Journal of Cellular Biochemistry 62:418–430 (1996)

Retinoblastoma Protein Family in Cell Cycle and Cancer: A Review

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Abstract Two genes, *p107* and *Rb2/p130,* are strictly related to *RB,* the most investigated tumor suppressor gene, responsible for susceptibility to retinoblastoma. The products of these three genes, namely pRb, p107, and pRb2/p130 are characterized by a peculiar steric conformation, called "pocket," responsible for most of the functional interactions characterizing the activity of these proteins in the homeostasis of the cell cycle. The interest in these genes and proteins springs from their ability to regulate cell cycle processes negatively, being able, for example, to dramatically slow down neoplastic growth. So far, among these genes, only *RB* is firmly established to act as a tumor suppressor, because its lack-of-function is clearly involved in tumor onset and progression. It has been found deleted or mutated in most retinoblastomas and sarcomas, but its inactivation is likely to play a crucial role in other types of human cancers. The two other members of the family have been discovered more recently and are currently under extensive investigation. We review analogies and differences among the pocket protein family members, in an attempt to understand their functions in normal and cancer cells. © 1996 Wiley-Liss, Inc.

Key words: tumor suppressor genes, retinoblastoma gene, p107, Rb2/p130, pocket protein, cell cycle

The retinoblastoma family consists of a group of genes which gives rise to a new understanding of the eukaryotic cell cycle homeostasis. These genes encode for proteins characterized by a peculiar "pocket" structure. At present, the family consists of three members, the most investigated one being the retinoblastoma (*RB*) gene, the prototype for the tumor suppressor genes, which codifies for a protein called pRb. The other genes of the family are *p107* and *p130,* also called *Rb2/p130,* which codify for p107 and pRb2/p130 proteins, respectively. The "pocket" name depicts the unique tridimensional structure characterizing these proteins, which is responsible for most of the specific and functionally relevant protein-protein interactions in which these molecules are involved.

The pocket family members were discovered initially by investigators working on oncoproteins produced by DNA viruses. In particular, a set of proteins associated with the adenovirus 5 E1A oncoprotein was identified and major bands representing these proteins were named according to their apparent molecular mass in SDS-PAGE [Yee and Branton, 1985; Harlow et al., 1986], including p60, p105, p107, p130, and p300. The subsequent characterization of these proteins first identified p105 as the product of the *RB* gene [Whyte et al., 1988], and then p60 as cyclin A [Giordano et al., 1989, 1991a; Pines and Hunter, 1990]. Later, genes encoding p107 [Ewen et al., 1991; Zhu et al., 1993] and pRb2/p130 [Mayol et al., 1993; Li et al., 1993; Hannon et al., 1993] were cloned using different strategies and these proteins were found related to pRb primarily at the level of the pocket structure. Now p300, together with CBP, defines a family of transcriptional adaptor proteins that are specifically targeted by the E1A oncoprotein [Arany et al., 1995; Yuan et al., 1996].

Abbreviations used: CBP, CREB-binding protein; cdk, cyclin-dependent kinase; C/EBP, CAAT/enhancer-binding protein; DP1, dimerization partner 1; NF-IL6, nuclear factor for IL6 expression; SCLC, small cell lung cancer; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

Received May 1, 1996; accepted May 8, 1996.

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GENES CODING FOR THE POCKET PROTEINS

The full-length human *RB* cDNA has been cloned by three different groups [Friend et al., 1987; Lee et al., 1987a; Fung et al., 1987]. It is formed by 4,757 nucleotides, containing an open reading frame that codes for a protein of 928 amino acids, with a computed molecular mass of 106,159 Da. The *RB* transcription unit consists of 27 exons in about 200 kb. The human *RB* gene is positioned on chromosome 13q14 [Bookstein et al., 1988].

Partial human *p107* cDNA has been cloned by Ewen et al. [Ewen et al., 1991]. The full-length cDNA has been cloned by Zhu et al. [Zhu et al., 1993] from the same group, and is formed by 3,960 nucleotides, containing an open reading frame that codes for a protein of 1,068 amino acids, with a computed molecular mass of 120,876 Da. The human *p107* gene is positioned on chromosome 20q11.2 [Ewen et al., 1991].

The human *Rb2/p130* cDNA has been cloned by Mayol et al. [Mayol et al., 1993] and by two other groups [Hannon et al., 1993; Li et al., 1993] using different cloning strategies. It is formed by 3,853 nucleotides, containing an open reading frame that codes for a protein of 1,139 amino acids, with a computed molecular mass of 128,402 Da. The *Rb2/p130* transcription unit consists of 22 exons spanning over 50 kb of genomic DNA [Baldi et al., 1996a]. The human *Rb2/p130* gene is positioned on chromosome 16q12.2 [Yeung et al., 1993; Li et al., 1993].

PROPERTIES OF THE POCKET PROTEINS

All three pocket proteins are localized mainly in the nuclear compartment of the cell [Lee et al., 1987b; Ewen et al., 1991; Baldi et al., 1995]. A schematic picture of pRb, p107, and pRb2/p130 is given in Figure 1, putting in evidence the zones of higher homology among the members of the family. Basically, the structures consist of (1) the N-terminal portion, (2) the pocket structure subdivided into domain A, spacer and domain B, and (3) the C-terminal portion, also called domain C. The pocket functional domains A and B are the most conserved and are responsible for most of the interactions involving either some endogenous proteins or viral oncoproteins. The functional modifications of the pocket proteins determined by these interactions is discussed below.

POST-TRANSLATIONAL REGULATION DURING THE CELL CYCLE

The *RB* gene product is a well-known substrate for either kinase or phosphatase activity, thus undergoing extensive and regular changes in its phosphorylation status throughout the cell cycle. In asynchronous cells, pRb is present at various degrees of phosphorylation which is well depicted as a microheterogeneous pattern typically evident in SDS-PAGE analysis; the more the molecule is phosphorylated, the more slowly it migrates. For this reason the apparent molecular mass of pRb ranges between 105 and 115 kDa, when estimated by SDS-PAGE [Lee et al., 1987b; Whyte et al., 1988; Buchkovich et al., 1989]. Canonically, active pRb is the underphosphorylated gene product and phosphorylation is believed to be a way to inactivate the protein without degrading it. Subsequently, pRb can be re-activated by a specific phosphatase activity [Ludlow et al., 1993; Afshari and Barrett, 1994; Durfee et al., 1993]. In G0 and early G1, pRb is underphosphorylated. In late G1, the protein becomes phosphorylated at the restriction point

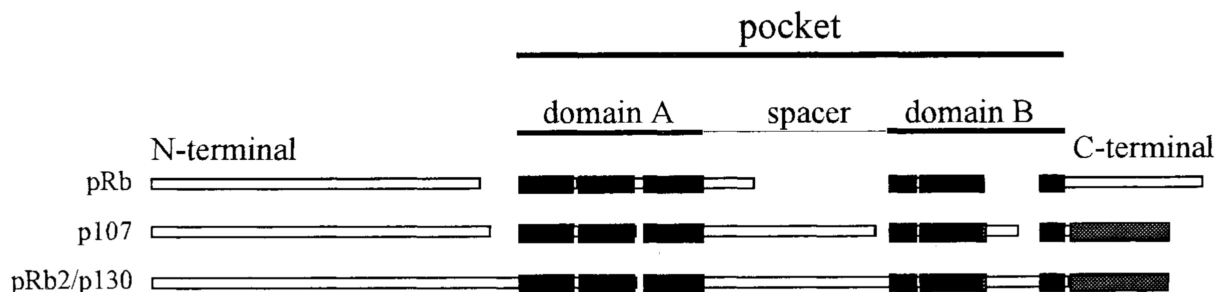

Fig. 1. The three proteins of the retinoblastoma family aligned according to their amino acid homologies in the pocket and in the C-terminal region. Black boxes indicate the homology regions in domain A and B of the pocket region among pRb, p107, and pRb2/p130. Gray boxes, only for p107 and pRb2/p130, indicate the homology regions in the C-terminal domain.

and phosphorylation increases in S phase and in the G2-M transition. The protein is found again to be underphosphorylated when the cell comes out from the mitotic process [Buchkovich et al., 1989; Chen et al., 1989; DeCaprio et al., 1989; Mihara et al., 1989] (Fig. 2). pRb is a substrate for specific cyclin/cdk complexes. In fact, several of them have been found to phosphorylate in vivo and in vitro pRb [Bandara et al., 1991; Hinds et al., 1992; Ewen et al., 1993; Harper et al., 1993; Grana et al., 1994]. With a closer look at the phases of pRb phosphorylation during the cell cycle, we can argue that D-type cyclin-dependent kinases might be responsible for early G1, cyclin E/cdk2 for mid/late G1, and cyclin A and cyclin B/Cdc2 for G2/M phosphorylation of pRb. After the cycle has been completed, cells generated by the mitotic process again display underphosphorylated pRb, because of the specific phosphatase activities. The stringent timing of the activity of the cyclin/kinase complexes on pRb is guaranteed further by the cdk-inhibitors [for a review see MacLachlan et al., 1995].

p107 also undergoes similar modifications during the cell cycle. The major complex responsible for its phosphorylation is the cyclin D1/cdk4 complex [Giordano et al., 1991b; Faha et al., 1992; Lees et al., 1992; Beijersbergen et al., 1995].

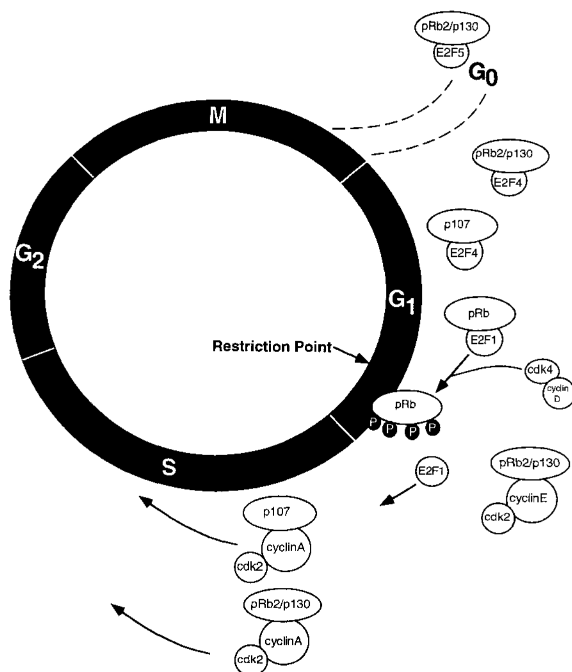

Fig. 2. Schematic representation of the interplay between the pocket proteins and the effectors which modulate their phosphorylation and their affinity for the E2F family of transcription factors. G0, G1, S, G2 and M indicate the respective phases of the cycle.

As far as pRb2/p130 is concerned, it displays evident cell cycle changes in phosphorylation, coupled to an extensive microheterogeneity in SDS-PAGE migration pattern [Baldi et al., 1995; Cobrinik et al., 1993]. It has been found associated with cyclin A and cyclin E and with cdk2 [Li et al., 1993; Hannon et al., 1993; Claudio et al., 1996]. From a functional point of view, cyclins A, D-type, and E overexpression rescue pRb2/p130-mediated growth arrest in SAOS-2 human osteosarcoma cells [Claudio et al., 1996].

INTERACTION WITH OTHER CELLULAR PROTEINS

The activity of pRb in cell cycle control is related essentially to its ability to bind to several proteins, thus modulating their activity. Among these, we should consider transcription factors.

The most investigated transcription factor which associates with the pRb pocket structure is E2F [Shirodkar et al., 1992; Cao et al., 1992; Chellappan, 1994; Sala et al., 1994; Jiang et al., 1995]. An E2F-binding site has been found in the promoter sequence of several growth promoting cellular genes, such as c-myb, c-myc, tymidine kinase, tymidine synthase, dihydrofolate reductase, DNA polymerase alpha, cyclin A, cyclin D1, Cdc2, and E2F itself [Horowitz, 1993]. Binding to the pocket region of pRb effectively sequesters the transcription factor, consequently blocking E2F-mediated growth stimulation [Chellappan et al., 1991; Hiebert et al., 1992; Schwartz et al., 1993; Helin et al., 1993a]. Underphosphorylated pRb binds to, sequesters, and neutralizes E2F in G0 and G1 phases. When pRb increases its level of phosphorylation, it decreases its affinity for E2F, whose release helps the cell cycle to proceed. E2F can complex the DP1 protein and it should be pointed out that E2F binding to pRb, indeed, takes place preferentially as E2F/DP1 [Girling et al., 1993; Helin et al., 1993b], an heterodimer functionally present in physiological conditions [Krek et al., 1993; Wu et al., 1995]. Besides the sequestering by pRb, the activity of the E2F/DP1 heterodimer is modulated further during the cell cycle by the phosphorylation of DP1. When the phosphorylation level of DP1 increases, the DNA binding activity of E2F/DP1 decreases proportionally [Bandara et al., 1994; Jooss et al., 1995]. Recently, other cDNAs encoding for E2F and DP1-related proteins have been cloned. These pro-

teins are named DP2 [Zhang, and Chellappan, 1995], E2F-2 [Ivey-Hoyle et al., 1993], E2F-3 [Lees et al., 1993], E2F-4 [Ginsberg et al., 1994; Sardet et al., 1995], and E2F-5 [Sardet et al., 1995; Hijmans et al., 1995] and contain structural homologies with E2F (now named E2F-1] in the region of the binding with the pocket structure of pRb [Krek et al., 1993] (Fig. 3).

Proteins p107 and pRb2/p130, sharing with pRb the characteristic of possessing the pocket structure, also share the ability to bind specifically to members of the E2F family [Cao et al., 1992; Lees et al., 1992; Shirodkar et al., 1992; Cobrinik et al., 1993; Beijersbergen et al., 1994; Ginsberg et al., 1994; Hijmans et al., 1995]. Several data sources suggest that pRb, p107, and pRb2/p130 associate with distinct E2F species in a puzzling and temporally modulated schedule. E2F-1, E2F-2, and E2F-3 complex with pRb, but do not interact with either p107 or pRb2/p130 [Chittenden et al., 1993; Dyson et al., 1993]. On the other hand, E2F-4 undergoes complex formation with both p107 and pRb2/p130 in vivo [Ginsberg et al., 1994; Beijersbergen et al., 1994; Vairo et al., 1995]. In G0 the major partner of E2F-4 is pRb2/p130. In G1, p107 replaces pRb2/p130 in this complex [Vairo et al., 1995]. The recently cloned E2F-5 has been isolated because of its ability to preferentially bind to pRb2/p130 [Hijmans et al., 1995]. In any case, the E2F-1 promoter results under E2F-dependent negative control during the cell growth response, being in G0 and early G1 transcriptionally repressed through E2F sites. Moreover, the presence of an E2F DNA-binding complex containing pRb2/p130 correlates with E2F-1 gene repression, and overexpression of pRb2/p130 inhibits transcription from the E2F-1 promoter. Also, D-type cyclin dependent kinase activity specifically activates the E2F-1 promoter by relieving E2F-mediated repression, resulting, on the other hand, in inhibition by the coexpression of *p16^INK4A^* [Johnson, 1995]. However, the role of pRb/E2F interplay is not easy to summarize [Weintraub et al., 1995].

A recently discovered and functionally important protein-protein interaction involves pRb and NF-IL6, a member of the C/EBP family of transcription factors [Chen et al., 1996]. When U937 large-cell lymphoma line is induced to differentiate along a monocyte/macrophage lineage, pRb is noted to interact with NF-IL6 via its SV40 large T-binding domain, enhancing NF-IL6 binding activity in vitro to its cognate DNA sequences. This indicates a potential novel biochemical function of underphosphorylated pRb which upregulates the activity of specific transcription factors important for differentiation, simultaneously downregulates, in a synergistic manner, transcription factors, such as E2F-1, which promote progression through the cell cycle.

Among the other proteins showing specific interaction with pRb, the c-Abl proto-oncoprotein, whose tyrosine kinase activity is regulated in the cell cycle through a specific interaction with pRb, should be mentioned [Welch and Wang, 1993]. Binding takes place between the domain C of underphosphorylated pRb, which lies outside the pocket, and the ATP-binding lobe of the c-Abl tyrosine kinase domain, resulting in inhibition of the kinase activity in G0 and

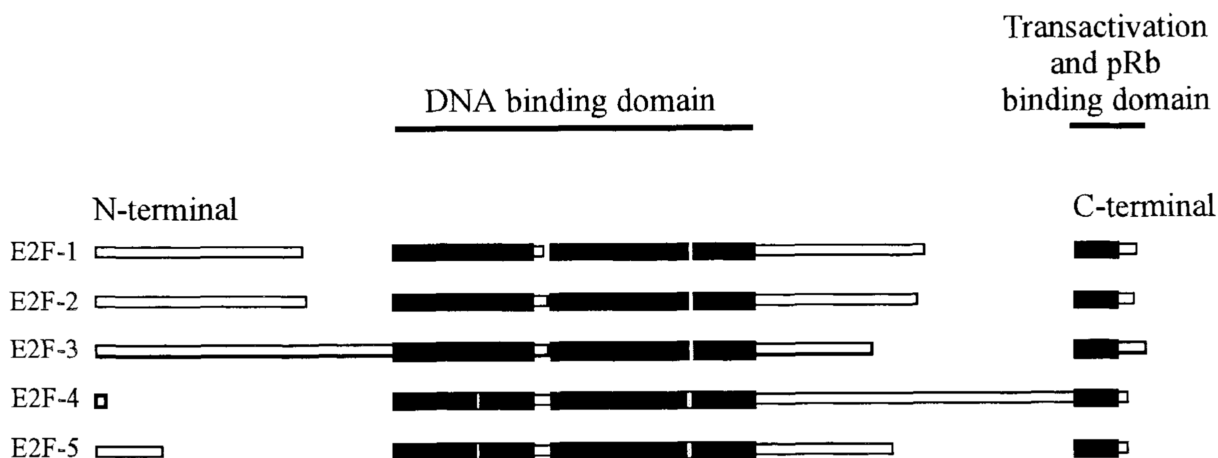

Fig. 3. The five E2F family proteins aligned according to their amino acid homologies (black boxes) at the level of the DNA binding domain and of the transactivation/pRb binding domain, which is located in the C-terminal region.

G1 cells. This interaction is not affected by the viral oncoproteins E1A, large T or E7. pRb phosphorylation at the G1/S boundary correlates with release of c-Abl and with activation of the tyrosine kinase in S phase cells.

ROLES OF THE POCKET PROTEINS IN SOME PHYSIOLOGICAL CONDITIONS

Differentiation

Checkpoints regulate progression during the cell cycle and are mandatory for many essential physiological functions. For example, a cycling cell cannot undergo differentiation, and these checkpoints may also be responsible for the withdrawal of a cell from the cycle, in order to commit it to terminal differentiation [Chen et al., 1995].

The pocket proteins, because of their ability to negatively regulate cell proliferation and to their precise relationship with the checkpoints, have been found often to be involved in differentiative processes. Differentiation in several human leukemia cell lines by means of exogenous agents, such as retinoic acid or phorbol esters, leads to accumulation of underphosphorylated pRb which is considered the form responsible for its growth suppressive properties [Chen et al., 1989; Buchkovich et al., 1989; DeCaprio et al., 1989; Akiyama and Toyoshima, 1990]. Also in human fibroblasts, terminal withdrawal from the cell cycle during senescence is associated with pRb dephosphorylation [Stein et al., 1990].

Differentiation along a monocyte/macrophage lineage requires the NF-IL6 transcription factor [Natsuka et al., 1992], which upregulates its transcriptional activity after binding to underphosphorylated pRb [Chen et al., 1996]. This is the same form that binds to and inactivates E2F-1, whose transcriptional effects push the cell to progress through the cell cycle [Nevins, 1992; Shirodkar et al., 1992; Cao et al., 1992]. Thus it can be argued that pRb can serve as a mediator for signals leading to either cell growth or differentiation.

A peculiar and well-defined role is played by pRb in terminal differentiation. A clear example comes from muscular tissue, where pRb plays a pivotal role in inducing and maintaining the differentiated phenotype in muscle cells, due to its interaction with MyoD [Caruso et al., 1993; Gu et al., 1993]. It has been demonstrated also that MyoD induces the *RB* promoter activity in a way distinct from its myogenic function [Martelli et al., 1994]. An involvement of the pocket protein family, and mainly of pRb2/p130, in muscle and neuronal differentiation comes from the characterization of E2F transcription factor complexes found during the in vitro induced differentiation of C2-C12 myotubes and p19 neuronal cells [Corbeil et al., 1995]. These data are further confirmed by an immunohistochemical study of the expression of the *Rb2/p130* protein in normal adult tissues. The highest levels of expression for the protein were found in muscle and neuronal cells (Baldi et al., manuscript in preparation). Moreover, in the promoter region of Rb2/p130 gene several putative consensus sequences have been found for transcription factors involved in several pathways of differentiation [Baldi et al., 1996a; for review see Giordano and Kaiser, 1996b].

Embryonic Development

In mouse embryo, the expression of *RB* mRNA is ubiquitous, with a peak around day 13 post coitum. The highest level of expression is observed in liver and brain [Bernards et al., 1989]. Immunohistochemical detection in SCID mice reveals that pRb becomes detectable around day 10.5 post coitum, appearing mainly in the phosphorylated form. The underphosphorylated form appears in significant amount only later in development. These authors find the highest amounts of protein to be in retina, megakaryocites, liver hematopoietic islands, osteoblasts, and in other specialized tissues [Szekely et al., 1992].

p107 expression is also subjected to developmental regulation at the level of mRNA accumulation in mouse embryos. Prominent expression of a 4.9 kb *p107* transcript is detected in fetal heart and liver, whereas only low levels of the transcript were present in most other tissues examined [Kim et al., 1995].

In the case of the *Rb2/p130* gene, the protein, evaluated in Western blot in a total embryo lysate, appears at day 10 post coitum, peaks around day 13 and remains high until late in gestation. In the adult mouse, the highest levels of *Rb2/p130* mRNA expression are found in muscle, kidney, and liver [Pertile et al., 1995].

In order to define more accurately the role of pRb in mouse development and in tumorigenesis, a mouse strain with an insertional mutation in exon 20 of the *RB* locus was generated. Homozygous mutants do not survive day 16 post coitum, showing major developmental abnormalities at the level of hematopoietic and nervous tissues. Reinsertion of a *RB* minitransgene corrects the developmental anomalies

[Lee et al., 1992]. Another engineered mouse strain, in which one allele of *RB* is disrupted, brings to viable *RB* heterozygous mice with a predisposition to pituitary neoplasms, but, interestingly enough, not to retinoblastoma [Jacks et al., 1992].

Strains of mice deficient for each of the other two *RB* family members have been generated. Preliminary studies on these mice revealed no obvious abnormalities [Lee et al., 1996; Cobrinik et al., 1996]. However, $Rb^{+/-}; p107^{-/-}$ mice have a pronounced growth retardation and increased mortality rate during the first 3 weeks after birth. Embryos homozygous for both *Rb* and *p107* die at 11.5 days of gestation, 2 days earlier than embryos homozygous for *Rb* alone. On the other side, mice having simultaneous inactivation of the *p130* and *p107* genes exhibited deregulated chondrocyte growth, shortened limbs, and neonatal lethality.

INTERACTION WITH VIRAL ONCOPROTEINS

As mentioned before, all the members of the pocket family share the ability to interact physically with the E1A oncoprotein via the pocket region [Whyte et al., 1988]. pRb also interacts specifically with the SV40 large T [DeCaprio et al., 1988] and with the human papillomavirus E7 oncoproteins [Dyson et al., 1989b]. Like pRb, p107 and pRb2/p130 also bind to key transforming sequences of these viral oncoproteins [Dyson et al., 1989a; Ewen et al., 1991; Marsilio et al., 1991; Giordano et al., 1991; Dyson et al., 1992; Mayol et al., 1993; Ludlow and Skuse, 1995].

These viral oncoproteins seem designed to actively compete with the E2F family members in binding to the pocket proteins. E1A, for example, is able to dissociate E2F-1 from the pocket of pRb, despite the fact that the pocket segments responsible for the binding of E1A end E2F-1 may be different [Fattaey et al., 1993]. The removal of the E2F family members from the pocket structure and their consequent release in free form, also helps E2F-mediated transactivation. This greatly enhances cells' ability to proceed through the cell cycle to enter the S phase, where viral DNA can be replicated with very high efficiency.

The oncoprotein E7 actively disrupts E2F-p107 complexes in a cell cycle-dependent manner [Zerfass et al., 1995]. pRb2/p130 is shown to repress E2F activity which is restored by either E1A or E7 from the high-risk, but not from the low-risk papillomavirus, the latter being unable to associate with pRb, p107, and pRb2/p130 [Dyson et al., 1992].

POCKET PROTEINS AND CANCER

Over the past years, unscheduled mitosis has been considered to be a major characteristic of either benign or malignant neoplastic diseases. For this reason, cancer development has been epitomized as being ideally related to loss of control in the cellular strategies that regulate the cell cycle. Despite the fact that the complex and intricate mechanisms which drive the malignant transformations are being more and more understood, unprogrammed cellular replication still plays a key role in the conceptual schemes we use to understand, prevent, diagnose and cure cancer.

For these reasons we will now analyze the actual and the potential relationships between pocket proteins and cancer.

Growth Suppressive Properties

Because of their ability to interact with the cell cycle control machinery and their growth suppressive effects, pocket proteins have been highly considered for use in the control of neoplastic growth. As noted before, pRb, p107, or pRb2/p130 overexpression can drive cancer cells to growth inhibition or arrest [Huang et al., 1988; Bookstein et al., 1990b; Takahashi et al., 1991; Zhu et al., 1993; Antelman et al., 1995; Claudio et al., 1994]. However, some peculiar tumor cell lines do not seem to be equally responsive to any pocket protein [Muncaster et al., 1992]. For example, the SAOS-2 human osteosarcoma cell line, which possesses a truncated non-functional pRb molecule [Shew et al., 1990], is definitely sensitive to pRb or p107 growth suppressive properties [Zhu et al., 1993]. On the other hand, while pRb2/p130 overexpression slows down SAOS-2 growth, this protein has also been able to inhibit the proliferation in the T98G human glioblastoma multiforme [Claudio et al., 1994] and in the MCF-7 human mammary adenocarcinoma cell lines (Claudio et al., manuscript in preparation), while neither pRb nor p107 showed any inhibitory effect. This could be because of the fact that T98G and MCF-7 cell lines display both homozygous deletion of the $p16^{INK4A}$ gene [Lukas et al., 1995], a cdk4 and cdk6 inhibitor [Serrano et al., 1993]. Because of this, it is reasonable that pocket proteins, even overexpressed, could be extensively phosphorylated by specific cyclin/cdk complexes, thus be-

coming functionally inactive in blocking the cell cycle. Conversely, pRb2/p130, which has been found coupled to cdk2 [Hannon et al., 1993; Claudio et al., 1996] could overcome $p16^{INK4A}$ homozygous deletion, bringing about effective growth inhibition.

Tumor Suppression

The *RB* gene; responsible for the susceptibility to retinoblastoma, is the prototype of a class of genes whose inactivation appears to be causally related to cancer and, therefore, these genes are referred to as tumor suppressor genes [Klein, 1987; Hansen and Cavenee, 1988; Weinberg, 1991, 1995; Sang et al., 1995]. Gross alterations in the *RB* gene are shown to occur regularly either in sporadic or in inherited forms of retinoblastoma, where deletion of chromosome 13 segments, containing the *RB* locus, are reported frequently [Cavenee et al., 1983; Dryja et al., 1986; Knudson, 1989] and are considered as essential steps in the pathogenesis of these tumors [Lee et al., 1988; Hansen and Cavenee, 1988]. Alterations in the *RB* gene structure, leading to either loss or functional inactivation of the gene product, have been observed also in more common human tumors, such as, for example, SCLC [Harbour et al., 1988; Hensel et al., 1990], non-SCLC [Shimizu et al., 1994], breast cancers [T'Ang et al., 1988; Varley et al., 1989; Fung and T'Ang, 1992; Steeg, 1992; Cox et al., 1994; Gottardis et al., 1995; Pietiläinen et al., 1995], osteogenic sarcomas [Benedict et al., 1988; Hansen, 1989; Reissmann et al., 1989], leukemias [Cheng et al., 1990; Ahuja et al., 1991; Ginsberg et al., 1991; Kornblau et al., 1992, 1994; Neubauer et al., 1993; Weide et al., 1993, 1994; Paggi et al., 1995], prostate [Bookstein et al., 1990a,b; Phillips et al., 1994], and bladder [Horowitz et al., 1990; Wolff et al., 1994] carcinomas and malignant gliomas [Venter et al., 1991; Godbout et al., 1992; Kyritsis and Saya, 1993; Paggi et al., 1994].

Deletion or functional inactivation for *p107* or *Rb2/p130* has not been described in human tumors yet, so their precise role in tumor suppression is still to be explained [Shirodkar et al., 1992; Riley et al., 1994]. One possible hypothesis is that these genes, which have functions similar to *RB*, despite concrete evidence that claims their functions are not overlapping, could be semi-redundant in vivo. This theory would establish a quite selective tissue-specificity for each pocket protein. So, frequent inactivation of *RB* is restricted to a subset of human tumors, such as retina and soft tissues sarcomas, and is a more rare event in other histotypes [Horowitz et al., 1990]. In this way, *p107* and *Rb2/p130* could be selective for tumor suppression in still unidentified, but specific, cells or tissues. As outlined above, pocket proteins, despite their deep structural similarities, display different binding properties with either endogenous or exogenous proteins, from the E2F family members to the viral oncoproteins.

It is interesting to note, however, that *p107* maps to the human chromosome region 20q11.2, a locus which is not commonly found damaged in human neoplasms [Ewen et al., 1991]. On the other hand, *Rb2/p130* maps to the region 16q12.2, an area frequently altered in human cancers, as breast, ovarian, hepatic, and prostatic carcinomas. This could, at least partially, support the involvement of *Rb2/p130* as a tumor suppressor in human cancers [Yeung et al., 1993]. Moreover, hereditary cylindromatosis, a rare autosomal dominant disease characterized by the development of multiple neoplasms originating from the skin appendages, is linked to loci on chromosome 16q12-q13 [Biggs et al., 1995]. The *Rb2/p130* gene, therefore, is one of the plausible candidate tumor suppressor genes causing this disease.

Transformation or Progression?

A large amount of experimental work has been done to understand the specific role of tumor suppressor genes in the development of cancer. As far as pocket genes are concerned, we will essentially discuss only data on *RB*, the only well-established tumor suppressor gene.

Many different groups which correlate *RB* with cancer induction, promotion, or progression report results that, on first analysis, are difficult to summarize. For example, we find that no particular importance is given by some investigators to *RB* in thyroid [Holm and Nesland, 1994] and in renal [Walther et al., 1995] tumors. Other groups report that loss of *RB* function may influence the pathogenesis and progression of lymphomas and leukemias [Ginsberg et al., 1991] and prostatic tumorigenesis [Phillips et al., 1994]. However, there is increasing evidence indicating the involvement of viruses in selected human tumors, such as papillomavirus-induced anogenital cancers, hepatitis B and C virus-associated hepatocellular carcinomas, nasopharyngeal carcinomas and

lymphomas linked to Epstein-Barr virus infection, and human T cell leukemia virus-associated adult T cell leukemias. So, in selected tumors, pRb is considered responsible for virus-induced tumorigenesis [Hoppe-Seyler and Butz, 1995; Saenz Robles et al., 1994]. In the future all these findings could be extended to the other pocket proteins, because of their ability to interact with viral oncoproteins in a way comparable with pRb. It is worthwhile, at this point, to outline the potential role of pRb2/p130 in the nasopharingeal carcinoma [Claudio et al., 1994]. Aside from the viral cooperation, it has been demonstrated also that pRb is implicated in cyclin-mediated tumorigenesis [Peters, 1994; Lukas et al., 1995].

Other investigators claim, however, that *RB*, at least, plays a pivotal role in the development of advanced primary bladder carcinoma and could have a role in certain renal cell carcinomas [Ishikawa et al., 1991], in breast cancer [Fung and T'Ang, 1992], in the passage through low-to high-grade lymphoproliferative diseases [Ludwig et al., 1993], in aggressive plasma cell dyscrasias [Corradini et al., 1994] and, generally, in hematological malignancies [Zhu et al., 1995].

All these results could match well with the concept of a supposed tissue-specificity we discussed above for all the pocket proteins. Moreover, it is important to consider species-specificity. Heterozygous deletion of the *RB* gene does not elicit the development of retinoblastoma in mice, putting, however, these animals in a tumor-prone situation [Jacks et al., 1992]. Basically this means that, while the *RB* gene in humans is strictly connected with the pathogenesis of retinoblastoma, this could not be the case for other mammalian species [Harlow, 1992]. The specific role of pRb could be mainly related to the type and amount of other proteins interacting inside or outside the pocket structure. Undoubtedly *RB* plays a role in tumor progression, since many experimental data correlate *RB* lack-of-function with progression in the multi-step process of carcinogenesis.

At this point, one could generalize that the same behavior could be predicted for the two other members of the family, p107 and pRb2/p130. So far, despite much experimental effort, no corroborating data give evidence for a tumor suppressor activity of these two gene products, and the information that knock-out of their genes in mice give a null phenotype, is not encouraging.

Very recent data are coming from the analysis of the expression of pRb2/p130 in the LAN-5 human neuroblastoma, a cell line able to undergo morphological and biochemical differentiation in the presence of retinoic acid [Tonini et al., 1991]. During the time-course of the differentiation process, as predicted by data published in other differentiating systems [Chen et al., 1989; Akiyama and Toyoshima, 1990; Whyte and Eisenman, 1992], pRb appeared down-regulated and present essentially in its under-phosphorylated form. The other pocket protein pRb2/p130, on the other hand, showed a marked increase by Western blot during retinoic acid-induced differentiation (Paggi et al., unpublished results). In accordance with these data in vitro, immunohistochemical results of paraffin-embedded sections of 77 human lung cancers, including epidermoid, small-cell lung cancer, carcinoid and adenocarcinoma, the pRb2/p130 amount was significantly inversely correlated with morphologically evaluated tumor malignancy [Baldi et al., 1996b], so that efforts are presently underway to identify pRb2/p130 as a potential and interesting marker for differentiation.

CONCLUSIONS

Studies in this field are expanding the knowledge of the connections between the fundamental pathways governing normal cell growth, differentiation, and embryogenesis and those responsible for oncogenesis. The role of growth regulatory genes, as well as the interplay with tumor suppressors, is far from being completely understood. For example, we recognize that data on cooperative, redundant, or selective roles of the pocket proteins in regulating cell cycle and cancer proliferation have been pointed out, but also that, on the other hand, a final explanation is yet to be reached.

We hope that further dissection and analysis of the mechanisms regulating cell cycle in normal and cancer cells will lead to new significant synthetic processes, allowing us to gain a better knowledge of these biological mechanisms, and improving practical approaches to successfully conquer human malignancies.

ACKNOWLEDGMENTS

Research in our laboratories is supported by P.F. CNR-ACRO, AIRC, and Ministero della Sanità grants to M.G.P. and NIH grant RO1 CA60999-01A1, and The Tobacco Research

Council to A.G., and assisted by Sbarro Institute for Cancer Research and Molecular Medicine. A.B. is on leave of absence from the Department of Anatomic Pathology, School of Medicine, University "Federico II," Naples, Italy. F.B. is an AIRC postdoctoral fellow. We would like to thank Candace M. Howard for her contribution to the writing of this manuscript. The authors also thank P.G. Natali, A. Felsani, and R. Tecce for critically reviewing the manuscript.

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