the mesophilic temperature range; and achieve COD reductions up to 90 percent. Three of these plants process beet sugar wastes, two process distillery wastes, and one processes citric acid wastes. The first full-scale, two-phase plant was built in 1980 in Belgium for the stabilization of liquid wastes generated during flax retting and has a capacity of 350 kg of COD per day; 87 percent COD reductions are obtained. The gas production rate is about 4.0 volumes(n) per liquid volume per day, and the yield is about 0.40 m^3(n) per kilogram of COD added. Three other full-scale, two-phase plants have been installed in West Germany to stabilize wastes from beet sugar, starch-to-glucose, and potato chip factories (*46*). Their capacities are 15,000, 20,000, and 32,000 kg of COD per day, respectively. A 9000-kg-COD-per-day plant for brewery wastes is under construction. Two-phase digestion process technology is available for license in the United States and Canada (*47*).

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Retinoblastoma: Clues to Human Oncogenesis

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Two major recent advances in human cancer research have been the detection of putative human oncogenes (*1*) and the delineation of tumor-specific chromosomal aberrations that might allow the expression of these oncogenes (*2*). In this article we discuss chromosomal evidence that supports the role of a diploid pair of "suppressor" alleles at the retinoblastoma locus in the development of this human tumor (*3–5*). This diploid retinoblastoma gene (wild-type alleles Rb+/Rb+) located in chromosomal region 13q14 (*6–8*) apparently functions in a fundamentally different way from the postulated mechanisms by which putative human cancer oncogenes are thought to produce tumors (*1*). In the case of the Rb gene it would appear that loss of function (Rb+/Rb+ → rb−/rb−) rather than gene activation or alteration as proposed for oncogenes is associated with the appearance of malignancy. Specific chromosomal changes in the retinoblast resulting in homozygosity or hemizygosity for the "mutant" or inactive allele appears to be a key mechanism leading to tumor formation (*3–5*). In addition, specific nonrandom chromosomal changes found in retinoblastoma suggest a potential role for an "expressor" gene (possibly an oncogene) in the etiology of this tumor. The evidence for both a suppressor and an expressor system in retinoblastoma will be presented in this article.

Genetics of Retinoblastoma

Retinoblastoma and certain other childhood tumors most likely arise from embryonal cells and could result from as

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few as two events, the first of which can be inherited. Solid childhood tumors appear to occur in two distinct groups of patients. Those in one group carry a germinal "mutation," have multifocal tumors (in the case of retinoblastoma, tumors develop in both eyes), and are at high risk for developing a second primary malignancy. Those patients in the second group do not carry a germinal mutation, have unilateral disease, and are at no increased risk for developing a second primary malignancy. In addition, the average age at tumor diagnosis for patients without the germinal mutation is significantly greater than for patients carrying the mutation. These two clinical subgroups are particularly obvious among retinoblastoma patients but are also easily identifiable in two other childhood tumors, namely, Wilms' tumor and neuroblastoma (*9*).

Retinoblastoma occurs in hereditary, nonhereditary, and chromosomal deletion forms. As used in this article, hereditary Rb refers to clinical situations in which there is a positive family history of the tumor, or in the case of sporadic disease, where the individual is bilaterally affected. No chromosomal defect is found in peripheral lymphocytes. The term nonhereditary Rb is applied to individuals with unilateral disease, no family history of the tumor, and no chromosomal abnormality in peripheral lymphocytes. It is estimated, however, that approximately 15 percent of patients with sporadic unilateral disease actually have the hereditary form of Rb. In the chromosomal deletion form of the tumor, the peripheral lymphocytes have a deletion of chromosomal region 13q14. Since affected individuals with all forms of Rb frequently survive and reproduce, Rb is a particularly useful model in which to study the genetic mechanisms involved in tumorigenesis. The locus for hereditary Rb has been assigned to chromosomal region 13q14 (*8*), the precise region of least common overlap in the 13-deletion form of the tumor (*6, 7*).

Possible genotypes at the Rb locus are outlined in Table 1. If only one eye is involved and there is no family history of retinoblastoma, the tumor is usually nonhereditary and the constitutional genotype is presumed to be Rb+/Rb+. When both eyes develop retinoblastoma or when there is a positive family history of the tumor, a mutant or inactive allele of germinal origin is assumed with a genotype of Rb+/rb− and dominant inheritance is the rule. Approximately 2 to 3 percent of patients with retinoblastoma have the 13-deletion form of the tumor and a genotype Rb+/−. In addition, a significant percentage of patients with retinoblastoma may have a chromosome deletion mosaicism involving 13q14 (*10*). Consequently the frequency of a microscopically visible deletion including 13q14 as the first "hit" could be considerably underestimated (*10*).

Table 1. Retinoblastoma genotypes at the Rb locus in 13q14.

Genotype	Sporadic unilateral	Hereditary bilateral	13 Deletion
Constitutional	Rb+/Rb+	Rb+/rb−*	Rb+/−
Tumor	rb−/rb− or rb−/−	rb−/rb− or −/rb−	rb−/− or −/−

*The genotype referred to when a patient is said to carry the retinoblastoma "gene."

Although pedigrees of affected families show typical dominant inheritance of the tumor, the inheritance of one inactive or deleted allele (Rb+/rb− → rb−/rb− or Rb+/− → rb−/−) is not in itself dominant at the cellular level (*3–5*). A second event (Rb+/rb− or Rb+/− → rb−/rb− or rb−/−) is required for retinoblastoma to develop. It is the near certainty of this second event occurring in at least one retinoblast that accounts for the dominant pattern of tumor inheritance observed clinically. The presence of the Rb gene (Rb+/rb−) in an individual increases his risk of developing this tumor by more than 100,000 times (*9*). The retinoblast is not the only target for this extremely potent human cancer gene, however, as detailed below.

Summary. The retinoblastoma gene can be considered a model for a class of recessive human cancer genes that have a "suppressor" or "regulatory" function. The loss or inactivation of both alleles of this gene appears to be a primary mechanism in the development of retinoblastoma. Such a mechanism is in direct contrast to that of putative human oncogenes which are thought to induce tumorigenesis following activation or alteration. The high incidence of second primary tumors among patients who inherit one inactive retinoblastoma allele also suggests that this cancer gene plays a key role in the etiology of several other primary malignancies. Finally, the observation that extra nonrandom copies of specific chromosomal regions occur in some of these tumors provides circumstantial evidence that an "expressor" gene (possibly an oncogene) may be involved in retinoblastoma development.

Evidence the Rb "Gene" (Rb+/rb−) Is a Generalized Human Cancer Gene

Patients with hereditary retinoblastoma (constitutional genotype Rb+/rb−) are at an increased risk to develop second nonocular malignancies (*11*). Among survivors with hereditary Rb, osteosarcomas are diagnosed 2000 times more frequently in the skull after radiotherapy and 500 times more frequently in the extremities than would be expected in the general population (*11*). In contrast, the frequency with which osteosarcoma is diagnosed among survivors of nonhereditary retinoblastoma (constitutional genotype Rb+/Rb+) does not differ from that in the general population.

Recently, Abramson *et al.* have graphically defined the full clinical impact of the Rb+/rb− constitutional genotype (*12*). In their large series with long-term thorough follow-up, more than 50 percent of individuals who survived hereditary retinoblastoma were dead from a second primary malignancy within 30 years after diagnosis of the retinoblastoma (*12*). Thus, individuals who carry the retinoblastoma "gene" have a high probability of developing lethal tumors before age 35 years. The Rb gene, Rb+/rb−, therefore, must be considered a powerful generalized human cancer gene.

Theories of Oncogenesis

In 1971 Knudson developed the hypothesis that retinoblastoma is a tumor caused by two mutational events (*13*). He observed that patients with bilateral retinoblastoma were first diagnosed at a significantly earlier age than those patients with unilateral disease. He postulated that in the bilateral, hereditary form of the disease, the first mutation was of germinal origin and thus was present in all cells. Therefore, a relatively short time was required for the second tumorigenic mutation in the somatic cell (retinoblast) to occur, accounting for the early age of diagnosis. Knudson concluded that in the unilateral, nonhereditary cases, both mutations must occur in a single retinoblast to explain the later

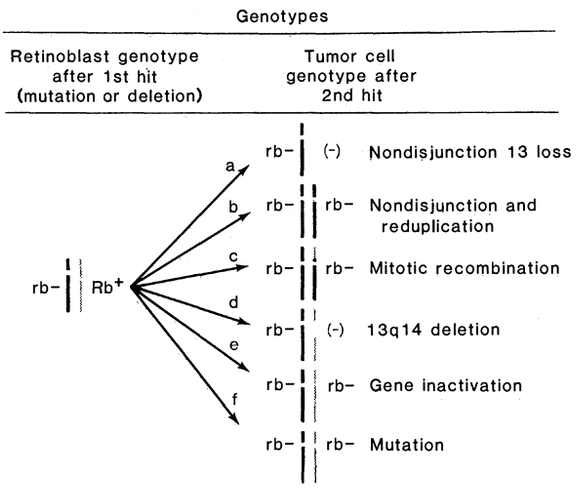

Fig. 1. Several mechanisms that would produce hemizygosity or homozygosity at the Rb locus are depicted. The "first hit," which could have occurred previously at the germinal level or independently at the somatic level within the retinoblast, is shown at the left. It could include a point or frameshift mutation that could not be detected microscopically (*3*), or a deletion that could be microscopically detected (*6, 7*) resulting in the inactivation of one Rb allele (rb− or −). A loss of the normal Rb allele might then occur as the second event leading to hemizygosity at the Rb locus (a) in which only the inactivated or deleted allele remained (*3, 4*). A nondisjunctional loss as the second event also could be followed by reduplication of the remaining rb− allele (b) producing homozygosity at the Rb locus (*5*). A mitotic recombination which includes the rb− allele in the recombination (c) is also a possibility (*5*) as is a microscopic deletion (*4, 18*) or a submicroscopic deletion (d). Inactivation of the remaining Rb+ allele can also be a rare occurrence resulting from the translocation of 13q14 onto an inactive X chromosome (e). In such case the structural Rb+ allele would still be present but would not be functional (*19*). Finally, the second event might be a point or frameshift mutation of the remaining Rb allele (f).

age of diagnosis generally observed for these patients.

Although Knudson's theory of tumorigenesis was widely accepted as the best explanation of the clinical observations, certain clinical irregularities existed. The presence of the germinal "mutation" or retinoblastoma "gene" (Rb+/rb−) did not always lead to tumor formation in both eyes. In addition, the occurrence of skipped generations and familial cases in which only one eye was affected in each patient lead Matsunaga to postulate "a tissue resistance" model for retinoblastoma. In his proposal, the two mutations of Knudson were assigned unequal values (*14*). Matsunaga assumed that the first mutation was the more potent "dominant" mutation and that the second event was a host error in differentiation. He likewise postulated that neither of these events involved chromosome 13 (*14*). Although a role for host factors in the expression of retinoblastoma still exists, Sparkes *et al.* demonstrated that the retinoblastoma gene for the hereditary form of retinoblastoma was indeed on chromosome 13 by assigning the Rb gene to 13q14 (*8*). Recently, Strong and her co-workers published an extensive pedigree which indicated that a balanced translocation involving 13q14 could explain the unusually large number of individuals in some pedigrees who transmit the gene (balanced translocation carriers) but who do not develop the tumor (*15*).

In 1973 Comings proposed a general theory of tumorigenesis (*16*) in which he combined elements of the oncogene theory of Todaro and Huebner (*17*) and the "two-mutation" theory of Knudson (*13*). He suggested that all cells contain structural transforming genes (some of which could be putative oncogenes) and these, when active, release the cell from its normal constraints on growth. Comings postulated that these genes were normally active during embryogenesis, but that in the process of differentiation they were "turned off" by diploid pairs of regulatory genes (alleles). He proposed that a mutation in both alleles of a regulatory gene accounted for release of suppression and subsequent transformation of the cell. Only very recently has a body of evidence appeared in support of Comings' concept of homozygous inactivation of diploid suppressor genes playing a role in human tumorigenicity (*3–5*). The particulars of that evidence are summarized below.

Evidence for a Rb Locus in 13q14 Common to All Forms of Rb

Considerable evidence from chromosomal and gene mapping studies indicates that a common retinoblastoma locus exists for both the hereditary and 13-deletion forms of retinoblastoma. The loci for both forms are closely linked to the locus for esterase D (EsD) (*7, 8*), a gene dose-dependent human polymorphic enzyme assigned to 13q14 (*8*). Consequently, since both Rb loci are closely linked to the EsD locus and predispose to multifocal retinoblastoma, they likely are identical.

At present only circumstantial evidence is available to support the assignment of the locus for sporadic nonhereditary Rb to chromosomal region 13q14. A deletion including region 13q14 has been observed in tumor cells from patients with both sporadic nonhereditary and hereditary retinoblastoma (*4, 18*). This would suggest that this same Rb locus is involved in the tumorigenic events in both of these forms of the tumor. Since the locus for both the hereditary deletion and nondeletion forms is known to be in 13q14, it is likely that this is also the site of the events leading to sporadic nonhereditary retinoblastoma.

Evidence That the Rb Gene Is Recessive

Substantial evidence supports the concept that the two independent genetic events proposed by Knudson to be sufficient for tumorigenesis may consist of the loss or inactivation of both wild-type alleles at the Rb locus in 13q14. The first evidence came from one of our patients with retinoblastoma who had a deletion of 13q14 that could not be observed microscopically (*3*). The deletion, defined by the concurrent loss of one EsD allele and the appearance of retinoblastoma (*3*), was beyond microscopic detection even at the 2000-band resolution level (*2*). However, this patient had bilateral retinoblastoma, and showed half-normal EsD activity in red cells, fibroblasts, and lymphoblastoid cells, a combination that suggested a genotype of Rb+/− secondary to a germinal, nonmicroscopically observable chromosomal deletion in 13q14.

In each of two distinct tumor clones from this patient a 13 chromosome was missing from the karyotype. Since both 13 chromosomes in all the nontumor cells examined appeared cytogenetically normal, the only method available to determine which 13 chromosome had been lost in the tumor was a measurement of EsD activity. Repeated analysis of the tumor cells failed to reveal any detectable EsD activity, an indication that the normal, nondeleted chromosome 13 was missing in each tumor line. Consequently the only 13 chromosome remaining in the tumor cells contained the "submicroscopic" deletion of both the Rb and EsD genes (*3*). Using restriction fragment length polymorphisms located on chromosome 13, Cavenee *et al.* have confirmed our interpretation that these tumor cells contain only one 13 chromosome (*5*). In addition they ruled out the possibility that portions of a

second chromosome 13 might exist as part of the unidentified markers present in the karyotype (*5*).

Since neither of the two independently derived retinoblastoma clones in this patient contained any genetic material from the Rb locus, the argument could be strongly made that loss of homologous alleles at the Rb locus (in this case Rb+/ $- \rightarrow -/-$) can be two genetic events sufficient for tumorigenesis. In these tumor cells, hemizygosity at the Rb locus would be present as shown in Fig. 1, mechanism a, in which the first genetic "hit" is a submicroscopic deletion of 13q14 and the second hit is the nondisjunctional loss of chromosome 13. These data also suggest that the Rb gene is recessive, since loss of one Rb allele was insufficient for tumor development, whereas the loss of both Rb alleles was associated with tumor formation. In addition, the recessive nature of the Rb gene also can be inferred from translocation carriers whose cells contain a germinal 13 translocation including 13q14 (*15*); in these carriers the presence of one extra copy of chromosomal region 13q14 was sufficient to prevent the appearance of retinoblastoma (*15*).

Another source of evidence supporting a structural or functional loss of both Rb alleles in the development of retinoblastoma comes from chromosomal studies of tumor cells. Data from our laboratory on nonrandom chromosomal changes in retinoblastoma cells are summarized in Table 2 [see also (*4*)]. Loss of chromosome 13 or a deletion of 13q14 is one of three nonrandom events found in the tumor cells. Such a loss would result in hemizygosity at the Rb locus as illustrated in Fig. 1, mechanism a.

Although the tumor genotype at the Rb locus in these cases could not be proved with certainty to be rb−/−, the nonrandom loss of chromosome 13 is circumstantial evidence in support of such a concept, especially in patients with the hereditary form of the disease. Further evidence comes from Balaban and her colleagues who have found chromosomal deletions that include chromosomal region 13q14 in patients with both hereditary and nonhereditary retinoblastoma (*18*). Such a deletion as the second hit also could lead to homozygosity for inactivity at the Rb locus (Fig. 1, mechanism d).

The final evidence suggesting the recessive nature of the retinoblastoma gene comes from Cavenee *et al.* (*5*), who used restriction fragment length polymorphisms located on chromosome 13 to examine the events affecting this chromosome in seven retinoblastomas. In each case the tumor cells contained two or, in one case, three cytogenetically normal forms of chromosomes 13. However, by comparing the various restriction fragment length polymorphisms in the constitutional 13 chromosomes to those in the tumors from the same individual, they were able to show in three of these cases that there had probably been a nondisjunctional loss of one chromosome 13 with duplication of the remaining chromosome 13 (Fig. 1, mechanism b). In another case a mitotic recombination event had occurred in one 13 chromosome with the breakpoint being proximal to 13q14 leading to homozygosity at 13q14 (Fig. 1, mechanism c).

These findings further suggest that the loss of all or part of a 13 chromosome with duplication of the remaining chromosome may be a frequent mechanism for the development of homozygosity (rb−/rb−) at the Rb locus. Rarely, inactivation of the remaining Rb+ allele could result from the translocation of 13q14 onto an inactive X chromosome as has been described (*19*), and is shown in Fig. 1 as mechanism e. In this latter case, the structural Rb+ allele would still be present but not transcribed. Finally, the second event could be a point or frameshift mutation of the remaining Rb allele (Fig. 1, mechanism f), but such an event cannot yet be detected.

When the tumors described by Cavenee *et al.* (*5*) are added to our cases (Table 2) and to those of Balaban *et al.* (*18*), it is apparent that the loss of genetic information in 13q14 is a frequent occurrence in tumor cells. Various retinoblastomas that contain two normal-appearing 13 chromosomes are being examined in collaboration with Cavenee to determine whether some of these tumors also have developed homozygosity of 13q14 (Table 2). On the basis of the data (*5*), it might be predicted that at least 50 percent of the tumors listed in Table 2 will be shown by chromosomal analysis or restriction fragment length polymorphism to have lost a 13 chromosome or to have become homozygous for a portion of chromosome 13, including the Rb locus. Such results in turn would provide further evidence that the development of hemizygosity or homozygosity at the Rb locus is a frequent occurrence in the tumor and a major factor in tumorigenesis (Fig. 1). In addition, the results indicate that a chromosomal mechanism plays a major role in the second tumorigenic event proposed by Knudson (*13*). The fact that region 13q14 (the region of the Rb locus) is a "hot spot" for chemically induced chromosomal aberrations (*20*) may contribute to the unexpected high frequency of this chromosomal mechanism.

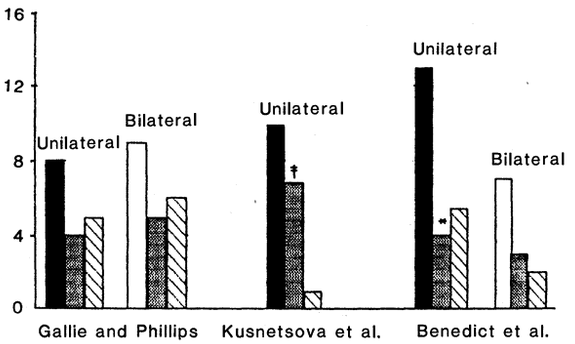

Fig. 2. Nonrandom chromosomal duplications in retinoblastoma (iso6p and +1q). Results from three studies involving these specific chromosomes are presented: Gallie and Phillips (*22*), Kusnetsova *et al.* (*21*), and Benedict *et al.* (*4*). The bar representations are as follows: black bar, total unilateral cases; white bar, total bilateral cases; gray bar, tumors containing an iso6p chromosome; striped bar, tumors with an extra copy of 1q. ‡Includes one tumor containing an extra 6q− chromosome. *Includes one tumor with an extra chromosome 6. The vertical axis shows the number of cases from the total unilateral or bilateral tumors studied by each group which had the specific nonrandom chromosomal changes.

Table 2. Nonrandom chromosomal changes in 20 retinoblastoma clones.

Tumor	Disease*	Chromosomal change†		
		−13‡	iso6p	+1q
LA-RB63-1B	B	+		
LA-RB66	B	+		+
LA-RB69A	B	+	+	
LA-RB69B	B	+		
LA-RB78	U	§	+	
LA-RB81	U	+	+	
LA-RB63-1A	B		+	
LA-RB73-1A	B		+	+
LA-RB75	U		+	
LA-RB80B	U		‖	
LA-RB59	U			+
LA-RB64	U			+
LA-RB65	U			+
LA-RB67	U			+
LA-RB80A	U			+
LA-RB62	U			
LA-RB70A	U			
LA-RB70B	U			
LA-RB72	U			
LA-RB73-1B	B			

*U, unilateral; B, bilateral. †Total karyotypic patterns of these tumors and the statistical evaluation of the specific chromosomal changes have been described (*4*). ‡Indicates a loss of chromosome 13. §Tumor clone showed a deletion of 13q14. ‖Tumor clone showed an extra chromosome 6 rather than a duplication of 6p (iso6p).

Additional Nonrandom Chromosomal Changes

Two other nonrandom chromosomal changes in retinoblastoma (an iso6p and a +1q) have been reported in three large cytogenetic studies of retinoblastomas (*4, 21, 22*) (Fig. 2). The first and perhaps most significant additional cytogenetic finding is the presence of an iso6p in several retinoblastomas from patients with both hereditary and nonhereditary disease. This marker chromosome appears to be fairly specific for retinoblastoma. It provides two extra copies of the short arm of chromosome 6 (6p) since in each of the cases illustrated at least two normal 6 chromosomes were also present. One tumor reported by Kusnetsova *et al.* (*21*) contained an extra 6q− chromosome, and one of the retinoblastomas studied by us (*4*) also had an extra chromosome 6. Therefore, 9 out of 17, 7 out of 10, and 7 out of 20 retinoblastomas reported by Gallie and Phillips (*22*), Kusnetsova *et al.* (*21*), and Benedict *et al.* (*4*), respectively, contained extra copies of 6p.

If there were an "expressor" cancer gene (perhaps a putative human oncogene) on 6p, the increase in gene dose of such an expressor gene in these tumors could play a role in tumorigenesis, perhaps as the "second event" proposed by Knudson. Should such an expressor for retinoblastoma exist, it would suggest that the genotype rb−/rb− or rb−/− in 13q14 may not be absolutely required for tumorigenesis. Rather, inactivation of only one Rb allele could be the first germinal or somatic event. In turn, the duplication of 6p resulting in a dose-dependent increase in the expressor gene might be the second event sufficient for tumor development.

The third nonrandom chromosomal change found in retinoblastoma is an extra copy of the long arm of chromosome 1 (1q) observed in two of the three reports summarized in Fig. 2. Gallie and Phillips found a triplication of 1q in 11 of 17 tumors. They reported that a common site of triplication was the region 1q25 to q32 (*23*). We also saw a trisomy for 1q in 7 of 20 of our retinoblastoma clones (Table 1 and Fig. 2). In contrast, Kusnetsova and her colleagues reported an extra copy of the long arm of chromosome 1 in only one of the tumors they examined (Fig. 2). However, they found a 1p+ chromosome in four of their tumors (*21*). Thus, these or other abnormal chromosomes stated to contain a translocation could have included a portion of 1q (*21*). Although trisomy of 1q also could result in a gene dose increase of an "expressor" gene, this chromosomal change is not specific for retinoblastoma since trisomy 1q has been described in several other tumors, including carcinoma of the breast (*24–26*), cervix (*26, 27*), testes (*28*), and ovary (*26, 29, 30*). Moreover, trisomy 1q has been reported to be a secondary chromosomal change as the tumor progresses rather than a primary event in both ovarian and hematological malignancies (*29*). Therefore, it may be that trisomy for 1q plays a role in regulating malignant progression rather than in producing the tumor.

Finally, double-minute chromosomes have occasionally been seen in primary retinoblastoma karyotypes (*4*). These chromosomal regions could carry important amplified genes involved in tumor development as proposed for the double-minute chromosomes seen in neuroblastomas (*31*).

Rb Gene: Prototype for a Suppressor Class of Human Cancer Genes

A number of other human tumors may fit the retinoblastoma model of tumorigenesis. These include Wilms' tumor, familial renal cell carcinoma, neuroblastoma, and small cell carcinoma of the lung.

Wilms' tumor, like retinoblastoma, is a childhood tumor which has hereditary, nonhereditary, and chromosome deletion forms. When it is dominantly inherited, the tumor is usually bilateral and multifocal. Like Rb, the average age at diagnosis is statistically lower for the hereditary compared to the nonhereditary form of the disease. Since the loci for aniridia and Wilms' tumor are linked in chromosomal region 11p13, aniridia is frequently a marker for the deletion form of Wilms' tumor (*32, 33*) as is 50 percent EsD activity for deletion Rb. A deletion of chromosomal region 11p13 also has been reported in Wilms' tumor cells from individuals who have neither aniridia nor a constitutional deletion of 11p13 (*34, 35*). Such patients, therefore, are analagous to those patients with the nonhereditary form of retinoblastoma and a deletion including 13q14 in their tumor cells (*4, 18*).

The parallels between retinoblastoma and Wilms' tumor suggest that a deletion in region 11p13 may be one of two genetic hits required for Wilms' tumor development. If it can be shown that the nondeleted 11 chromosome is lost in some of the tumors from patients with aniridia and Wilms' tumor who have a constitutional interstitial deletion of 11p13, we could infer that the Wilms' gene may also be a recessive gene similar to the Rb gene.

Familial renal cell carcinoma also appears to follow the retinoblastoma model. Although this is an adult tumor, it has an earlier average age of diagnosis than nonhereditary renal cell carcinoma and frequently the tumor occurs in both kidneys or with multiple primary tumors in one kidney (*36, 37*). In one family a balanced translocation was described with the constitutional breakpoints at 3p12 and 8q24 (*36*). The cancer developed in ten family members over three consecutive generations. This translocation was detected in all family members who survived their renal cancer, whereas the 12 individuals who did not develop renal cancer had normal karyotypes. Thus, familial renal cell carcinoma without apparent constitutional chromosomal abnormalities may be similar to the hereditary form of retinoblastoma. Individuals with a translocation from chromosome 3 to chromosome 8 who develop renal cell carcinoma in contrast may be analogous to those individuals with the deletion form of retinoblastoma (Table 1). One obviously would have to assume that in the translocation from chromosome 3 to 8 a mutation or submicroscopic deletion occurred at the breakpoint.

A specific, acquired translocation (3p; 11p) was also found in tumor cells from one patient with familial renal cell carcinoma who had a normal constitutional chromosomal pattern (*38*). This case therefore may be analogous to patients with hereditary retinoblastoma in whom a deletion including 13q14 was observed in their tumor cells (*18*), as shown as mechanism d in Fig. 1. It would be particularly informative to examine tumor material from the family described by Cohen *et al.* (*36*) with a constitutional 3;8 translocation to determine whether hemizygosity developed at the site of the renal cell carcinoma gene as suggested for the Rb gene (Fig. 1, mechanism a).

Neuroblastoma is another childhood tumor that has similarities to retinoblastoma. Familial cases have been reported (*39*), and there is a statistically significant number of tumors that have shown a deletion of chromosome 1 including bands p32 to pter (*40*). Although a constitutional deletion has not been identified to date in patients with neuroblastoma, statistical analysis of incidence figures fits a two-mutation hypothesis for its origin as does retinoblastoma and Wilms' tumor (*9*).

In addition, there are other tumors in which specific deletions have been found, including the short arm of chro-

mosome 3 in small cell carcinoma of the lung (*41*). Several other tumors also appear to have two patterns of tumor development, one attributable to a dominantly inherited gene and the other which is nonhereditary (*9*). Consequently, there is considerable information to support the fact that there is a group of human genes which function in a manner similar to the retinoblastoma gene.

Other Evidence for Suppressor Genes in Human Cancer

Evidence has been presented for the recessive nature of the Rb gene and for the structural loss or inactivation of the wild-type Rb+ allele as a second event resulting in tumor formation. Therefore, it is likely that the diploid pair of wild-type alleles at the Rb locus (Rb+/Rb+) normally have a regulatory or suppressor role, perhaps acting as a suppressor of a transforming gene which functions normally during embryogenesis (*16*). It is entirely feasible, however, that the normal Rb+ alleles function as a suppressor of a factor which can be active beyond embryogenesis, especially since the Rb gene also predisposes to the development of several other tumor types with high frequency in adults (*12*). Although numerous molecular mechanisms could be postulated to account for the regulatory or suppressor activity of the Rb gene, there are no data to justify an extensive discussion of these possibilities.

Genes that suppress tumorigenicity have been well documented in the normal human genome. Most of this information has been derived from chromosomal and tumorigenicity analysis following intraspecific or interspecific hybridization of malignant × nonmalignant cells (*42*). Briefly, tumorigenicity is generally suppressed in intraspecific human "malignant × normal" hybrids that retain both sets of parental chromosomes. When specific chromosomes are lost in such hybrids, the ability to produce tumors is restored. Chromosome 13 has been implicated as a suppressor chromosome in human × hamster hybrids (*43*), although the relationship, if any, between the suppression of tumorigenicity in this latter system and the proposed "suppressor" function of the Rb gene is unknown.

Furthermore, suppression appears to be dependent on chromosome dosage in certain hybrid systems. For example, tumorigenicity is suppressed in hybrids between human malignant HT1080 fibrosarcoma cells and normal human cells (*44*). However, this suppression is overcome by an additional complement of chromosomes from the malignant HT1080 parent (*44*). Such a dosage effect also could be present in the development of retinoblastoma if the loss of both Rb+ alleles or a loss of one Rb+ allele combined with the gain of a specific "expressor" gene is required for tumor formation.

Finally, it should be noted that a balance of expressor and suppressor chromosomes has been implicated as the basis of tumorigenesis in nonhuman systems (*45, 46*). If an equal or greater number of suppressor chromosomes were present in a given cell compared to a number of expressor chromosomes, suppression of tumorigenicity appeared to be the rule (*45, 46*). However, if more expressor than suppressor chromosomes were present, such cells produced tumors (*45, 46*). If chromosome 6p is shown to contain genetic information for the expression of retinoblastoma, these earlier studies (*45, 46*) may be relevant to the human situation, since a balance between an "expressor" gene on 6p and the suppressor gene on 13q14 could be a key to retinoblastoma development. In addition, amplification of a gene contained in the double-minute chromosome occasionally seen in retinoblastoma (*4*) could also be a factor in retinoblastoma development.

Whatever the specific function of the Rb gene in 13q14 may be, there is no doubt that the gene plays a major role in the development of retinoblastoma as well as second primary malignancies of other specific tissue types in those individuals who inherit the Rb gene. Therefore, ample reason is provided to attempt to clone this gene. DNA libraries of chromosome 13 have already been constructed and several polymorphisms on chromosome 13 have been identified (*5*). In addition, appropriate target DNA's such as that from the tumor which contains only a submicroscopic deletion including the Rb and EsD gene are available (*3*) and probes for EsD are being constructed to expedite this cloning.

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