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Invited Review

The retinoblastoma gene family and its role in proliferation, differentiation and development

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Summary. The retinoblastoma gene family is composed of three members: the retinoblastoma gene, one of the most well studied tumor suppressor genes and two related proteins, p107 and pRb2/p130. These three proteins share many structural and functional features and play a fundamental role in growth control. Intense investigation of these proteins has identified a series of similar cell-cycle regulators and transcription factors with which they interact. Although the precise function of the retinoblastoma gene product and its relatives remains unknown, recent data suggests that they play parallel roles in controlling cell cycle progression and promoting cellular differentiation. In this review, we will attempt to clarify some of the molecular mechanisms by which these three related proteins cooperate to control cellular proliferation and differentiation.

Key words: Tumor suppressor gene, Retinoblastoma gene family, Cell cycle, Development

RB family and cell proliferation

According to Knudson's "two-hit" hypothesis, many types of human cancers are thought to be caused by a loss of heterozygosity of tumor suppressor genes (Knudson, 1971, 1984). The role of tumor suppressor genes in the negative control of cell proliferation has become more and more intriguing within the last decade. Because inactivating mutations in these genes are associated with deregulated growth in tumor development, their gene products are likely to play important roles in the regulation of cell growth. The first tumor suppressor gene cloned was the retinoblastoma susceptibility gene Rb-1 (Friend et al., 1986; Fung et al., 1987; Lee et al., 1987). It encodes a nuclear phosphoprotein (pRb), that is found to be mutated in a wide range of human malignancies (Weinberg, 1991).

Many different types of genetic lesions, such as large-scale deletions, and splicing mutations resulting in the deletion of an exon, are involved in inactivation of the RB function (Horowitz et al., 1989; Kaye et al., 1990). In addition, point mutations and small deletions in the promoter region of RB1 have also been found (Bookstein et al., 1990a). After the cloning of the Rb-1 gene, several groups showed that mutations in both alleles of this gene were also present in several others human tumors, including osteosarcomas, bladder carcinomas, prostate carcinomas, breast carcinomas, small cell lung carcinomas, cervical carcinomas and leukemias (Horowitz et al., 1990). Since a large subset of human tumors carry mutations in the RB1 gene, and RB transcripts are present in all normal tissues examined, it is logical that this tumor suppressor gene plays an important role in the control of normal cell proliferation. This is further confirmed by the observation that introduction of a normal Rb-1 gene into cell lines lacking the functional gene reverses tumorigenicity, induces senescence, or alters cell proliferation (Huang et al., 1988; Bookstein et al., 1990b; Goodrich et al., 1991).

The most important indication of how pRb exerts its growth-suppressing properties came from the observation that the phosphorylation state of pRb oscillates regularly throughout the cell cycle (Chen et al., 1989; De Caprio et al., 1992). In G0 and early G1 phases of the cell cycle, pRb is found in a hypophosphorylated form, and as the cell cycle continues, pRb is phosphorylated in at least three steps: first in mid-G1, then in S, and then again near the G2-M transition. The protein is extensively dephosphorylated as the cells emerge from M and re-enter G0 or G1 phase (Hollingsworth et al., 1993). The viral oncoprotein E1A, T antigen and E7 bind specifically to unphosphorylated or hypophosphorylated pRb (De Caprio et al., 1988; Whyte et al., 1988; Dyson et al., 1989), suggesting that the hypophosphorylated pRb possesses a division-suppressing activity that can be turned off in G1 by phosphorylation or by association with viral oncoproteins (Sang et al., 1995). The endogenous kinase that phosphorylates pRb has not been clearly identified and it is possible that different cyclin-CDK complexes

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phosphorylate pRb during the different stages of the cell cycle. Many, and perhaps all, of the phosphorilations of pRb are carried out by complexes of cyclins and cyclin-dependent kinases (Dulic et al., 1992; Koff et al., 1992; Dowdy et al., 1993; Ewen et al., 1993; Grana et al., 1993; Kato et al., 1993). The ability of pRb/p105 to exert its growth-suppressive activity is due to its interaction with transcription factors, such as the family of the E2F/DP molecules, which function is to promote the transcription of genes required for cell-cycle progression (Chellapan et al., 1991; Chellapan, 1994).

pRb binds to E2F/DP heterodimers during the G1 phase of the cell cycle and blocks their transcriptional activities (Hiebert et al., 1992). Since E2F binding sites were found in the promoters of multiple growth-responsive and growth-promoting cellular genes, such as c-myc, c-myb, dihydrofolate reductase (DHFR), thymidine kinase (TK), thymidine synthetase, DNA polymerase α, RB1, cyclin A, cyclin D1, cdc2 and E2F itself, the Rb-E2F interaction is thought to block the activity of E2F as a transcription factor for genes whose products are required for the DNA synthesis (Chellapan et al., 1991). Cell-cycle-dependent phosphorylation of pRb, as well as complex formation with a number of DNA tumor viral oncoproteins (such as Adenovirus E1A, SV40 T antigen, and Papillomavirus E7) impair its ability to bind to E2F/DP complexes. This eventually results in the entry of the cells into the S phase of the cell cycle (see Fig. 1) (Nevins, 1992).

Two other proteins, p107 and pRb2/p130, previously identified by their interaction with adenovirus E1A oncoproteins, have been cloned and have been shown to be structurally and, in many aspects, functionally related to pRb/p105 (Ewen et al., 1991; Hannon et al., 1993; Li et al., 1993; Mayol et al., 1993). Sequence analysis of these two proteins reveals that they share large regions of homology with pRb, especially in two discontinuous domains which make up the "pocket region" (Mayol et al., 1993). The pocket domain is required for the binding of the three members of the Rb-family with the DNA tumor viral oncoproteins and for the complex formation with the E2F family of transcription factors (Moran,

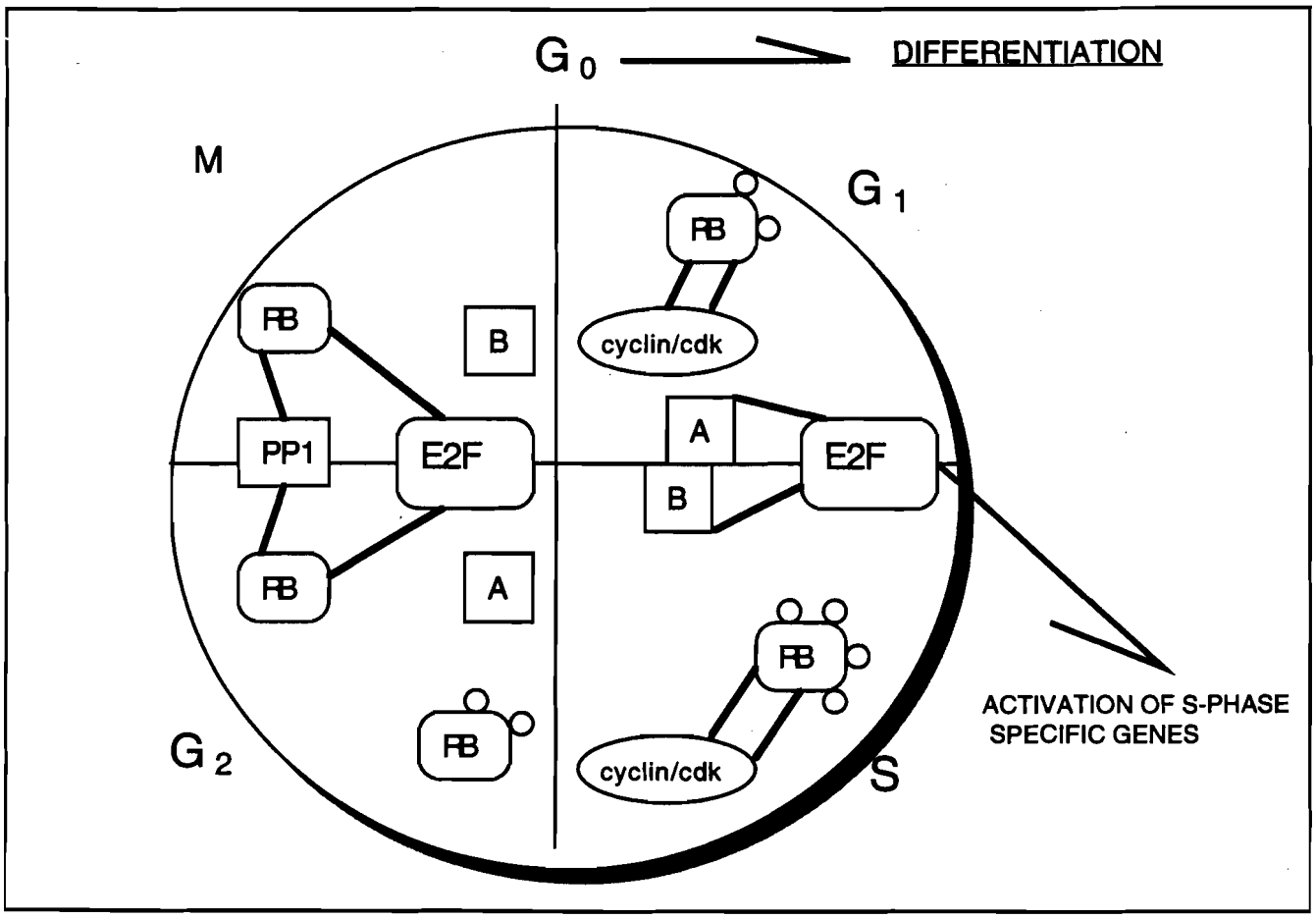

Fig. 1. The retinoblastoma gene in the cell cycle: the phosphorylation status of pRb is indicated by ○. Also indicated are the cyclin-cdk complexes that bind to pRb at the different stages of the cell cycle, the catalytic subunit of protein phosphatase type 1 (PP1 and see text) and the E2F transcription factor family (A and B represent hypothetic targets of the E2F family, that drive the entering in the S phase of the cell cycle.

1993).

Outside of these domains, pRb2/p130 and p107 still share regions of homology, but both differ remarkably from pRb. The structural identities are reflected in similar functional properties. In fact, it has been shown that these two new proteins have, like pRb, growth suppressive properties (Zhu et al., 1993, 1995; Claudio et al., 1994). For example, overexpression of either pRb or p107 can suppress the growth of SAOS-2, an pRb -/- and p107+/+ cell line of human osteosarcoma. pRb2/p130 has the same effects as those of pRb and p107 on SAOS-2 cells, however, it also inhibits proliferation of the human glioblastoma cell line T98G, which is resistant to the growth suppressive effects of both pRb and p107, suggesting that although the different members of the RB family may complement each other, they are not fully functionally redundant (Claudio et al., 1994).

All three RB family members inhibit cell cycle progression in the G1 phase (Goodrich et al., 1991; Zhu et al., 1995; Claudio et al., 1996). The mechanisms by which these three proteins exert their control on cell cycle progression are not fully understood, but are likely to involve pathways similar to that used by pRb, including formation of complexes with the cell-cycle machinery and members of the E2F family of transcription factors. In fact, both pRb2/p130 and p107 display a cell cycle regulated phosphorylation pattern (Beijersbergen et al., 1993; Baldi et al., 1995), and form complexes with different members of the E2F family of transcription factors with a varying temporal order of complex formation (Shirodkar et al., 1992; Cobrinik et al., 1993; Hijmans et al., 1995; Jiang et al., 1995; Vairo et al., 1995).

p107 maps to a chromosome region not commonly found altered in human neoplasias (Ewen et al., 1991). On the contrary, Rb2/p130 has been mapped to human chromosome 16q12.2, an area that has been found frequently altered in several human neoplasias including breast, ovarian, hepatic, and prostatic carcinomas, suggesting an involvement of the Rb2/p130 gene in human cancer as a tumor suppressor gene (Yeung et al. 1993). In addition, the gene responsible for familial cylindromatosis, a rare autosomal dominant disease characterized by multiple neoplasms originating from the skin appendages, has been recently localized to chromosome 16q12-q13, thereby further lending credence to the hypothesis that the Rb2/p130 gene is a tumor suppressor gene (Biggs et al., 1995). Moreover, preliminary studies on the differential expression of the three members of the retinoblastoma gene family in lung cancer suggest an important role for Rb2/p130 in the pathogenesis and progression of this kind of neoplasia (Baldi et al., 1996a). Further studies on the genetic structure and the protein expression of both p107 and pRb2/p130 in several tumor cell lines, as well as various human tumor specimens, will be necessary in order to identify possible, spontaneously occurring, genetic alterations.

RB family in development and differentiation

Many recent studies have pointed to a role for the retinoblastoma gene product, pRb, in differentiation and development, in addition to its well established role as a cell cycle regulator at the G1 phase of the cell cycle (De Luca et al., 1995). Direct evidence for this hypothesis came from two genetic approaches in murine systems: RB gene knock-out and RB "gain of function" studies (Clarke et al., 1992; Jacks et al., 1992; Lee et al., 1992; Bignon et al., 1993). In the first case, the phenotype of the RB(-/-) animals shows gross defects in hematopoietic and central nervous system development. RB(-/-) mice died in utero at day 14 of gestation. Therefore, the RB(-/-) phenotype is lethal, not due to increased susceptibility to neoplastic transformation but rather due to a failure of some essential tissues to terminally differentiate. On the other hand an Rb "gain-of-function" created by ubiquitously overexpressing Rb in mice, resulted in a dwarf phenotype. Therefore, uncontrolled expression of Rb gene induces premature cell growth arrest and altered normal differentiation patterns in the entire animal.

Results from these studies and others support the notion that Rb1 is influential in governing the transition between cell growth and differentiation (Bernards et al., 1989; Coppola et al., 1990; Zhou et al., 1991; Richon et al., 1992; Gu et al., 1993; Hamel et al., 1993; Slack et al., 1993; Condorelli et al., 1995; Kranenburg et al., 1995). Of particular interest is the spectrum of expression of pRb2/p130 and p107 in some terminally differentiated tissues. For instance in the nervous system and in muscle high levels of expression for Rb2/p130 have been found, while p107 is expressed at very low levels (Baldi et al., manuscript submitted). Considering the fundamental role of pRb in differentiation and development, it is conceivable that the other two members of the retinoblastoma gene family may serve a similar function. Therefore, it is possible to construct a simple model in which, during terminal differentiation, the increased concentration of unphosphorylated pRb or pRb-related proteins bind to and inactivate the products of genes involved in cell growth progression. Another possible mechanism is that pRb or its related proteins bind to and enhance cell-type specific transcription factors, as it happens for pRb and MyoD in skeletal muscle cells in culture (Gu et al., 1993). Our data showing that some terminally differentiated tissues, such as muscle and neural tissue display a strong positivity for pRb2/p130, while low levels of expression were found for p107, supports the hypothesis that different levels of expression of these two proteins can contribute to the maintenance of the terminal differentiation status in these particular cells (Baldi et al. manuscript submitted). In developing mouse embryos, the Rb2/p130 protein is expressed as early as day 10 of gestation and reached a peak of expression around day 13 of gestation, implying a developmental regulation of the Rb2/p130 gene in murine ontogeny (Pertile et al., 1995). Although

targeted disruption of a single Rb allele does not lead to an overt developmental phenotype, disruption of both alleles of Rb1 in mice is lethal in utero at approximately day 14 of gestation. These results indicate that Rb does not have a measurable essential cell cycle function in early embryonic development, nor does it have an essential role in the ontogeny of most tissues, which have by and large completed their development and terminal cell differentiation events by day 14 of gestation. Since the levels of pRb2/p130 protein remained markedly elevated until at least day 17 of gestation, targeted disruption of the Rb2/p130 gene may result in a more pronounced aberrant phenotype for populations of cells which differentiate relatively late in gestation, such as those of the neural lineages.

The presence in the promoter region of Rb2/p130 of putative consensus sequences for transcription factors involved in several pathways of differentiation (Baldi et al., 1996b), further strengthens the hypothesis that it has a role in constraining cell proliferation and promoting terminal cell differentiation. Therefore, an universal scenario can be drawn in which pRb and its related proteins bind to several transcription factors to regulate not only the cell growth, but also the differentiation. A thorough understanding of p107 and pRb2/p130's function in cellular growth control and morphogenesis will undoubtedly evolve as gene engineering technologies are employed to manipulate expression of these genes.

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