in adult control (sham-operated) animals (see Fig. 2, a to c).

In fluorographs of labeled extracellular soluble proteins from intact control PNS and CNS tissue (Fig. 2, a to c), the synthesis of the 37-kD protein appeared to be low. However, within 10 to 14 days after a hemisection of the spinal cord (Fig. 2e) and after a crush-induced lesion of the optic nerve (Fig. 2f), the synthesis of the protein was increased to rates similar to those in crushed sciatic nerve (Fig. 2d). The 37-kD proteins in the PNS and CNS had an identical molecular weight and isoelectric point of *p*H 5.4, and their synthesis increased with a similar time course, which suggests that these proteins are homologous. When the protein-stained gels (Fig. 2, g to i) corresponding to the fluorographs in Fig. 2, d to f, were compared, the 37-kD protein was detected only in the sciatic nerve (Fig. 2g). In the distal stump of injured rat sciatic nerve, the protein accumulated to approximately 2 to 5 percent of the total extracellular protein (*7*). Despite its high rate of synthesis, the 37-kD protein did not accumulate in rat spinal cord (Fig. 2h) or optic nerve (Fig. 2i). Even prolonged synthesis of the protein at its maximum rate for at least 3 months after crushing the optic nerve did not increase its concentration of this protein.

The rapid disappearance of the 37-kD protein from the extracellular space in spinal cord and optic nerve may be due to proteolytic degradation, because uptake (or reuptake) and intracellular accumulation of the protein in these CNS tissues was not observed. A similar degradative mechanism was either absent or inhibited in the peripheral nerve.

The effect of permanent denervation and axonal regeneration on the synthesis of the 37-kD protein in rat sciatic nerve was also examined. In one group of adult Sprague-Dawley rats, sciatic nerves were unilaterally transected with a permanent separation of the proximal and distal nerve stumps. In another group, sciatic nerves were unilaterally crushed. The nerve stumps distal to the site of lesion were removed between 1 and 8 weeks after injury, and extracellular soluble proteins were labeled with [^{35}S]methionine and analyzed by 2D PAGE and fluorography as described for developing nerves. The relative rate of [^{35}S]methionine incorporation into the 37-kD protein was estimated by densitometric scanning of the fluorographs. The synthesis of the protein increased to similar maximum rates in both crushed and transected sciatic nerves within 1 week after injury. In nerves with crush lesions that allowed subsequent axon elongation from the proximal into the distal nerve stump, the rate of 37-kD protein synthesis declined slowly to rates near control within 8 weeks after the lesion. However, in a transected sciatic nerve where regeneration of axons into the distal stump was prevented, the synthesis of the protein did not return to the low control rates but remained at a maximum rate for at least 8 weeks after injury. Further, experiments on the exact spatial distribution of the sites of synthesis of the 37-kD protein in longer sciatic nerves from rabbits (*10*) revealed that it is synthesized exclusively in those segments of a severed nerve where the axons degenerate (*1*). These results confirm the earlier observation (*7*) but suggest further that regenerating axons in sciatic nerve may provide a signal to decrease the synthesis of the 37-kD protein in nerve. The determination of the cellular origin of the protein should be important in exploring the nature of this signal.

The expression of a 37-kD protein during development as well as after injury of the PNS and CNS suggests that it plays a role in de novo nerve growth and in nerve repair. The potential to increase the rate of synthesis and secretion of the 37-kD protein after axotomy was retained in both the mature PNS and CNS. However, the protein accumulated only in the PNS. The lack of accumulation of the protein in the adult mammalian CNS may represent one of the critical molecular differences between PNS and CNS that limit the ability of central axons in mammals to regenerate. Although the function of the 37-kD protein in nerve development or regeneration is still unknown, the time course and spatial distribution, as well as the possibility that its synthesis is axon-regulated, favor a function related to some aspects of nerve growth and maturation that require contact between axons and their sheath cells (*11*).

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Genetic Origin of Mutations Predisposing to Retinoblastoma

Abstract. *Retinoblastoma is one of several human tumors to which predisposition can be inherited. Molecular genetic analysis of several nonheritable cases has led to the hypothesis that this tumor develops after the occurrence of specific mitotic events involving human chromosome 13. These events reveal initial predisposing recessive mutations. Evidence is presented that similar chromosomal events occur in tumors from heritable cases. The chromosome 13 found in the tumors was the one carrying the predisposing germline mutation and not the homolog containing the wild-type allele at the* Rb-1 *locus. These results suggest a new approach for identifying recessive mutant genes that lead to cancer and a conceptual basis for accurate prenatal predictions of cancer predisposition.*

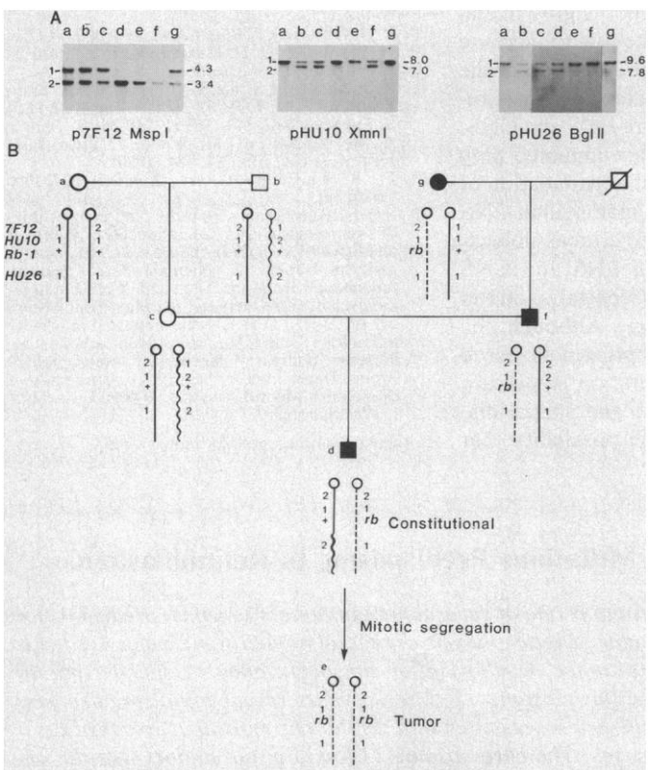
Retinoblastoma, a childhood tumor of embryonic neural retina, occurs in heritable and nonheritable forms. Genetic and physical mapping data implicate the *Rb-1* locus on human chromosome 13 in the development of both forms of the tumor (*1–6*). Knudson has proposed that the conversion of a normal retinal cell to a malignant retinoblastoma cell requires two events (*7–9*). In this model, heritable

cases inherit the first of these events as a germline mutation; the second event occurs somatically in the cell that becomes the tumor. Evidence suggests that these mutational events occur at the two homologous *Rb-1* loci and that the net result of the second event is to expose the initial mutation (*10*). The elimination of the normal allele at the *Rb-1* locus would occur most frequently by mitotic nondisjunction or recombination, resulting in a tumor cell that is homozygous over much of its chromosomes 13 (*10*). Similar mechanisms have been indicated in Wilms's tumors (*11–14*). However, rigorous proof that the chromosome remaining in the tumor carries the original predisposing mutation has not been presented. The model predicts that the chromosome 13 that is lost during tumorigenesis (thereby revealing the initial predisposing germline mutation at the *Rb-1* locus) is the normal homolog. We have tested this prediction by comparing constitutional genotypes of affected and unaffected parents to the constitutional and tumor genotypes of their affected children. We show here that the chromosomes 13 remaining in tumors from two hereditary retinoblastoma cases were derived from the affected parents.

Our approach was similar to that which led to the observation of somatic loss of germline heterozygosity in nonhereditary retinoblastomas (*10*). Recombinant DNA probes have been isolated (*15, 16*) that reveal variant restriction endonuclease recognition sequences at loci on human chromosome 13. The fragment-length alleles at each of these loci behave in family inheritance studies as would be expected for codominant Mendelian alleles (*15, 16*). Thus, heterozygosity at any of these loci in a child allows discrimination between the parental chromosome 13 contributions.

A chromosome 13–specific DNA probe (pHU26) identified a locus that maps to 13q22 at which the proband (KS2H) was constitutionally heterozygous (Fig. 1A). The tumor tissue (Rb-KS2H) from this patient showed only the longer allele at this locus. His unaffected parent, KS2C, was constitutionally heterozygous at this locus, whereas his affected parent, KS2F, was homozygous for the longer allele. The proband must, therefore, have inherited the shorter allele at the locus from his unaffected parent, and it was the chromosome carrying this allele that was lost in the tumor. Thus, in this case, the chromosome remaining in the tumor was the one inherited from the diseased parent and must be the one carrying the initial predisposing mutation at *Rb-1*. In this family, the proband inherited the predisposition to retinoblastoma from his father, KS2F, who had inherited it from his mother, KS2G (Fig. 1B). The proband obtained the shorter allele from his unaffected mother and the longer allele from his affected father. It is this latter chromosome that must contain the mutant *Rb-1* locus, and it was this chromosome that was retained in the child's tumor.

We obtained corroborating evidence of this interpretation by examining other loci on chromosome 13 that are defined by probes p7F12 (mapped to q12) and pHU10 (mapped to q13), in several other



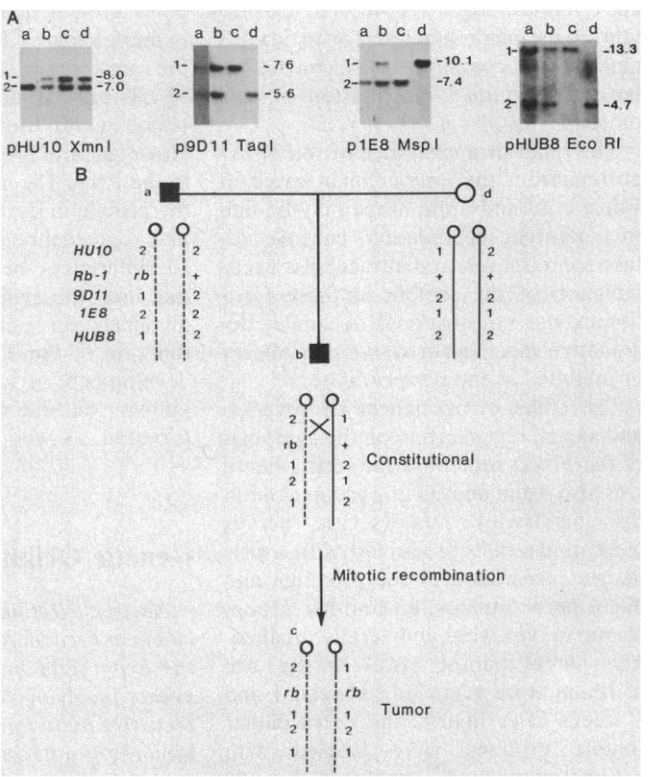


Fig. 1 (left). Loss of germline heterozygosity in a hereditary retinoblastoma tumor. (A) DNA was isolated from peripheral blood leukocytes from each of the indicated individuals and from primary tumor biopsy from the proband KS2H. The DNA was digested with the indicated restriction endonucleases, separated by electrophoresis through 0.8 percent agarose gels, transferred to nylon membranes (Zetapor; AMF-Cuno), and hybridized to the indicated probes homologous to loci on human chromosome 13. The family members are designated: a, KS2A; b, KS2B; c, KS2C; d, KS2H; e, Rb-KS2H (tumor); f, KS2F; and g, KS2G. (B) Pedigree Rb-KS2 and inferred chromosome 13 haplotypes at the *7F12, HU10, Rb-1*, and *HU26* loci. Filled symbols represent individuals with retinoblastoma; dashed line, nonrecombinant chromosome; straight and wavy line, recombinant chromosome. Fig. 2 (right). Loss of germline heterozygosity by mitotic recombination in a hereditary retinoblastoma tumor. (A) DNA was isolated from primary fibroblasts (b) or lymphoblastoid lines (a and d) and from tumor tissue (c) that had been minimally passaged in immunodeficient mice. DNA was digested and analyzed as described (legend to Fig. 1). The family members are designated: a, 468L; b, 462F; c, Rb-462 (tumor); and d, 469L. (B) Pedigree and inferred chromosomal haplotypes. Filled symbols represent individuals with retinoblastoma. The "X" indicates the probable point of mitotic crossover.

members of this family (Fig. 1A). Assignment of the alleles at each of these loci, in combination with those for pHU26, and a consideration of the allelic combinations from the grandparents (KS2A, KS2B, and KS2G), parents (KS2C and KS2F), child (KS2H), and child's tumor (Rb-KS2H) made it possible to infer chromosomal haplotypes (Fig. 1B). The proband (KS2H) inherited a nonrecombinant chromosome from his paternal grandmother (KS2G) through his father (KS2F) and a recombinant chromosome from his mother (KS2C). It appears that the chromosome retained in the tumor (Rb-KS2H) was inherited from his affected grandmother (KS2G) through his affected father (KS2F).

In a second case (Fig. 2), constitutional tissues were obtained from the proband (462F), his affected father (468L), and his unaffected mother (469L), as well as from his tumor tissue (Rb-462). Four heterozygous loci (Fig. 2A) were observed in constitutional DNA from 462F which were defined by pHU10 (maps to q13), p9D11 (maps to q22), p1E8 (maps to q22), and pHUB8 (maps to q22). Each of the loci distal to the *Rb-1* locus became homozygous in the tumor and each of the remaining alleles was derived from the affected parent (468L). However, the locus defined by probe pHU10, which maps proximal to the *Rb-1* locus, retained heterozygosity. These results are consistent with mitotic recombination between the *HU10* and *Rb-1* loci such that both chromosomes retained by the tumor are homozygously mutant at the *Rb-1* locus (Fig. 2B). Mitotic recombination has been shown to occur during oncogenesis of nonheritable retinoblastoma (*10*), and the present results suggest that this chromosomal event also occurs in heritable cases. In cases that were uninformative for loci proximal to the *Rb-1* locus but were homozygous at distal loci, we have ascribed such loss of heterozygosity to nondisjunctional loss of the wild-type homolog with subsequent reduplication of the mutant chromosome (*10*). Such a process would require several specific events, whereas mitotic recombination would accomplish the same result while demanding only a single event.

The results reported here provide molecular evidence supporting the model for the involvement of recessive mutations in tumorigenesis (*7–10*). This model predicts that the normal chromosome 13 is lost through aberrant events during the mitotic division of a predisposed retinal cell. In both hereditary cases of retinoblastoma that we examined, the chromosome lost during tumorigenesis was the one inherited from the phenotypically normal parent, whereas the retained chromosome was inherited from the affected parent.

Loss of heterozygosity for chromosome 13 is a frequent event in nonfamilial retinoblastoma (*10*). We have now extended these observations to hereditary cases, indicating that both types of retinoblastoma result from similar mechanisms involving the same genetic locus. A possible alternative interpretation is that both of the hereditary tumors resulted from interstitial deletions of the normal chromosome 13 homolog in the tumor such that one allele at each of the marker loci is removed. We consider this to be unlikely because of the rarity with which such events have been observed in retinoblastoma tumor karyotypes, and the fact that our second case (Fig. 2) clearly contained at least part of each parental chromosome. In addition, the genetic distances between the markers reported here are substantial (*17*) and presumably any such deletion would have to be quite large. Although satisfactory karyotypes were not obtained from the tumor Rb-KS2H, no deletion of chromosome 13 was apparent in the constitutional karyotypes of any of the family members. Karyotypic analysis of the tumor Rb-462 showed two chromosomes 13 that were present in their entirety. Another, more trivial explanation for these results is that point mutations occurred within the restriction sites that define the polymorphic regions of these loci. Since we observed loss of heterozygosity at more than one locus, at least in our second case, we also believe this to be unlikely.

The ability to identify which chromosome 13 of an affected parent carries a mutation predisposing to retinoblastoma may have predictive value in genetic counseling. Risk could be more precisely ascertained than is possible at present if the aberrant chromosome could be identified, as described here, in an already diseased sibling. Subsequent prenatal analysis of the chromosomes 13 in a second sibling, or in a child of the first sibling, would allow accurate prediction of predisposition to tumor formation. This information would help identify the children who should be frequently examined and the children who are free of such genetic predisposition.

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