cent per year from 1962 to 1968 (Fig. 3). Such growth rates are impossible in a species with delayed reproduction and single births without continued addition of breeding-age females. Young females pupping for the first time appear to be the primary colonists. Thereafter, they tend to return to the same location to give birth each year (*8*).

It appears that continuous dispersal from Baja California cannot have been sufficient both to hold down the growth rate there and to account for the uniformly high rates in California during the last 15 years. The growth of the combined Californian and Mexican populations was about 8.3 percent per year between 1965 and 1977. We calculated the number of pregnant females that must have emigrated each year to hold the increase in pup production on Guadalupe to 5.4 percent if the true species growth rate was 8.3 percent. In the unlikely event that all the presumed surplus females reached the California islands to give birth, pups born to immigrants plus reproduction by females born on the islands could have been responsible for the growth of pup numbers in California until about 1973 but not thereafter. Calculated immigration cannot account for the observed number of pups born in California in recent years if the true species growth rate is only 8.3 percent. Immigration from Baja California probably contributes to population growth in California, but how much can be established only through extensive tagging data. If the number of immigrating females has been relatively constant over the years, their impact on population growth would progressively lessen as the indigenous population grows.

Approximately 11,900 elephant seal pups were born in California in 1982 (Table 1). If the population growth observed on the Baja California islands between 1965 and 1977 had continued at the same rate since 1977, pup production there would have been about 11,200 in 1982, for an aggregate total of some 23,000 births. It has been stated that the Baja California population has stabilized (*12*), but there are no recent census data to verify this.

The California elephant seal population has been doubling every 5 years for more than two decades. Processes associated with increased density must eventually slow the rate of growth. Crowding could lead to mother-pup separation, disruption of harem structure and breeding success, and crushing of pups by adult males. Females may increasingly be forced to use exposed beaches where losses of pups to storms are high. Increased density may lead to delayed maturity, a greater fraction of nonbreeding mature females, and lower adult and pup survival. Growth and maturation of the southern elephant seal (*Mirounga leonina*) accelerated when its population was reduced by man (*16*); the reverse process seems equally likely.

When the population growth rate will begin to decline is not known. Le Boeuf and Panken (*4*) stated in 1977 that "the Año Nuevo rookery seems to have reached its carrying capacity," yet population growth there continued at the same exponential rate as before at least through 1980 (Fig. 3). There are still insufficient data to define the optimal population of northern elephant seals in California.

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References and Notes

1. G. A. Bartholomew and C. L. Hubbs, *Mammalia* **24**, 312 (1960).
2. G. A. Bartholomew and R. Boolootian, *J. Mammal.* **41**, 366 (1960).
3. B. J. Le Boeuf, D. G. Ainley, T. J. Lewis, *ibid.* **52**, 370 (1974).
4. B. J. Le Boeuf and K. J. Panken, *Proc. Calif. Acad. Sci.* **41**, 267 (1977).
5. Harbor seal (*Phoca vitulina richardsi*), California sea lion (*Zalophus californianus*), northern fur seal (*Callorhinus ursinus*), Steller sea lion (*Eumetopias jubatus*), and Guadalupe fur seal (*Arctocephalus townsendi*). All but the last breed on San Miguel Island, giving it the greatest known diversity of pinniped species.
6. G. A. Antonelis, S. Leatherwood, D. K. Odell, *Fish. Bull.* **79**, 562 (1981); M. L. Bonnell *et al.*, in *Summary of Marine Mammal and Seabird Surveys of the Southern California Bight Area, 1975–78* (Report PB81-248-171, National Technical Information Service, Springfield, Va., 1978), part 1, vol. 3; H. R. Huber, R. J. Boekelheide, T. McElroy, R. P. Henderson, C. Strong, D. G. Ainley, *Studies of Marine Mammals at the Farallon Islands, California, 1980–1981* (Contribution 239, Point Reyes Bird Observatory, Stinson Beach, Calif., 1981); B. J. Le Boeuf and K. T. Briggs, *Mammalia* **41**, 167 (1977); D. K. Odell, *J. Mammal.* **55**, 81 (1974); R. T. Orr and T. C. Poulter, *Pac. Discovery* **15**, 13 (1962); *Proc. Calif. Acad. Sci.* **32**, 377 (1965).
7. D. W. Rice, K. W. Kenyon, D. Lluch B., *Trans. San Diego Soc. Nat. Hist.* **14**, 73 (1965).
8. J. Reiter, K. J. Panken, B. J. Le Boeuf, *Anim. Behav.* **29**, 670 (1981).
9. San Miguel Island data by R. L. De Long and G. A. Antonelis, National Marine Fisheries Service (NMFS), Seattle, Wash., and B. S. Stewart (1979); G. A. Antonelis and B. S. Stewart (1980); E. C. Jameyson, NMFS, and B. S. Stewart (1981); B. S. Stewart and D. P. DeMaster (1982). San Nicolas Island data by B. S. Stewart (1980, 1981, and 1982).
10. The logarithmic growth model appropriate to this annually reproducing species is $\log \hat{y} = \log a + x \log(1 + i)$, where $\hat{y}$ is estimated pup number in year x, i is annual rate of increase, and a is a constant.
11. D. G. Kleinbaum and L. L. Kupper, *Applied Regression Analysis and Other Multivariate Methods* (Duxbury, Boston, Mass., 1978), p. 58.
12. B. J. Le Boeuf and M. L. Bonnell, in *The California Islands: Proceedings of a Multidisciplinary Symposium*, D. M. Power, Ed. (Santa Barbara Natural History Museum, Santa Barbara, Calif., 1980), p. 475.
13. B. J. Le Boeuf and R. S. Peterson, *Science* **163**, 91 (1969).
14. C. H. Townsend, *Zoologica* **1**, 159 (1912).
15. D. K. Odell, *J. Mammal.* **51**, 81 (1974).
16. J. A. Estes, *J. Fish. Res. Board Can.* **36**, 1009 (1979).
17. We thank W. Ehorn and his staff at Channel Islands National Park for assistance on San Miguel Island; R. Dow, Point Mugu Naval Air Station, for help in visiting San Nicolas Island; and many enthusiastic Air Force helicopter pilots for support. Comments on the manuscript were made by B. Collier, T. A. Ebert, D. Goodman, B. J. Le Boeuf, C. J. Walters, R. Hilborn, A. R. E. Sinclair, R. L. De Long, and G. A. Antonelis. Funded by Headquarters, Space Command, U.S. Air Force, as part of an assessment of environmental impact of the space shuttle.

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Gene for Hereditary Retinoblastoma Assigned to Human Chromosome 13 by Linkage to Esterase D

Abstract. *Evaluation of three families with hereditary retinoblastoma demonstrates close linkage of the gene for this tumor with the genetic locus for esterase D. These results assign the gene for the hereditary form of retinoblastoma to band q14 on chromosome 13, the same region which is affected in the chromosome deletion form of this eye tumor, and therefore suggest a common underlying mechanism in the pathogenesis of these two forms of retinoblastoma.*

Retinoblastoma is the prototype of human developmental tumors (*1*). When there is a positive family history of the tumor, it usually follows an autosomal dominant inheritance pattern. The majority of cases occur sporadically. A small subgroup of retinoblastoma patients has been found to have a deletion of band 13q14.

We have previously assigned the locus for esterase D (ESD) to chromosomal region 13q14 by deletion mapping (*2*). This assignment raised the possibility that the hereditary nondeletion form of retinoblastoma might be controlled by a gene in band 13q14 close to the ESD locus. Our present studies verify this hypothesis by demonstrating tight linkage between the loci for hereditary retinoblastoma and ESD.

We were able to evaluate ESD enzyme activity and electrophoretic types in 35 families in which the inherited form of retinoblastoma occurs. Families were ascertained through contacts with ophthalmologists who were caring for these patients; all persons were given eye examinations to determine the presence or

absence of retinoblastoma. Heparinized blood samples were sent by overnight transport at ambient temperature to our laboratory in Los Angeles, where the electrophoretic types of ESD were determined by a modification of the method described by Hopkinson *et al.* (*3*), who showed that there are two common allelic forms of this enzyme, type 1 and type 2. Three families were informative for linkage analysis; that is, the affected parent carried both ESD types so that the transmission of the disease gene and ESD type to the children could be followed (Fig. 1). The ESD electrophoretic types are also noted in Fig. 1; all samples had normal enzyme activities. All probands had normal G-banded chromosomes.

Genetic linkage analysis was performed with the use of program LIPED (*4*), which calculates, for each pedigree, the odds of linkage versus no linkage at specified values of recombination fraction θ (a measure of the distance between two loci). The θ value at which the log of the odds (lod score) is at a maximum is the best estimate of θ among those θ values considered. Information from several pedigrees is combined by summing lod scores at each θ value. Quadratic interpolation can then be used to obtain the maximum likelihood of θ ($\hat{\theta}$) and its corresponding lod score ($\hat{Z}$). By convention, linkage is considered "proven" if a lod score is greater than 3.0 (odds 1000:1 in favor of linkage). This rigorous significance level is required because the prior probability of autosomal linkage is about 1/22; for the posterior probability of a type I error to be less than .05, α must be about .001 (*5*).

Lod scores were calculated at 36 combinations of θ(male), θ(female) (six values of θ were 0.01, 0.05, 0.10, 0.20, 0.30, and 0.40) for each pedigree and then summed across the three pedigrees; $\hat{\theta}$ and $\hat{Z}$ were then estimated. This analysis was performed under four sets of conditions: on the assumption of (i) a penetrance of 0.8 and 0.9 for the retinoblastoma gene and (ii) the first affected individual in pedigree B to be, or not to be, a new mutation. If she is not a new mutation, one of her parents carries, but does not express, the gene. Her deceased mother had a history of reduced vision in one eye; however, we do not believe this represents conclusive evidence of regressed retinoblastoma. The extended pedigree was, therefore, analyzed with her father coded as "unaffected," and her mother as "unknown." In this way, the probability of her father being a carrier was about one-fifth or one-tenth (depending on the penetrance used) the probability of her mother being a carrier.

When the genotype is unknown for persons in the pedigree, the program LIPED computes the probabilities of possible genotypes, with population gene frequencies being supplied by the investigator. We provided the following ESD gene frequencies that are appropriate to the Caucasian background of the families: ESD type 1, 0.9; ESD type 2, 0.1 (*3*). For the retinoblastoma gene, we supplied a frequency of 0.001 to indicate that the gene is "rare."

Table 1 shows the summed lod scores calculated under the various assumptions. Only scores at $\theta_m = \theta_f$ are shown, since the maximum score occurred when θ was equal for males and females. The results prove linkage (lod score $\hat{Z}$ greater than 3) between hereditary retinoblastoma and the ESD locus, regardless of the penetrance chosen, if we assume the first affected person in pedigree B is a new mutant. If we assume she is not a new mutant, the evidence for linkage is reduced but still "highly suggestive." The maximum likelihood estimate of the recombination fraction, $\hat{\theta}$, in all cases is 0.0, that is, very close linkage. This agrees with the observation that there are no proven recombinants in the three pedigrees.

The new gene map information described here indicates that the ESD locus is close to the genetic locus for the inherited nondeletion form of retinoblastoma. Thus, it is possible in informative families to use the ESD electrophoretic type to predict who may be at risk to develop retinoblastoma before the disease has become manifest in that individual, allowing prenatal or preclinical detection (or both) and early institution of therapy.

Ideally, it would be useful to have a genetic marker that is more polymorphic than ESD and is also closely linked to the retinoblastoma locus. Thus, efforts should be directed toward demonstrating greater polymorphism for the ESD locus (for example, by isoelectric focusing or with temperature-sensitive assays) and toward identifying a polymorphic restriction endonuclease site located very close to the retinoblastoma locus.

We have now shown that the chromosome deletion associated with retinoblastoma includes the ESD locus and that the locus for the hereditary nondeletion form of retinoblastoma is closely linked to the ESD locus. This strongly suggests the hypothesis that the hereditary form of retinoblastoma is caused by a submicroscopic change at the same site that is affected in the constitutional chromosome deletion form of the disease. Therefore, we suggest that a "mutation" at this site may be necessary for the

Table 1. Summed lod scores for RB-ESD under the four sets of assumptions.

Set	Assumption		θ						$\hat{Z}$	$\hat{\theta}$
	Penetrance	Pedigree B new mutant	0.01	0.05	0.10	0.20	0.30	0.40		
1	0.8	Yes	3.06	2.81	2.49	1.80	1.07	0.39	3.12	0.0
2	0.9	Yes	3.43	3.17	2.82	2.06	1.25	0.46	3.50	0.0
3	0.8	No	2.58	2.33	2.01	1.36	0.64	0.13	2.64	0.0
4	0.9	No	2.69	2.43	2.08	1.33	0.59	0.07	2.76	0.0

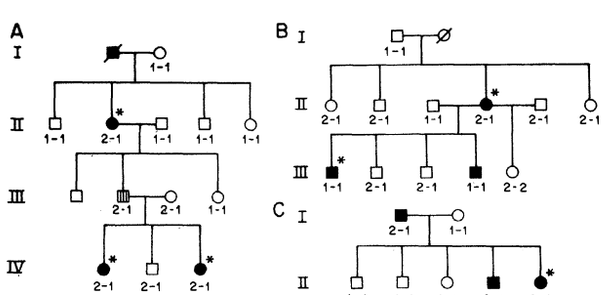

Fig. 1. Pedigrees of the three informative families (A, B, and C) are noted. The darkened symbols indicate persons with retinoblastoma; an asterisk (*) by the symbol indicates that the tumor is unilateral. The tumor in the affected persons developed in early childhood and had a typical presentation for retinoblastoma. All unaffected persons at risk have had negative eye examinations, and their current ages for each pedigree are: A.IV, 5 years; B.III, 21, 19, and 13 years; and C.II, 13, 11, and 9 years. The male with the hatched symbol in pedigree A.III is an adult with no evidence of tumor or residual scar that would suggest a regressed tumor; however, he must carry the gene because his mother is affected and his two daughters have retinoblastoma. The family of pedigree B has been reported previously (*8*). The numbers under the individual symbols indicate the ESD genotype.

development of retinoblastoma in at least these two forms of the disease. This interpretation is also consistent with the theory of Knudson (*6*) who believes the manifestation of retinoblastoma depends upon the presence of at least two mutations. However, these results speak against the contention of Matsunaga (*7*) who has argued that the major retinoblastoma gene is probably not located on 13q.

While these studies do not give further insight into the normal function of the retinoblastoma gene, they may, however, assist in the eventual isolation of this gene and the identification of its function.

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References and Notes

1. F. Vogel, *Hum. Genet.* **52**, 1 (1979).
2. R. S. Sparkes *et al.*, *Science* **208**, 1042 (1980).
3. D. A. Hopkinson, M. A. Mestriner, J. Cortner, H. Harris, *Ann. Hum. Genet.* **37**, 119 (1973).
4. J. Ott, *Am. J. Hum. Genet.* **28**, 528 (1976).
5. N. Morton, *ibid.* **7**, 277 (1955).
6. A. G. Knudson, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **68**, 820 (1971).
7. E. Matsunaga, *Hum. Genet.* **56**, 53 (1980).
8. S. J. Funderburk, R. S. Sparkes, M. C. Sparkes, L. Field, *Am. J. Hum. Genet.* **32**, 107A (1980).
9. Supported in part by NIH grant HD-05615 and HD-04612 (R.S.S.) and NIH grant EY-02715 (to A.L.M. and W.F.B.). Part of this work was performed at the Clayton Center for Ocular Oncology in conjunction with the Clayton Foundation for Research (A.L.M. and W.F.B.). We thank Drs. Robert Ellsworth, David Abramson, and David Kitchin and Mrs. Lee Sturgis for their help in ascertaining informative families. We also thank the many physicians who sent blood samples on families who were found not to be informative for ESD.

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Patient with 13 Chromosome Deletion: Evidence That the Retinoblastoma Gene Is a Recessive Cancer Gene

Abstract. *Although a constitutional chromosomal deletion including 13q14 has been found to date in all retinoblastoma patients whose esterase D activity is 50 percent of normal, one female patient has been found who has 50 percent esterase D activity in all normal cells examined but no deletion of 13q14 at the 550-band level. Therefore, she has the smallest constitutional chromosomal deletion within 13q14 that is associated with susceptibility to retinoblastoma. Two stem lines were identified in a retinoblastoma from this patient, and each one had a missing 13 chromosome. No detectable esterase D activity was found in the tumor, indicating that the normal nondeleted 13 chromosome was lost in both stem lines. Thus the data from this patient not only show that there is a total loss of genetic information at the location of the retinoblastoma gene within the tumor, but also imply that recessive genes may play an important role in the development of certain human tumors including retinoblastoma.*

A small percentage of patients with hereditary retinoblastoma have an associated constitutional deletion of chromosomal region 13q14. Although retinoblastoma is a rare tumor, it is important because the genetic susceptibility to retinoblastoma may provide a unique opportunity to examine the molecular basis for the development of a specific human tumor.

Esterase D (ESD), an enzyme of unknown function that is found in most tissues, has also been assigned to chromosomal region 13q14 by deletion mapping; that is, the gene producing it occurs at the same location as the retinoblastoma gene (*1*, *2*). The activity of this enzyme depends on the "dose" of the gene (*1*). Thus, the tissues of individuals with a deletion of one 13 chromosome, which includes q14, show only 50 percent of the ESD activity found in normal individuals. Esterase D is also an electrophoretic polymorphism with the two common alleles being types 1 and 2. The close proximity of the loci for the enzyme and deletion retinoblastoma should enable one to identify individuals with a deletion of 13q14 beyond the resolution of chromosomal banding. In addition, examination of the tumor chromosomal patterns and ESD levels within a patient with retinoblastoma should provide a clue to the mechanism of human tumorigenesis. In this report we describe data from such a patient indicating that the retinoblastoma gene is a recessive gene at the cellular level.

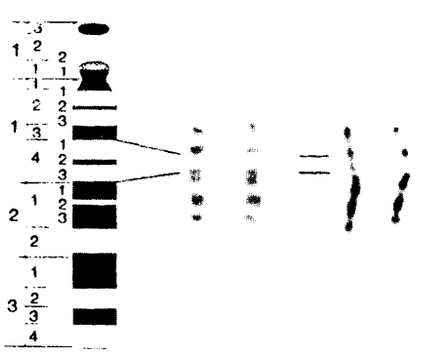

Fig. 1. High-resolution G banding showing the normal banding pattern of 13q14. The diagram of the 13 chromosome at the left indicates segments 14.1, 14.2, and 14.3. All three bands are present in the two 13 chromosomes shown from representative metaphases of peripheral blood lymphocytes (center) and a lymphoblastoid line (right). Thus, there is no evidence of any chromosomal deletion of 13q14 in this patient at approximately the 550-band level (*3*).

The patient is a 3-year-old female who was admitted to the Childrens Hospital of Los Angeles with bilateral retinoblastoma. Except for the presence of the tumor, no other physical or developmental abnormalities previously associated with the 13q14 syndrome (*2*) were found. The ESD activity in her red blood cells was 50 percent of normal (Table 1). Since both of her parents had normal ESD activity, the probability that she inherited a null allele for the enzyme was extremely small. Evaluation of a large number of additional markers gave no evidence of nonpaternity.

The probability of separate mutations occurring simultaneously in the ESD and retinoblastoma genes is remote; therefore, we suspected that the patient had a deletion within band 13q14. However, an examination of more than 40 early metaphase spreads from her peripheral blood lymphocytes showed that at the 550-band level (*3*), all three bands of 13q14 were present (Fig. 1). Since ESD analysis was done on the red blood cells, whereas the chromosomal analysis was performed on peripheral lymphocytes, the possibility of a mosaicism for 13q14 deletion could not be excluded. Therefore, we carried out the ESD assay and