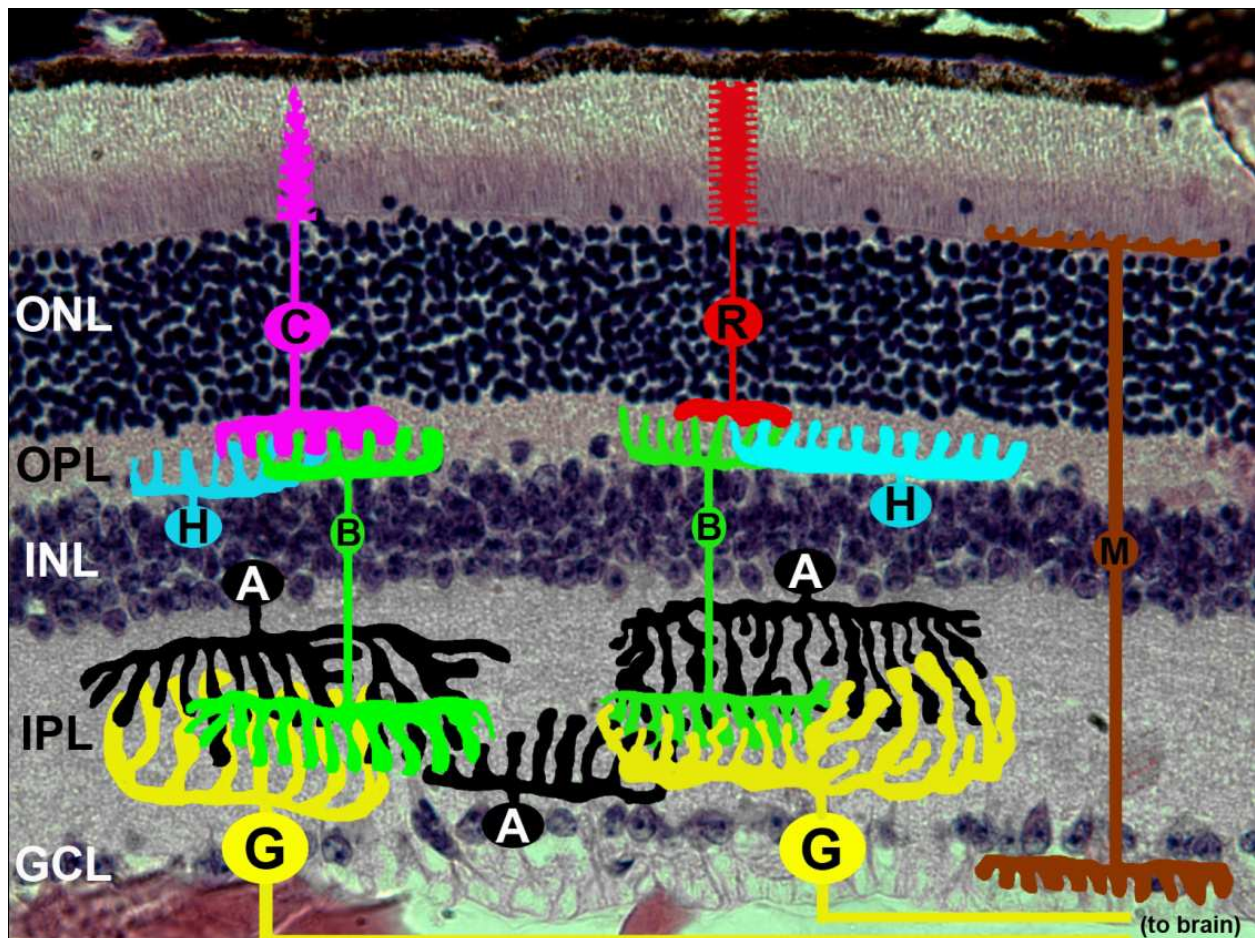
Fig. 1. Structure and connectivity of the mature retina. Animated neurons are drawn on top of a photomicrograph of a hematoxylin & eosin stained adult retina. Rod and cone photoreceptors are located in the ONL, horizontal, amacrine and bipolar cell interneurons and Müller glia are located in the INL, and RGCs and displaced amacrine cells are in the GCL. Light enters the eye and is first processed by the outer segments of rod and cone photoreceptors in the ONL. This information is then passed to the OPL, where connections between photoreceptors and bipolar cells are made, and signals are modulated by horizontal cells. Finally, bipolar cell axons pass visual information to RGC dendrites in the IPL – signaling that is refined by amacrine cells. Information is finally transmitted by RGC axons to the brain for further processing. (A,amacrine cell, B,bipolar cell; C,cone photoreceptor; G, retinal ganglion cell; GCL, ganglion cell layer; H, horizontal cell; INL, inner nuclear layer; IPL, inner plexiform layer; M, Müller glia; ONL, outer nuclear layer; OPL, outer plexiform layer; R, rod photoreceptor).

The GCL also contains some displaced amacrine cells. The three nuclear layers are separated by two synaptic layers; the inner plexiform layer (IPL), which separates the GCL/INL and the outer plexiform layer (OPL), which separates the INL/ONL.

In the embryo, the retina begins as a small bilateral outpocketing of the rostral diencephalon, first emerging at around embryonic day (E) 8.5 in mice (Svoboda and O'Shea 1987; Wawersik and Maas 2000; Fuhrmann 2010). As the evaginated diencephalon comes into contact with the surface ectoderm, it induces formation of the lens placode in the overlying epithelium. At E9.5, the lens vesicle signals to the adjacent diencephalic neuroepithelial cells to invaginate and

form an optic cup (Svoboda and O'Shea 1987; Fuhrmann 2010). The inner and outer layers of the optic cup form the neural retina and retinal pigmented epithelium (RPE), respectively. Thus, by E10.5 the neural retina is morphologically distinct, and is comprised of actively proliferating, progenitor cells (Kagiyama et al. 2005; Hirashima et al. 2008). Based on lineage tracing and clonal analyses, all seven retinal cell types are generated from this common pool of multipotent retinal progenitor cells (Turner and Cepko 1987; Holt et al. 1988; Wetts and Fraser 1988; Fekete et al. 1994; Alexiades and Cepko 1997). There is also, however evidence for the existence of some restricted retinal cell lineages (Alexiades and Cepko 1997; Li et al. 2004; Pearson and Doe 2004; Cayouette et al. 2006). Currently, the proportion of retinal progenitors that are multipotent versus lineage-restricted is not known (Cayouette et al. 2003; Cayouette et al. 2006).

1.2 Retinal cell fate specification and differentiation

Retinal cell differentiation commences at around E10.5 in mouse and is not complete until postnatal day (P) 12, preceding in a medial-to-lateral gradient (Young 1985a; Wallace 2011). The seven retinal cell types are generated in a stereotyped, overlapping sequence and can be grouped into two major, overlapping phases; a prenatal and postnatal phase (Young 1985b; Young 1985a; Stiemke and Hollyfield 1995; Cepko et al. 1996) (Figure 2). In the prenatal phase, RGCs first begin to differentiate, starting at approximately E10.5, followed closely by horizontal cells and cone photoreceptors, and slightly later by amacrine cells. This prenatal phase of retinal histogenesis peaks around E15.5 and continues until approximately P2 in mice. In the postnatal phase of retinal cell differentiation, which peaks around P0, rod photoreceptors, bipolar cells and Müller glia are generated (Cepko et al. 1996; Livesey and Cepko 2001; Wallace 2011). Strikingly, the order of cellular differentiation is grossly conserved across vertebrate species despite a wide variance in the overall length of the differentiation period that ranges from 25 hours in *Xenopus* [i.e. stage 28-stage 40; (Holt et al. 1988)] to approximately 3 weeks in rodents (Young 1985b; Rapaport et al. 2004).

Over the course of cellular differentiation, temporal cues are thought to gradually reduce developmental plasticity such that progenitor cells become biased towards a smaller number of cell fates (Competence Model), resulting in the stereotyped order of cellular differentiation (Cepko et al. 1996; Livesey and Cepko 2001). In both multipotent and lineage biased progenitors, transcription factors act combinatorially to specify distinct retinal cell fates at different developmental times (Inoue et al. 2002). Thus, by changing the repertoire of transcription factors that are expressed and active, unique temporal identities are specified and appropriate differentiation programs are initiated (Cepko et al. 1996). Many transcription factors that participate in retinogenesis act in a combinatorial manner to specify retinal cell fates (Hatakeyama et al. 2001; Inoue et al. 2002). As a consequence, misexpression of any one transcription factor does not necessarily induce the generation of the cell types that would be predicted based on its pattern of expression and loss-of-function phenotype. For example, *Math3* and *Mash1* are expressed in bipolar cells, a cell type that is absent in *Math3;Mash1* double mutants, but misexpression of either of these two basic-helix loop-helix (bHLH) proteins alone promotes rod rather than bipolar genesis (Tomita et al. 2000). In contrast, misexpression of *Math3* or *Mash1* in conjunction with the homeodomain protein *Chx10* leads to bipolar cell genesis (Hatakeyama et al. 2001). Similarly, amacrine cells are specified by the combined activities of the bHLH protein NeuroD in conjunction with the homeodomain proteins Pax6 or Six3 (Inoue et al. 2002), whereas Math3 can specify a horizontal cell fate in combination with Pax6 or Six3, and a bipolar cell fate in combination with Chx10 (Hatakeyama et al. 2001; Inoue et al. 2002).
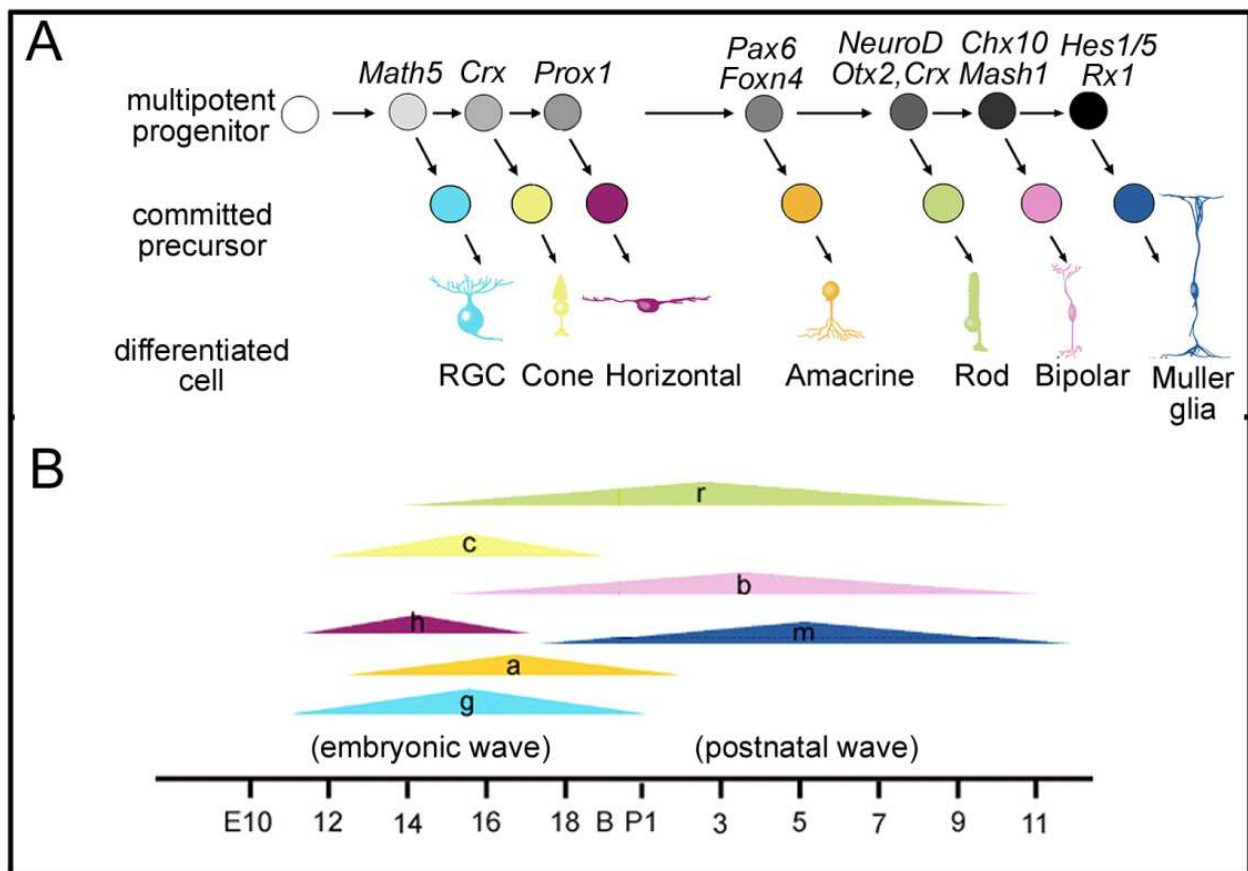
Fig. 2. Sequential generation of retinal cells during development.
(A) The competence model of retinal cell differentiation. Progenitors are gradually restricted in their developmental potential, resulting in the differentiation of different retinal cell types as developmental time proceeds. RGCs are born first, quickly followed in an overlapping manner by cones, horizontal cells, and amacrine cells (embryonic phase), and rod photoreceptors, bipolar cells and Müller glia (postnatal phase). (B) A schematic illustrating that retinal cell neurogenesis occurs in roughly two distinct but overlapping waves.

1.3 Retinal cell migration

The three-layered structure of the retina is reminiscent of the six-layered organization of the neocortex. However, while neocortical neurons are born in non-overlapping waves and migrate radially to sequentially populate the layers in an 'inside-out' manner (i.e., deep-layers formed first, followed by more superficial layers) (Caviness 1982; Caviness et al. 1995; Takahashi et al. 1999), the timing of differentiation and the migratory routes of retinal neurons are more complex. For example, during the early-phase of retinal cell differentiation, RGCs, horizontal cells, amacrine cells and cone photoreceptors have overlapping birthdates, but distinct destinations: RGCs and some amacrine cells migrate to the GCL, horizontal cells and some amacrine cells migrate to the INL and cone photoreceptors migrate to the ONL (Baye and Link 2008; Galli-Resta et al. 2008). How are these distinct migratory routes established? The logic behind radial cell migration begins with an understanding of how retinal progenitor cells divide. Most retinal progenitors

have a radial morphology and maintain contact with both the apical and basal surfaces of the retinal neuroepithelium (Figure 3). During the cell cycle, retinal progenitors undergo interkinetic nuclear migration in a cell cycle-dependent fashion, such that mitotic divisions, and hence neuronal birth, occur at the apical surface of the retina (Turner et al. 1990). Horizontal cell precursors are an exception to the rule, as they undergo non-apical mitoses near their final location in the INL (Figure 3, 4) (Godinho et al. 2007), while other cell types are all thought to be born at the apical surface. As RGCs differentiate, they lose their apical attachment, retaining a basal extension that becomes an axon and is thought to help drag RGCs into the GCL (Poggi et al. 2005; Zolessi et al. 2006) (Figure 3). In contrast, amacrine cells lose both apical and basal contacts upon differentiation, and must somehow respond to unknown environmental cues as they migrate into their final positions in the INL and GCL (Galli-Resta et al. 2008) (Figure 3). Finally, cone photoreceptors remain at their apical site of birth – where the future ONL will develop (Figure 3). Similarly, in the second phase of differentiation, rod photoreceptors remain in the apical compartment post-differentiation, whereas bipolar cells and Müller glia must migrate into the INL (Figure 4).

The retina also has a unique three-dimensional architecture in the tangential plane, with the cell bodies of cone photoreceptors, amacrine cells, horizontal cells and RGCs migrating tangentially to position themselves at regular intervals to allow complete sampling of the visual field. These non-random cellular arrays are known as mosaics and evenly tile the retinal field (Galli-Resta et al. 2008). Individual cellular mosaics are characterized by minimal distances between like-cells – a spacing that is achieved by processes that include self-avoidance or isoneuronal repulsion and repulsion of like-neighbours or heteroneuronal repulsion (Grueber and Sagasti 2010). While the molecular cues that establish retinal cell mosaics are poorly understood, individual retinal mosaics are known to develop cell autonomously and are not influenced by the mosaics of other cell types (Rockhill et al. 2000).

1.4 Retinal cell death during development

Apoptosis is also known as programmed cell death, and is a process whereby a cell induces its own death through a well characterized caspase-mediated signalling pathway. During development, neurogenesis and apoptosis are both required to occur in a balanced fashion so that appropriate numbers of each retinal cell type are present in the mature organ. Strikingly, in the CNS, 20-70% of neurons undergo apoptosis (Burek and Oppenheim 1996). While it is still not known why organisms generate extra cells and then have to delete them, the process of apoptosis has been conserved throughout evolution and is essential for proper tissue morphogenesis and to determine the final size of tissues and organs (Burek and Oppenheim 1996; Bahr 2000; Buss and Oppenheim 2004). During embryonic retinal development there are three waves of cell apoptosis. First, during the optic cup stage (E10-E11), apoptosis is primarily observed in the presumptive RPE and optic stalk (Pei and Rhodin 1970; Silver and Hughes 1973). Next, a second wave of apoptosis is observed during the stage of optic fissure closure (E11 to E12), with apoptotic retinal progenitor cells observed at the fissure site (Silver and Robb 1979; Hero 1990). The third wave of apoptosis happens during the optic nerve enlargement stage (E15.5-E17.5). At this stage, neuroepithelial cells close to the optic nerve head undergo degeneration (Silver and Robb 1979). Cell apoptosis is also observed in all three layers in postnatal stage retinae, occurring in a central to peripheral gradient. Studies in rat retinae indicate that around 5%, 50%, and

51% of the ONL, INL, and GCL populations undergoes programmed cell death, respectively (Voyvodic et al. 1995). Cell apoptosis starts in the GCL, and lasts from P2 to P11 with a peak at P2-P5 (Young 1984). Two phases of apoptosis are then observed in the INL, with amacrine cell degeneration peaking at P3-P8, followed by bipolar and Müller glial cell degeneration peaking at P8-P11. Photoreceptor cell apoptosis lasts for two weeks, with a peak from P5 to P9 (Young 1984).
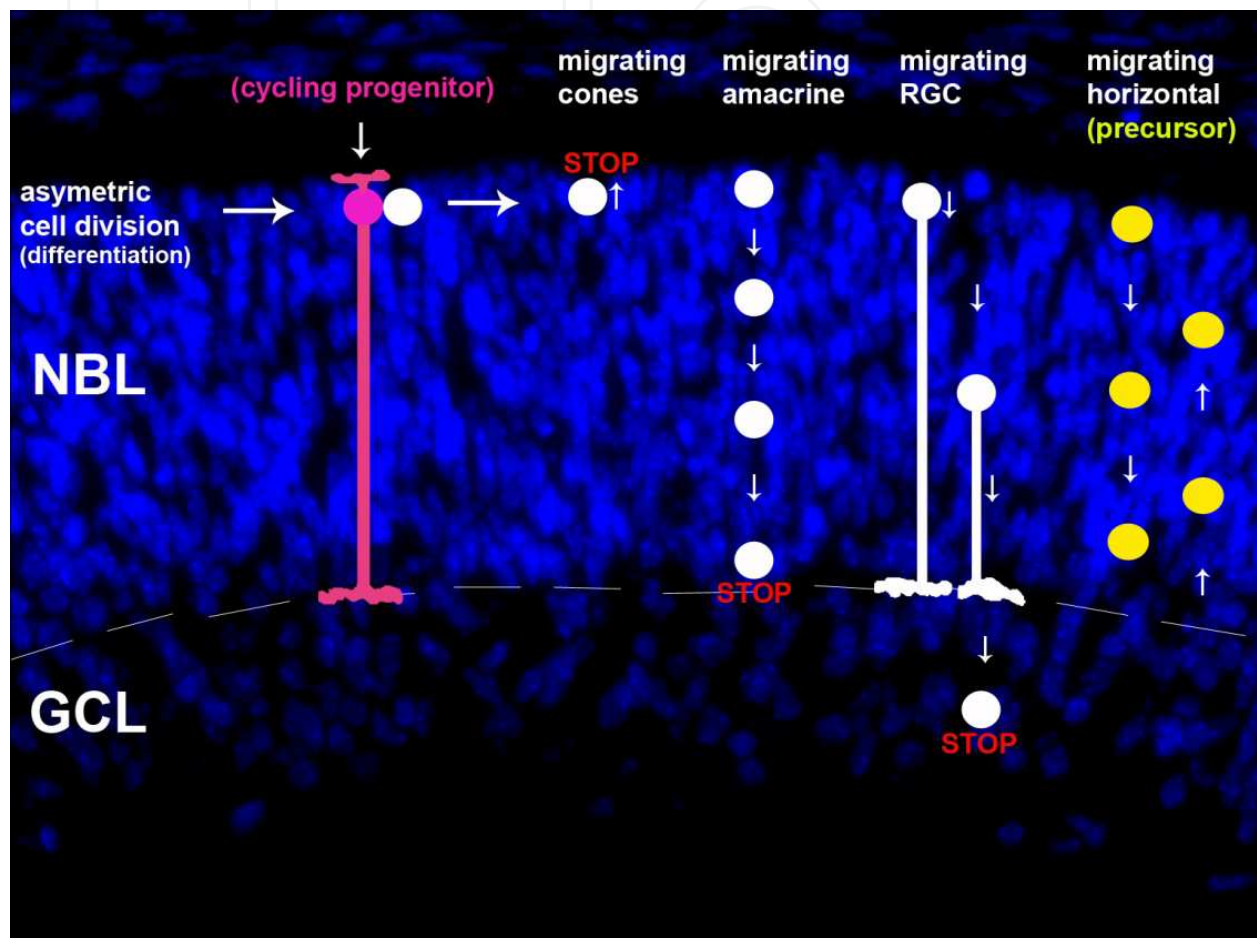

Fig. 3. Site of neuronal birth and migration patterns for retinal neurons born in the early embryonic phase of retinal cell differentiation.
Cells are schematized on top of a photomicrograph of an E15.5, DAPI stained retina. Cycling progenitors (pink) maintain contacts with the apical and basal surface of the retinal neuroepithelium. Retinal progenitors then divide asymmetrically at the apical surface of the neuroblast layer (NBL), giving rise to immature neurons that must migrate to their correct laminar positions within the retina. Cone cells differentiate at the apical surface, where they will remain and form the future ONL (not shown). Amacrine cells differentiate at the apical surface, lose their apical and basal contacts, and then migrate through the NBL to reach the INL (and some go to the GCL). RGCs differentiate at the apical surface and lose their apical contact but maintain their basal contact – an extension that will help RGCs migrate into the forming GCL. During this time, horizontal cell precursors continue to migrate along the apico-basal axis of the NBL, not undergoing terminal differentiation and finding their final position until postnatal stages.
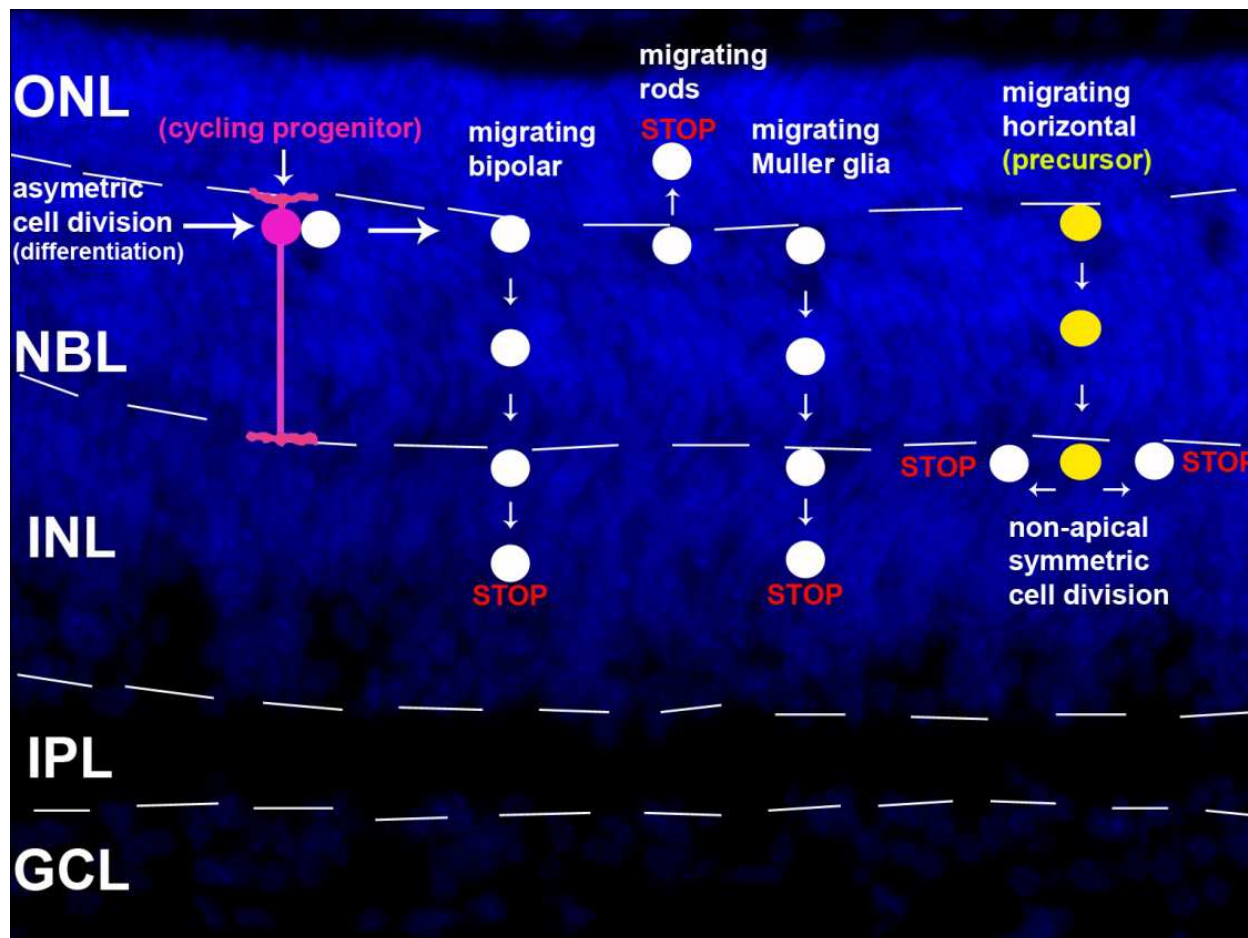
Fig. 4. Site of neuronal birth and migration patterns for retinal neurons born in the late postnatal phase of retinal cell differentiation.

Retinal cells are schematized on top of a photomicrograph of a P3, DAPI stained retina. Cycling progenitors (pink) divide asymmetrically at the apical surface of the NBL to eventually give rise to immature neurons that must migrate to their correct laminar position within the retina. Bipolar cells migrate through the NBL basally and into the middle of the forming INL. Rods remain at the apical surface of the retina, where the ONL is developing. Müller glia migrate in a similar fashion to bipolar cells to reach the INL. Horizontal cell precursors migrate into the apical surface of the forming INL, undergo a terminal symmetric division and migrate in a tangential fashion to find their final position in the INL.

The molecules involved in the apoptotic pathway have been extensively studied in several systems, including the retina (Burek and Oppenheim 1996). While a comprehensive summary is beyond the scope of this review, apoptotic molecules identified in the retina include death receptors, excitotoxic factors, proapoptotic Bcl-2 family proteins, proteases, DNases and transcription factors (for review, see (Isenmann et al. 2003)).

This review will summarize what is currently known and what are some unanswered questions about established tumor suppressor genes and their roles in different stages of retinal histogenesis.

2. Tumor suppressor genes regulate retinal progenitor cell proliferation

2.1 Introduction to the cell cycle

All tissues, including the retina, are genetically programmed to acquire an optimal size, which is determined both by the total number of cells and the sizes of individual cells (Gomer 2001). A major open question is how retinal progenitors know when to switch from making one cell type to the next so that appropriate numbers of each of the seven cell types are generated. In the retina, the choice of cell fate is intimately coupled, albeit not absolutely linked, to the timing of cell cycle exit. Given the central role that tumor suppressor genes play in regulating cell cycle exit, it is therefore not surprising that they are emerging as key regulators of the normal cell cycle in the developing retina. A general introduction to the cell cycle and the tumor suppressor genes that operate in this pathway is highlighted below.

During the cell cycle, dividing cells must replicate their DNA (S-phase) and then segregate a 2N complement of chromosomes into each daughter cell during mitosis (M-phase). S-phase and M-phase of the cell cycle are separated by two Gap phases (G1 before S and G2 before M), in which key checkpoints regulate entry into the next cell cycle phase. An important checkpoint in G1 is the restriction point, where the decision is made to either commit to another round of cell division or to exit the cell cycle and enter G0 (i.e., stop dividing). Two major classes of regulatory molecules control cell cycle progression - the cyclins and cyclin-dependent kinases (CDKs), which together form functional complexes (Nigg 1995). In general, CDKs are constitutively expressed whereas cyclin expression is more tightly regulated and restricted to specific cell cycle stages (Nigg 1995). Progression past the restriction point in G1 is regulated by complex formation between CDK4 or CDK6 with members of the Cyclin D family (D1/D2/D3). Active Cyclin D-CDK4/6 complexes phosphorylate the retinoblastoma protein (Rb), an important tumor suppressor gene that controls G1 progression (Paternot et al. 2010). When Rb is in its hypo-phosphorylated form, it is bound to E2F transcription factors, an interaction that is dissociated by hyperphosphorylation of Rb, releasing the E2F proteins so that they can initiate the transcription of genes required in S-phase of the cell cycle (Figure 5).

The CDK/cyclin complexes that operate in G1 phase of the cell cycle are negatively regulated by cyclin dependent kinase inhibitors (CDKIs), which block cell cycle progression. CDKIs fall into two families: 1) Cip/Kip (p21[Cip1], p27[Kip1] and p57[Kip2]) and 2) INK4 (p16[INK4a], p15[INK4b], p18[INK4c], and p19[INK4d]) (Besson et al. 2008). In the Cip/Kip family, *p57[Kip2]* and *p27[Kip1]* are considered tumor suppressor genes as mutations in these genes are associated with tumor formation in humans (Lee and Kim 2009; Guo et al. 2010). In contrast, *p21[Cip1]* is not often mutated in human cancers, but its deletion is associated with tumor formation in mouse models, suggesting that it does have tumor suppressor properties ((Franklin et al. 2000; Poole et al. 2004; Gartel 2009) and references therein). Moreover, *p21[Cip1]* mediates cell cycle exit induced in response to DNA damage by *p53*, a well known tumor suppressor that is frequently mutated in human cancers (Gartel 009). Finally, in the INK4 family, *p16[INK4a]* and *p19[INK4d]* are known tumor suppressor genes that block the assembly of Cdk4/6-cyclin D complexes, thereby inhibiting progression through G1 into S phase of the cell cycle (Besson et al. 2008; Wesierska-Gadek et al. 2010).
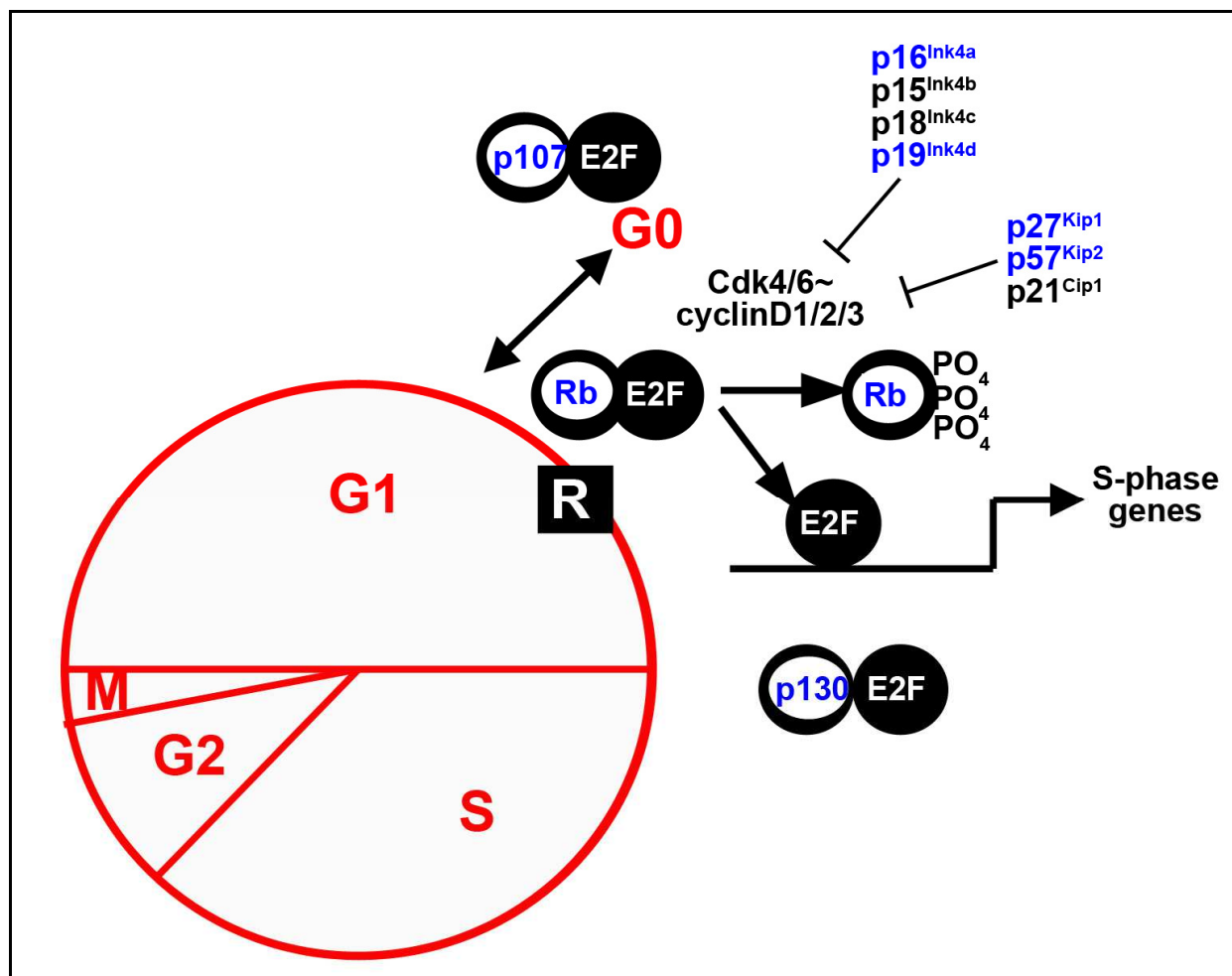
Fig. 5. Tumor suppressor genes and the cell cycle. Schematic illustration of the major phases of the eukaryotic cell cycle.

Progression through the restriction point (R) in G1 is controlled by Cdk4/6~cyclinD1-D3 complexes, which phosphorylate the Rb protein. When Rb is hypophosphorylated, it is bound to E2F transcription factors. When Rb is hyperphosphorylated, it releases E2F transcription factors, which can induce the expression of genes required for progression into S-phase of the cell cycle. The related pocket proteins p107 and p130 also complex with E2F transcription factors. The activities of Cdk4/6~cyclinD1-D3 complexes are negatively regulated by CDKIs of the Cip/Kip and INK4 families. Cell cycle regulators that are known tumor suppressor genes are listed in blue.

2.2 CDKIs regulate retinal progenitor cell proliferation

Of the Cip/Kip family members, only *p27Kip1* and *p57Kip2* have been studied extensively in the developing retina. During embryogenesis and in the early postnatal period, p27Kip1 and p57Kip2 are each expressed in a few scattered progenitor cells in the outer neuroblast layer (ONBL) as well as in a few postmitotic cells that are forming the INL. However, p27Kip1 and p57Kip2 are not co-expressed in the retina, suggesting that they function in distinct populations of retinal cells (Dyer and Cepko 2000; Dyer and Cepko 2001). The few cells that express CDKIs in the ONBL are thought to be those that are exiting the cell cycle and differentiating. Indeed, withdrawal of the growth factor EGF from cultured retinal

progenitor cells induces *p27^Kip1* expression in association with cell cycle exit and cellular differentiation (Levine et al. 2000). In gain of function experiments in E14.5 retinal progenitors, *p27^Kip1* promote premature cell cycle exit and differentiation (Dyer and Cepko 2000; Levine et al. 2000; Dyer and Cepko 2001). Conversely, in *p27^Kip1* mutants, uncommitted retinal progenitor cells, but not committed precursor cells, divide excessively during late retinogenesis (Levine et al. 2000; Dyer and Cepko 2001). Similar results were obtained in *Xenopus* retina. In gain-of-function experiments, *p27^Xic1* promotes cell cycle exit while loss-of-function conversely promotes retinal progenitor cell proliferation (Ohnuma et al. 1999). *p57^Kip2* is similarly sufficient and required for cell cycle exit of retinal progenitors, but it functions during an early stage of retinal development, with ectopic cell divisions observed as early as E14.5 in knock-out mice (Dyer and Cepko 2000; Dyer and Cepko 2001).

In the murine retina, the INK4 family member p19^INK4d is expressed in retinal progenitors at all embryonic and postnatal stages tested (Cunningham et al. 2002). Mutation of *p19^INK4d* results in increased retinal progenitor cell proliferation during early postnatal stages (Cunningham et al. 2002). Prolonged retinal progenitor cell proliferation is also observed in *p19^INK4d;p27^Kip1* double null retinae, including at P18, a stage when no BrdU+ proliferating cells are normally detected in the murine retina (Cunningham et al. 2002). The cause of the ectopic cell divisions was shown to be re-entry into the cell cycle by differentiated retinal neurons, including horizontal and amacrine cells (Cunningham et al. 2002).

The activities of p27^Kip1 and p19^INK4d are thus not temporally restricted in the retina – they function both in the embryonic and postnatal stages of retinal development to control cell cycle exit. In contrast, p57^Kip2 is only required during the embryonic early stage of retinal development to control cell cycle exit (Levine et al. 2000; Dyer and Cepko 2001).

2.3 *Rb* and its family members regulate retinal progenitor cell proliferation

The retinoblastoma protein (Rb) encoded by the *Rb1* gene was the first tumor suppressor gene identified (Chinnam and Goodrich 2011). In humans, mutation of *RB1* results in retinoblastoma, a devastating childhood tumor of the eye that robs children of their vision (DiCiommo et al. 2000). Rb prevents progression from G1 into S phase of the cell cycle by binding E2F transcription factors – an inhibition that is released by CDK4/6-cyclinD-mediated phosphorylation of Rb (Frolov and Dyson 2004; Burkhart and Sage 2008; Paternot et al. 2010). Rb is a pocket-protein that is highly related to two other family members, p107 and p130, which are similar in both sequence and function, also acting as nuclear phosphoproteins (Ewen et al. 1991; Hannon et al. 1993). Like Rb, p107 and p130 also act as negative regulators of cell proliferation through interactions with E2F transcription factors (Zhu et al. 1993; Claudio et al. 1994). It is now known that different Rb family proteins associate with different E2Fs at different times during the cell cycle (Bernards 1997).

In mouse models, animals heterozygous for an *Rb* null allele die at 6-8 months of age due to pituitary gland tumours, but display no evidence of retinal abnormalities, proliferation defects, or retinoblastoma (Clarke et al. 1992; Jacks et al. 1992). This is in keeping with evidence in humans that both *Rb* alleles must be mutated for tumors to arise (DiCiommo et al. 2000). In contrast, animals homozygous for an *Rb* null allele die in the embryonic period - between E12-E15 (Clarke et al. 1992; Jacks et al. 1992). While a detailed analysis of retinal development is not possible at these early stages, it is notable that no gross abnormalities or enhanced proliferation were observed in surviving *Rb* mutant embryos at E13.5 (Zhang et al. 2004). However, more S-phase cells were observed in retinae from homozygous *Rb* null

mutants that were explanted at E13.5 and cultured for 6 days *in vitro* (DIV - equivalent to early postnatal retinae *in vivo*) (Zhang et al. 2004). This suggested that *Rb* may only be required to regulate the cell cycle during early postnatal stages of retinal development, a model that was tested further with the advent of the Cre-LoxP system, and the ability to conditionally knock-out (cKO) *Rb* in the retina (Chen et al. 2004; MacPherson et al. 2004; Zhang et al. 2004). Using a Nestin-Cre driver, *Rb* cKO retinae were generated, and ectopic S- and M-phase progenitors were detected in the presumptive IPL/GCL at E18.5, although overall numbers of dividing cells were not significantly increased (MacPherson et al. 2004). Despite the increases in ectopic cycling and mitotic cells, paternal Nestin-Cre *Rb* cKO show no evidence of retinoblastoma but also die at early postnatal stages (MacPherson et al. 2004), likely due to the broad effect of deleting Rb in most of the developing brain.

One reason why *Rb* mutants may not display defects in retinal development may be because of compensation by *p107* and *p130* family members. Analyses of retinal development in *Rb;p107* double mutants was initially precluded by the death of these embryos by E11.5 (Robanus-Maandag et al. 1998). To circumvent this problem, chimeric embryos were generated with *Rb;p107* double mutant embryonic stem cells (Robanus-Maandag et al. 1998). *Rb;p107* double mutant chimeric embryos develop retinoblastoma by E17.5, with tissue hyperplasia originating in retinal cells committed to the amacrine cell lineage (Robanus-Maandag et al. 1998). Subsequently, the floxed *Rb* allele was used to analyze *Rb* mutations in combination with conventional $p107^{-/-}$ (Chen et al. 2004), $p130^{-/-}$ (MacPherson et al. 2004) or $p130^{-/-}; p107^{+/-}$ (Ajioka et al. 2007) mutations. Using a Pax6α-Cre driver, *Rb* cKO mice were generated, knocking out *Rb* in peripheral and not central retinal progenitors; this mutant allele was analyzed on a $p107^{-/-}$ background (Chen et al. 2004). At P0, in both *Rb* cKOs and *Rb* cKO; *p107* double knockouts, BrdU+ cells were not increased in number, but were ectopically positioned throughout the differentiated zones of the retina, where dividing cells are not usually detected (Chen et al. 2004). It was not until P8 (and up to P18) that *Rb* cKOs and *Rb* cKO; *p107* double knockouts displayed an overall increase in the number of BrdU+ cells (Chen et al. 2004; MacPherson et al. 2004). *Rb* cKO;*p107* double null mice also developed retinoblastoma as early as P8, with larger tumors apparent in older animals (Chen et al. 2004). Many of the ectopic BrdU+ cells were amacrine cells in *Rb* cKO and *Rb* cKO;*p107* double mutants, while tumors cells rarely expressed markers of photoreceptors, bipolar cells, or RGCs (Chen et al. 2004). Similar results were observed in *Rb* cKO;*p130* double knockout mice generated with a nestin-cre driver, which also developed retinoblastomas in adulthood, with tumors comprised of syntaxin+ and calretinin+ amacrine cells and not other retinal cell types (MacPherson et al. 2004). Thus, the Rb family of pocket proteins have an important role in regulating cell cycle exit in committed amacrine cell precursors, furthermore suggesting that amacrine cells may be the cell of origin for retinoblastoma.

While *Rb* cKO; *p107-/-* and *Rb* cKO; *p130-/-* double knockouts generated with Pax6α-cre and Nestin-cre drivers, respectively, both develop retinoblastomas derived from amacrine cell precursors, it was recently demonstrated that in *Rb* cKO;*p130-/-;p107+/-* animals that were generated with a Chx10-cre driver (commonly referred to as "p107 single" mice as they only express this one pocket protein), metastatic retinoblastoma develops in adults, with tumor cells derived from fully differentiated and synaptically coupled horizontal cells that have de-differentiated and re-entered the cell cycle (Ajioka et al. 2007). In contrast, in human retina, perinatal-derived retinoblastoma (most clinical cases of human familial retinoblastoma are detected during early infancy in children), which requires bi-allelic *Rb1*

mutation and/or inactivation, seems to arise from *Rb* mutant cells of the cone photoreceptor precursor lineage (Xu et al. 2009), rather than an amacrine or horizontal cell lineage as in mouse (Chen et al. 2004; Ajioka et al. 2007). The oncogenes *MDM2* and *n-Myc* are highly expressed in cone precursors and are required for the propagation of human retinoblastoma (Xu et al. 2009). It was also found that the cone-specific transcription factors *RXRγ* and *TRβ2* are also required for retinoblastoma proliferation and survival in several human retinoblastoma cell lines (Xu et al. 2009). Moreover, *RXRγ* was found to positively regulate the expression of the oncogene *MDM2* (Xu et al. 2009). Given the evidence found in this human study, it is difficult to say which of the currently generated mouse models of retinoblastoma most closely resemble (or successfully "model") bonafide human retinoblastoma. Further studies will be needed to understand the differences observed in mouse models and in human tumors with respect to the cell of origin for retinoblastoma.

2.4 *Zac1* and *Tgfβ2* regulate retinal progenitor cell proliferation

Zac1 is a tumor suppressor gene encoding a zinc finger transcription factor that promotes cell cycle arrest and apoptosis in cell lines, while germline mutations are associated with numerous carcinomas (Abdollahi et al. 1997; Spengler et al. 1997; Pagotto et al. 2000; Bilanges et al. 2001; Abdollahi et al. 2003; Koy et al. 2004). Similarly, we have shown that *Zac1* is required to elicit apoptosis and cell cycle arrest in the developing murine retina (Ma et al. 2007a). In *Zac1* null mutants, the retina becomes hypercellular in late retinogenesis, an increase in cell number that is associated with ectopic cell divisions in the early postnatal retina (Ma et al. 2007a). Conversely, in gain-of-function experiments, misexpression of *Zac1* blocks retinal cell proliferation (Ma et al. 2007a). Currently, it is not known how *Zac1* promotes cell cycle exit, either in tumor cells or in retinal progenitor cells (Spengler et al. 1997; Ma et al. 2007a). It seems unlikely that *Zac1* regulates retinal cell cycle exit through $p27^{Kip1}$ or $p57^{Kip2}$, as *Zac1* has a distinct requirement in late retinal progenitors, while $p57^{Kip2}$ functions in early progenitors, and $p27^{Kip1}$ functions throughout retinogenesis (Ma et al. 2007a).

We found that *Zac1* is required to induce expression of *transforming growth factor βII* (*TGFβII*) in the retina (Ma et al. 2007a). Notably, *TGFβII* is also a known tumor suppressor gene, and was shown to negatively regulate the proliferation of retinal progenitor cells and Müller glia (Close et al. 2005). Interestingly, previous reports have indicated that *TGFβ* regulates $p27^{Kip1}$ expression (Ravitz and Wenner 1997), whereas *Zac1* does not regulate $p27^{Kip1}$ expression (Ma et al. 2007a). However, Zac1 is known to associate with and modulate the activity of several transcriptional regulators, including p53 (Huang and Stallcup 2000; Huang et al. 2001). *p53* is a well known tumor suppressor that is required for the cellular response to TGFβ signaling in *Xenopus* mesoderm (Cordenonsi et al. 2003; Cordenonsi et al. 2007).

3. Tumor suppressor genes regulate retinal cell death

3.1 CDKIs regulate retinal cell death

Compensatory mechanisms exist in the retina to ensure that the final ratios of individual cell types remain constant, with excess proliferation often balanced by an increase in apoptosis. This is indeed the case in $p27^{Kip1}$ and $p57^{Kip2}$ mutants, where increases in cell death are thought to counterbalance the increased retinal progenitor cell proliferation observed in these mutants (Dyer and Cepko 2000; Levine et al. 2000; Dyer and Cepko 2001). While

19^{INK4d} knockout mouse retinae reported no change in apoptosis at postnatal stages (P10, P14, and P18), $p19^{INK4d};p27^{Kip1}$ double knockouts did show increased apoptosis at P10 and P14 (Cunningham et al. 2002). Thus, despite the increase in cell proliferation, cell number is not dramatically changed in $p19^{INK4d};p27^{Kip1}$ double null because apoptosis in these retinae is five times higher than in wild-type retinae (Cunningham et al. 2002). This cell death was unable to be rescued upon co-deletion of *p53*, as $p19^{INK4d};p27^{Kip1};p53$ triple knockout mouse retinae also showed increased levels of apoptosis at postnatal stages (Cunningham et al. 2002), indicating the underlying mechanism of death signalling is *p53*-independent.

3.2 *Rb* and its family members regulate retinal cell death

In all *Rb* mutant eyes, including in conventional *Rb* knockouts at E13.5 (Zhang et al. 2004), chimeric *Rb* knockouts (from E16 onwards) (Robanus-Maandag et al. 1998), and in *Rb* cKOs generated with Nestin-Cre (at E18.5) (MacPherson et al. 2004) or Pax6α-cre (from P0) (Chen et al. 2004; MacPherson et al. 2004), an increase in cell death was observed. Notably, this increase in cell death was no longer evident in E13.5 retinal explants from conventional *Rb* knockouts that were cultured for 12 DIV (similar to late postnatal stages *in-vivo*), suggesting that *Rb* is required to prevent apoptosis only during embryonic and early postnatal stages (Zhang et al. 2004). It was also reported that amacrine cells seem to be spared from apoptosis since they were not dramatically reduced in number in Rb mutants, unlike other retinal cell types (MacPherson et al. 2004). The mechanism behind the excessive cell death in Rb mutants is independent of *p53*, as *Rb* cKO;*p53* double knockout retinae displayed the same increase in apoptosis observed in *Rb* cKO single mutants (MacPherson et al. 2004). *Rb;p107* double chimeric knockouts (Robanus-Maandag et al. 1998) or *Rb* cKO;*p107* double knockouts generated with Nestin-cre (MacPherson et al. 2004) or Pax6α-cre (Chen et al. 2004) also displayed enhanced apoptosis from E17.5 onwards. The cells that were more prone to apoptosis in double knockout retinae was investigated in more detail, demonstrating that RGCs and other cell types undergo extensive cell death, but amacrine cells were selectively spared from apaoptosis (Chen et al. 2004). The authors of this study argue strongly that because amacrine cells are intrinsically death resistant in double *Rb* cKO;*p107* knockouts, they are capable of forming retinoblastomas, and that resistance to cell death may be a general feature of tumor formation. However, what remains to be determined is why amacrine cells are protected from cell death in Rb mouse mutants, while all other retinal cell types seem to undergo apoptosis when the pocket proteins are mutated.

3.3 *Zac1* regulates retinal cell death

Our analysis of *Zac1* mutant retinae revealed that there are fewer apoptotic cells in E18.5 explants cultured for both 4 and 8 DIV (Ma et al. 2007a). However, misexpression of *Zac1* was not sufficient to induce cell death in the murine retina (Ma et al. 2007a), although it was sufficient to induce apoptosis in the Xenopus retina (Ma et al. 2007b), as well as in mouse or human cell lines (Spengler et al. 1997; Varrault et al. 1998). Interestingly, an isoform of human *Zac1* lacking the first 2 zinc fingers (Zac1Δ2) has a reduced capacity to induce apoptosis and an increased ability to arrest cell cycle progression, suggesting that these two functions are carried out independently of one another (Bilanges et al. 2001). How *Zac1* induces apoptosis in the retina and elsewhere, remains to be determined.

4. Tumor suppressor genes and retinal cell fate specification

In the retina, the choice of cell fate is intimately coupled, albeit not absolutely linked, to the timing of cell cycle exit. This raises the question of how cell fate specification and cell cycle exit decisions are coordinated.

4.1 The CDKIs *p27*^{Kip1} and *p57*^{Kip2} influence cell fate decisions in the retina

Misexpression of *p27*^{Xic1} in *Xenopus* promotes Müller glial cell genesis, while conversely, inactivating *p27*^{Xic1} decreases Müller glial cell number (Ohnuma et al. 1999). *p27*^{Xic1} also has the ability to modulate the capacity of transcription factors to specify retinal cell fates in *Xenopus.* For example, *Xath5* has a more potent ability to induce an RGC fate when co-expressed with *p27*^{Xic1}, whereas *Xath5* promotes alternative "later" fates when cell cycle progression is stimulated by cyclin E1 (Ohnuma et al. 2002). In murine systems, *p27*^{Kip1} appears to function differently than its *Xenopus* homolog. For instance, misexpression of *p27*^{Kip1} in mouse or rat retinal cells increases the number of rod photoreceptors that differentiate (Levine et al. 2000; Dyer and Cepko 2001), suggesting that *p27*^{Kip1} may specify a photoreceptor cell fate. Providing further support for this idea, rat embryonic retinal progenitors express high levels of p27^{Kip1} in response to the withdrawal of EGF from the culture media, leading to cell cycle exit and preferential photoreceptor differentiation (Levine et al. 2000). However, if *p27*^{Kip1} is instead misexpressed together with Notch in mouse retinae, Müller glial cells are increased in number, indicative of a collaborative interaction between Notch and *p27*^{Kip1} in the specification of a Müller glial cell fate (Levine et al. 2000).

Despite these striking gain-of-function phenotypes, in mammalian loss-of-function experiments, conventional *p27*^{Kip1} knockout mouse display no overall changes in the final proportions of retinal cells that differentiate (Levine et al. 2000; Dyer and Cepko 2001). This indicates that while *p27*^{Kip1} is sufficient to alter cell differentiation in mammalian retinae, it seems to not be required to do so. This is in contrast to *p57*^{Kip2}, which does appear to have an essential role in cell fate specification in the mammalian retina (Dyer and Cepko 2000). In the mature mouse retina, p57^{Kip2} expression is restricted to a subset of amacrine cells (Dyer and Cepko 2000). In *p57*^{Kip2} knockouts, more calbindin+ amacrine cells are generated, while all other cell types are generated in proper numbers (Dyer and Cepko 2000). Currently, it is not known how *p57*^{Kip2} influences the genesis of calbindin+ amacrine cells, but it may be through collaborative interactions with transcription factors that are involved in specifying the identity of this amacrine cell subpopulation.

4.2 Rb pocket proteins influence cell fate decisions in the retina

The mutation of *Rb* by several genetic means results in a reduction in rod photoreceptor differentiation in the retina, including: 1) in conventional *Rb* knockout mice (E13.5 retinal explants cultured for 12 DIV) (Zhang et al. 2004), 2) following infection of floxed *Rb* retinae with a cre-expressing retrovirus (Zhang et al. 2004), and 3) in *Rb* cKO retinae generated with Nestin-cre (MacPherson et al. 2004) or Pax6α-cre (MacPherson et al. 2004) drivers. RGCs and bipolar cells were also reduced in *Rb* cKO retinae generated with the Pax6α-cre driver (MacPherson et al. 2004), and in *Rb* cKO;*p107* double knockout retinae generated with the Pax6α-cre driver, which also had fewer rod photoreceptors (Chen et al. 2004). The loss of photoreceptors, bipolar cells, and RGCs in *Rb* mutants is likely due to increased apoptosis (Chen et al. 2004; MacPherson et al. 2004). The only evidence that *Rb* is involved in controlling the terminal differentiation of a retinal cell type comes from the analysis of

starburst amacrine cells, which require Rb-mediated inhibition of E2f3a, a cycle regulator and oncogene, in order to differentiate (Chen et al. 2007).

4.3 *Zac1* and *TgfβII* regulate retinal cell fate specification through novel pathways

Zac1 mutants develop supernumerary rod photoreceptors and amacrine cells, acting in a cell autonomous fashion to block rod cell differentiation, while acting non cell autonomously to block amacrine cell development (Ma et al. 2007a). Mechanistically, we showed that *Zac1* regulates amacrine cell number via a negative feedback loop that acts as a cell number sensor (Ma et al. 2007a). We found that *Zac1* acts in amacrine cells late in retinogenesis to positively regulate TGFβII expression, which then acts as a negative feedback signal to limit amacrine cell production (Ma et al. 2007a). We found that members of the TGFβ family of ligands (TGFβII) and receptors (TGFβ receptors I and II) are expressed in amacrine cells in wildtype retinae but *Zac1* mutant retinae express significantly less TGFβII and consequently, phosphorylated Smad2/3, a downstream effector, are reduced (Ma et al. 2007a). After showing that retinal explants exposed to exogenous antibodies towards TGFβ receptor II also generate more amacrine cells, we showed that adding exogenous TGFβII to *Zac1* mutant retinal explants rescued the supernumary amacrine cell phenotype (Ma et al. 2007a). Finally, we analyzed the E18.5 retinae of conditional *TGFβ receptor II* knockout mice and also showed qualitatively that there are more amacrine cells present in mutant retinae relative to wildtype (Ma et al. 2007a). A Zac1-TGFβII negative regulatory loop thus controls amacrine cell differentiation in the retina, ensuring that appropriate numbers of these cell differentiate.

5.Tumor suppressor genes and retinal cell migration

5.1 Cell cycle regulators and retinal lamination

The role of CDKIs has been best studied in the cell cycle, but there is growing evidence that CDKIs regulate multiple other processes, including cytoskeletal dynamics and cell migration (Besson et al. 2008). In the developing nervous system, p27[Kip1] has been implicated in regulating the migration of neocortical neurons, using domains of the protein that are not involved in cell cycle regulation (Nguyen et al. 2006). Consistent with a potential migratory role for *p27[Kip1]* in the developing retina, in the mature *p27[Kip1]* knockout mouse retinae, bipolar, horizontal and possibly a few amacrine cell bodies are displaced from their regular positions within the INL (Levine et al. 2000). Also, in *p19[INK4d]*; *p27[Kip1]* double knockout mouse retinae, there is apparent displacement of Müller glia and rod photoreceptors from their normal positions in the INL and ONL, respectively (Cunningham et al. 2002). However, the dysplasia of Müller glial cells and rods is reported to be almost completely rescued in *p19[INK4d]*; *p27[Kip1]*; *p53* triple knockout retinae (Cunningham et al. 2002), which suggests that p53 contributes to the p19/p27-dependent migration of rods and Müller glia. It was also reported that *Rb* cKO retinae generated with a Chx10-cre driver have ectopic Pax6+ cells in the ONL (Donovan and Dyer 2004), indicating that *Rb* regulates the proper radial migration of amacrine and/or RGCs to their proper layer within the developing retina. Finally, our analysis of *Zac1* mutant retinae revealed that amacrine cells migrate aberrantly, forming an ectopic cellular layer between the GCL and INL (Ma et al. 2007a). This suggests that *Zac1* is required to regulate the proper migration of amacrine cells in the retina. In all cases, it is poorly understood how tumor suppressor genes regulate cellular migration at the molecular level.

5.2 PI3K/PTEN signaling and retinal lamination

Pten (phosphatase and tensin homolog) encodes a lipid and protein phosphatase that is a negative regulator of the PI3K pathway. *Pten* is also a potent tumour suppressor, with loss of 10q23 heterozygosity commonly found in malignant glioblastomas (Li et al. 1997) and somatic *Pten* mutations found in multiple carcinomas (Steck et al. 1997; Ali et al. 1999). In the nervous system, *Pten* mutations are associated with hypertrophy of the cerebellum, neocortex and hippocampus (Backman et al. 2001; Groszer et al. 2001; Kwon et al. 2006; Lehtinen et al. 2011). *Pten* mutants also display defects in cell migration, lamination, dendrite arborization and myelination in different CNS domains (Marino et al. 2002; Yue et al. 2005; Kwon et al. 2006; Fraser et al. 2008). When *Pten* was deleted in the retinal pigment epithelium (RPE), photoreceptor degeneration was observed (Kim et al. 2008). Strikingly, PI3K signalling promotes progenitor cell proliferation and decreased apoptosis in the retina (Pimentel et al. 2002), and influences cell migration in other systems (Rosivatz 2007). Recently, our lab conditionally deleted the tumor suppressor *Pten* from the developing retina using the Pax6α-Cre driver line (Cantrup et al., submitted). We found that amacrine cells and RGCs were disorganized and scattered within the normally cell-sparse IPL, suggesting that PTEN regulates the proper radial migration of both amacrine and RGC's within the developing retina. We also have evidence that PTEN regulates the tangential migration of a subset of amacrine cells. Studies are currently ongoing to determine the molecular and cellular mechanisms by which PTEN regulates cell migration.

6. Tumor suppressor genes not yet characterized in retinal development

6.1 *Runx1*

In other parts of the developing mouse brain, *Runx1* is required for the development of selective spinal cord interneurons (Stifani et al. 2008), hindbrain cholinergic branchiovisceral motor neuron precursors, some sensory neurons in the trigeminal and vestibulocochlear ganglia (Theriault et al. 2004), and olfactory receptor sensory neurons (Theriault et al. 2005). According to GENSAT (gene expression nervous system atlas from NCBI), *Runx1* is expressed in the forming RGC layer at E15.5 in mice. Conventional knockouts for *Runx1* die at E12.5 (Okuda et al. 1996), prohibiting a detailed analysis of mouse retinal development. However, floxed-*Runx1* mice have been made (Kimura et al. 2010) and could be used to generate retinal-specific knock-outs.

6.2 *Tsc1* and *Tsc2*

In cultured rat immature hippocampal neurons, overexpression of *Tsc1* and *Tsc2* together inhibits neurite outgrowth, while conversely, knockdown of either *Tsc1* or *Tsc2* induces neurite growth and increases neurite number (Tavazoie et al. 2005). In the developing mouse neocortex, *Tsc1* conditional knockouts display ectopic axons, an effect also observed in E14.5 organotypic cortical slice cultures in which *Tsc2* is knocked down (Tavazoie et al. 2005). Mouse conventional knockouts for *Tsc1* and *Tsc2* die at E10.5-11.5 (Rennebeck et al. 1998; Kobayashi et al. 2001), prohibiting a detailed analysis of mouse retinal development. Both *Tsc1*- and *Tsc2*-floxed mice have been made (Uhlmann et al. 2002; Hernandez et al. 2007) and could be utilized to study the effect of *Tsc1* or *Tsc2* during retinal development in mice. GENSAT reports a moderate/high mRNA expression of both *Tsc1* and *Tsc2* in the GCL, IPL, INL and RPE at P7 in the mouse retina.

6.3 *Apc*

In the developing mouse cerebral cortex, the conditional loss of *Apc* results in an impaired formation of the radial glial scaffold, which leads to the defective migration of cortical neurons and subsequent layer formation, and the aberrant growth of axonal tracts (Yokota et al. 2009). *Apc* has also shown to be required in the maintenance, differentiation, and migration of neurons derived from adult neural stem cells in the subventricular zone and hippocampus of mice (Imura et al. 2010). GENSAT reports a moderate expression of *Apc* mRNA in the GCL, and a lower relative expression in the rest of retina, and a very high expression in the RPE, all at P7. Mouse conventional knockouts for *Apc* die before gastrulation (around E.5) (Moser et al. 1995), prohibiting any analysis of mouse retinal development. *Apc*-floxed mice have been made (Kuraguchi et al. 2006) and could be utilized to study the effect of *Apc* during retinal development in mice.

6.4 *Nf1* and *Nf2*

The conditional inactivation of *Nf1* results in the selective increase of neural stem cell proliferation and subsequent gliogenesis in the developing brainstem, but not in the developing cerebral cortex (Lee da et al. 2010). Conditional deletion of *Nf2* in GFAP+ cells show increased glial cell proliferation and deletion in adult Schwann cells showed Schwann cell hyperplasia, and other characteristics of neurofibromatosis type 2 (Giovannini et al. 2000). *Nf2* has been deleted in the developing lens as well showing that it is required for proper cell-cycle exit, differentiation and cell polarity of developing mouse lens cells (Wiley et al. 2010). GENSAT reports a moderate ubiquitous expression of *Nf1* mRNA in the neural retina, and a very high expression in the RPE at P7. GENSAT also reports a moderate "salt & pepper" mRNA expression of *Nf2* in retinal progenitors in the neuroblastic layer, and a relative higher expression in the forming GCL at E15.5. Later at P7, there is high *Nf2* expression in both the GCL/INL, and high expression in the RPE. Mouse conventional knockouts for *Nf2* die before gastrulation (around E5) (McClatchey et al. 1997), prohibiting any analysis of mouse retinal development. *Nf2*-floxed mice have been made (Giovannini et al. 2000) and could be utilized to study the effect of *Nf2* during retinal development in mice. Mouse conventional knockouts for *Nf1* die at E12.5-13.5 (Brannan et al. 1994), prohibiting a detailed analysis of mouse retinal development. *Nf1*-floxed mice have been made (Zhu et al. 2001) and could be utilized to study the effect of *Nf1* during retinal development in mice.

6.5 *Cdh1*

In adult mice, it was shown that *Cdh1* regulates the proliferation of neural stem cells in the subventricular zone (Garcia-Higuera et al. 2008). *Cdh1* has also been conditionally knocked out in the developing lens and it generated microphthalmia, iris hyperplasia, and lens epithelial cell deterioration (Pontoriero et al. 2009). GENSAT reports a very high expression of *Cdh1* in the INL and RPE, a moderate expression in the GCL, and a lower expression in rest of the mouse retina, all at P7. To our knowledge, mouse conventional knockouts for *Cdh1* were never made. However, *Cdh1*-floxed mice have been made (Garcia-Higuera et al. 2008) and could be utilized to study the effect of *Cdh1* during retinal development in mice.

7. Summary

Formation of a functional nervous system requires that appropriate numbers of the correct types of neuronal and glial cells are first generated and then migrate to their final

destinations. In some regions of the CNS, including the retina, precisely regulated patterns of cell proliferation and migration result in the formation of discrete neuronal layers, each comprised of stereotyped proportions of neuronal subtypes. Defective neuronal layering results in severe functional and visual deficits. In the last few decades, great strides have been made towards understanding how neurons acquire their specific identities during development, revealing a central role for both intrinsic and extrinsic factors. In contrast, the molecular cues that orchestrate tissue morphogenesis are less well understood. Research in the area of tumor suppressor genes has provided key insights into the molecular mechanisms that: 1) control neuronal number by regulating specific patterns of progenitor cell proliferation and differentiation and, 2) guide the complex migratory routes of individual neuronal populations, resulting in the formation of discrete layers in the retina. Such studies may aid in the future design of new stem cell therapies in the clinic, where the current challenge is to direct appropriate numbers of cells to differentiate, migrate and integrate into correct retinal layers, thereby allowing functional recovery.

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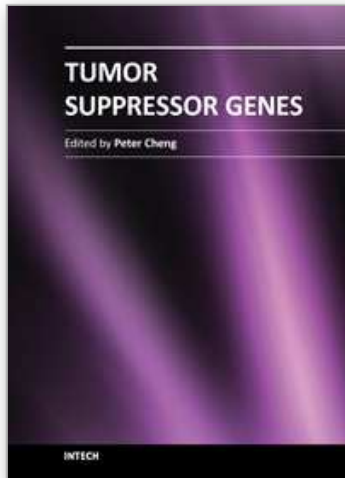
Tumor Suppressor Genes

Edited by Dr. Yue Cheng

Functional evidence obtained from somatic cell fusion studies indicated that a group of genes from normal cells might replace or correct a defective function of cancer cells. Tumorigenesis that could be initiated by two mutations was established by the analysis of hereditary retinoblastoma, which led to the eventual cloning of RB1 gene. The two-hit hypothesis helped isolate many tumor suppressor genes (TSG) since then. More recently, the roles of haploinsufficiency, epigenetic control, and gene dosage effects in some TSGs, such as P53, P16 and PTEN, have been studied extensively. It is now widely recognized that deregulation of growth control is one of the major hallmarks of cancer biological capabilities, and TSGs play critical roles in many cellular activities through signaling transduction networks. This book is an excellent review of current understanding of TSGs, and indicates that the accumulated TSG knowledge has opened a new frontier for cancer therapies.

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