Finding the Anti-Oncogene

Inheritance of certain growth-suppressing genes in a mutated form confers susceptibility to cancer. The first such gene to be isolated gives rise to a predisposition to retinoblastoma, an eye tumor

by Robert A. Weinberg

The roots of cancer lie in our genes. Cancer often begins when a carcinogenic agent—radiation or a chemical—damages the DNA of a critical target gene in a particular cell. The mutant cell then multiplies, and its descendants ultimately form the large aggregate of cells called a tumor.

This generalized scheme has been given focus and precision in the past decade with the identification of some of the genetic targets of carcinogens: the oncogenes. Once activated by a mutation, an oncogene promotes excessive or inappropriate cell proliferation; its activation represents one critical step in the creation of many types of cancerous growths.

Within the past few years a quite different class of cancer genes has been discovered. They act in normal cells not to promote proliferation but to suppress it. The loss of growth-suppressor genes from a cell removes a normal constraint on its growth. Such a genetically depleted cell may proliferate uncontrollably, and this too may lead to cancer. The discovery of growth-suppressor genes enriches understanding of the genetics of cancer and in time will lead to the reformulation of ideas about how the growth of normal cells is regulated.

A n understanding of growth-suppressor genes must begin with an understanding of their mirror images, the growth-promoting oncogenes. When oncogenes isolated from tumors are inserted into normal cells, the latter take on many traits of cancer cells [see "A Molecular Basis of Cancer," by Robert A. Weinberg: SCIENTIFIC AMERICAN, November, 1983]. Such gene-transfer experiments have shown that oncogenes act to deregulate the growth of the cells into which they are introduced and so must (by implication) be responsible at least in part for the aberrant behavior of the tumor cells from which they are extracted. Several lines of investigation have made it clear that such oncogenes are altered versions of normal genes, called proto-oncogenes, that act as central regulators of growth in normal cells. In the course of a lifetime any of a variety of mutations can convert one of these normal genes into a malignant oncogene.

The creation of a single oncogene may be necessary for the genesis of a tumor, but it is far from being sufficient. Tumorigenesis is a multistep process. The evolution of a line of tumor cells seems to depend, at the very least, on the accumulation of mutations altering a number of genes, among them oncogenes. The altered genes then function in concert to create full-fledged malignant growth.

To date, however, oncogenes have been detected in only 15 or 20 percent of human tumors. Human tumor cells may well carry other growth-promoting activated oncogenes, which remain elusive simply because current methods for detecting such genes are rather insensitive. It has seemed likely, however, that yet other cancer genes could not be identified because they operate on totally different principles. For example, if some tumors arise through the loss of growth-suppressor genes, the genes' influence would be felt only when they were absent.

Another basic principle distinguishes oncogenes from growth-suppressor genes. The oncogenes studied to date are invariably activated through somatic mutations; genetic changes occurring in one or another target organ and not in the germ cells. Mutant, activated oncogenes are therefore not transmitted from parent to offspring. In strong contrast, mutant forms of the growth-suppressor genes might indeed be found in germ cells—sperm or eggs—and be passed on from one generation to the next. A child acquiring a mutant growth-suppressor gene at conception would have a greater lifetime risk of the onset of cancer.

A window on the operation of such growth-suppressor genes has been opened by studies of retinoblastoma, a tumor of the eye. The tumor is fairly rare, afflicting about one in 20,000 infants and young children, but it serves as a model for many other, ostensibly unrelated types of cancer. What has been learned about the gene that causes it has already put in place several large pieces of the genetic puzzle of cancer causation.

Retinoblasts are the precursors of cells in the retina, the light-sensitive screen at the back of the eye; the particular cells that form a retinoblastoma seem to be ones ordinarily destined to become the photoreceptor cells called cones. Once a retinoblast differentiates to form a specialized retinal cell, it stops dividing and can no longer serve as a target for tumorigenesis. This would seem to underlie the age distribution of retinoblastomas, which are never seen in older children or adults.

Until the middle of the 19th century the onset of retinoblastoma signaled a uniformly fatal disease course. Tumors rapidly invaded the brain and led to the death of an afflicted child. With the invention of the ophthalmoscope by Hermann von Helmholtz in 1850, it became possible to look into the globe of the eye and detect the tumor long before it expanded to invade adjacent tissues. With early diagnosis came the possibility of cure (through surgical removal of the af-
lected eyeball), and this led in turn to the first cases of "familial" retinoblastoma; many patients survived to reach adulthood and have children, about half of whom contracted this otherwise very rare tumor. Retinoblastoma also continued to strike in its historic "sporadic" form, which is seen in children whose families have no history of the disease.

By the middle of this century the two types of retinoblastoma were recognized as being distinct manifestations of a single disease. For the geneticist they represented a bewilderment. The familial form of the disease clearly depended on the transmission of a gene from parent to offspring. Just how genes could be involved in triggering the sporadic disease was not clear, however. If genes were indeed involved, were they the same ones responsible for triggering the familial disease, or were different genes implicated in each form?

In 1971 Alfred G. Knudson, Jr., of the Institute for Cancer Research in Philadelphia proposed a simple genetic explanation for these apparently complex phenomena. His hypothesis recognized that mutated genes can come to a person through the two routes I have alluded to: as an inheritance from a parent or through the somatic mutations that occur accidentally in tissues during one's lifetime. He theorized that the origins of both types of retinoblastoma could be traced to changes in the same set of genes.

After studying the rate at which the disease appeared in young children, Knudson concluded that all tumor cells actually carry not one mutant gene but rather two such genes. In familial retinoblastoma, he argued, the first of the two required mutations is present in a critical gene from the moment of conception and so becomes disseminated to all the cells in a child's body, including all the cells of the retina. Such a mutated gene, which confers susceptibility to the tumor, might have been acquired from a genetically affected parent or from a genetic accident during the formation of a parent's egg or sperm. The second required mutation could then occur somatically, and thus locally in one of the many retinal cells that already carried a congenitally acquired mutation. In the contrasting nonfamilial, sporadic retinoblastoma, Knudson proposed, both of the required mutations occur somatically and locally within a single retinal cell, whose descendants then expand to form a tumor.

It is clear now that Knudson's formulation was essentially correct. At

SITE ON CHROMOSOME 13 was first implicated in retinoblastoma by Jorge J. Yunis of the University of Minnesota Medical School. When he stained chromosomes from retinoblastomas to highlight their banding, he noted that a segment of the long arm of one chromosome 13 was often missing. The computer image, made by Yunis, compares a normal chromosome 13 (left) with the abnormal one he first reported (see Illustration on next page). Part of a large light band near the top of the normal chromosome, including the orange subband, is deleted in the abnormal version. (The oval shapes at the top represent the telomeres, the ends of the chromosomes, which in micrographs seem not to be connected to the rest of the chromosome.)
the time, it crystallized thinking about retinoblastoma, but it did leave two major questions unresolved. First, what is the nature of the gene or genes that are acquired in mutant form from a parent or are altered by somatic mutations? Is one gene involved or several distinct genes? Second, what kinds of mutation intervene to create the cancer-causing alleles (specific versions of a gene)? Are they mutations of the kind that hyperactivate a proto-oncogene and thereby create an oncogene? Or do these mutations serve on the contrary to inactivate a gene and thereby wipe out its functioning?

The essential clues to solving these puzzles came from microscopic examination of the chromosomes present in normal cells and in retinoblastoma cells. When an experimenter traps cells in an early stage of cell division, their chromosomes can be viewed with special clarity. A good microscopist can even discern the detailed structure of a chromosome through the presence of individual bands arrayed in characteristic positions along its length.

The chromosomes of a tumor cell often look different from those of a normal human cell. The deviations may simply reflect the genetic chaos that accumulates in tumor cells during their toruous evolution from the normal to the malignant state. On occasion, however, microscopists can identify specific chromosomal changes that occur reproducibly in many tumors of a given type.

This was the case when Jorge J. Yunis of the University of Minnesota Medical School studied cells from a number of different retinoblastomas. The longer of the two arms (the q arm) of one chromosome, No. 13, was often found to have a deletion: one or another of the bands ordinarily seen on the chromosome was often missing. Most often it was some part of band 14. The association of 13q14 alterations with retinoblastoma was much too frequent to be attributed simply to random genetic accidents; instead it seemed the deletion must confer a growth advantage of some kind on the cells that spawn the tumor.

These data provided the beginnings of an answer to Knudson's puzzles: they showed that one of the mutation events associated with retinoblastoma is a deletion that could well lead to the total loss of a gene and thus to the loss of some critical function. Further chromosomal analysis showed that in certain children affected with familial retinoblastoma, the chromosome-13 deletions could be found not only in the tumor cells but also in normal cells throughout the child's body and in the body cells of one parent. When the deletions were found in sporadic cases, on the other hand, they were invariably confined to the tumor cells. The damaged chromosomes could be found, in other words, precisely the cells Knudson had predicted would carry the mutant genes responsible for the two forms of retinoblastoma.

The chromosome-13 gene ostensibly involved in triggering these tumors was given the name \textit{Rb}. It had been identified by the presence of a microscopically visible deletion, which at the molecular level involves the loss of a segment of DNA encompassing hundreds of thousands of the bases that constitute a strand of the genetic material. Yet such a large chromosomal lesion is only one of a number of mutational mechanisms by means of which the \textit{Rb} gene might be inactivated. Much smaller deletions that have no effect on the microscopically visible structure of the chromosome can be just as effective in knocking out gene function. Indeed, recent work has shown that changes affecting single DNA bases ("point mutations") may suffice.

At this stage one of Knudson's two critical target genes had been associated with a specific site on chromosome 13, but the identity of the second target was still elusive. It might be a different gene on one or another of the 22 chromosomes of the cell, all but two of which are present in two copies. Alternatively, the second target for mutation might be the second copy of the initially affected chromosome-13 gene, which still survived on the intact, paired chromosome 13.

Through a clever genetic trick, data were in hand by 1983 suggesting that the second genetic target for mutation was indeed to be found on the second chromosome 13. The evidence came from an indirect genetic analysis that traced the fate of another gene—one that is conveniently situated very near the \textit{Rb} gene on chromosome 13. By studying such a "marker" gene one can often predict the fate of a closely linked but invisible neighbor. Robert S. Sparkes of the University of California School of Medicine at Los Angeles found such a marker: the gene encoding an enzyme called esterase D. Rosalind Godbout, Brenda Callie and Rob-

PHOTOMICROGRAPHS of the normal and abnormal chromosome 13s are shown here, flanking a diagram of the normal chromosome. The deletion undergone by the abnormal chromosome (right) is indicated on the diagram by the blue bracket. A different deletion, observed in a chromosome from a different retinoblastoma, is bracketed in black. The two deletions overlapped somewhat, pinpointing the site of a putative retinoblastoma gene in a small region of subband 13q14.1, close to subband 13q14.2; q designates the long arm of a chromosome. (Chromosome 13 has a minute short arm: the segment above the constriction near the top of the chromosome.)
PEDIGREE of a family with familial retinoblastoma was published by Thaddeus P. Dryja and his collaborators. Affected members are indicated by solid circles (females) or squares (males). Five children in the second generation developed the tumor. One son who was unaffected had nonetheless inherited a mutated chromosome 13; two of his daughters were affected.

er B. Phillips of Toronto's Hospital for Sick Children found that in some retinoblastoma patients the esterase D gene was present in normal cells in two different versions, one on each of the paired chromosomes 13. In patients' tumor cells, however, the esterase D marker gene was often found in two identical copies. One of the two alleles (versions) had been lost and had been replaced by a duplicate of the other allele.

The esterase D gene itself has no functional role in retinoblastoma, but it served as an easily traced proxy for the neighboring Rb gene. If one of the two versions of the enzyme gene was lost and replaced by the survivor, the same fate was imputed to the neighboring Rb gene. It appeared, then, that if a retinal cell began with one normal and one mutated version of its Rb gene, it might sometimes end up with two mutated copies of the gene. More detailed analysis confirmed that possibility: tumor cells were often seen to carry two copies of the defective allele of the Rb gene. This finding provided the critical clue to the second step in tumor formation. It was the loss of the surviving, intact copy of the Rb gene.

Kodnson's theory could now be reformulated. The two hits required to trigger cancer involve the two copies of the Rb gene. Each hit inactivates one copy of the gene, creating an inactive, or "null," allele. Children born with one intact and one defective copy of the Rb gene might lose the intact copy through somatic mutation in one of their retinal cells, triggering cancer. Others, although born with two good copies, might through rare misfortune happen to lose both copies of the gene in a retinal cell early in life, leading to the same end result.

The children carrying a congenitally acquired, mutant Rb gene are fully normal save for their greatly increased risk of cancer. Although they have only one normal copy of the gene in virtually every cell of their body, their overall development is unremarkable. The single normal Rb allele is clearly sufficient to fulfill the gene's function in normal development; the defective Rb allele in every cell does not actively perturb this development. In other words, the mutation is "recessive" and is manifested only when the surviving, intact copy of the gene (which is "dominant" at the cellular level) is lost in one or another retinal cell.

Now, if the inactivation of a gene such as Rb serves to trigger the runaway growth of cancer, it follows that the gene in its normal incarnation must act to restrain cell growth. This implies the existence of a class of genes dedicated to the negative regulation of normal growth, which I prefer to call growth-suppressor genes. Because loss of these genes can lead to malignant growth and because many oncogenes act in a diametrically opposite way—by promoting aberrant growth—such genes as Rb have come to be called anti-oncogenes or tumor-suppressing genes. These terms are likely to survive, but they are imprecise. The normal function of the Rb gene is surely to suppress growth in general: any involvement in tumors is unintended. Imprecise or not, the term anti-oncogene has some logical basis. A gene such as Rb, when it is intact, may well function to oppose the action of an oncogene. By the same token, a cancer cell, which gains a growth advantage by developing oncogenes that actively promote cell growth, might further help itself to proliferate by getting rid of genes that have hitherto constrained its growth. Indeed, this may be a particularly frequent mechanism of cancer causation, since it is much easier to knock out a gene by crude genetic blows than to hyperactivate a gene by subtle mutational tinkering.

The loss of anti-oncogenes may be a quite common, if unrecognizable, part of the development of cancer. Examination of the chromosomes of various tumor types often reveals characteristic chromosomal aberrations. On occasion there is a loss of specific chromosomal segments, but in other instances more subtle genetic analyses have been required to pinpoint the loss of specific genes.

These genetic analyses, pioneered by Webster K. Cavenee (who is now at the Ludwig Institute for Cancer Research in Montreal), follow in outline the same strategy whereby the fate of the Rb gene was traced by examining the fate of its closely linked neighbor, the esterase D gene. In Cavenee's analyses the closely linked neighbors are specific DNA sequences that may be present in dissimilar (heterozygous) versions in normal tissue and in identical (homozygous) versions in tumor tissue. The progression from heterozygosity to homozygosity of these DNA segments provides a good indication of the fate of closely linked anti-oncogenes.

By now there is a substantial list of genes that appear to lose both functional copies during the creation of one or another type of tumor. Ductal breast cancer, for example, involves a gene on the long arm of chromosome 13; Wilms' tumor of the kidney involves a gene on chromosome 11, and small-cell carcinoma of the lung apparently results from defects in a gene on chromosome 3. In each case it appears that both copies of a critical gene are frequently either lost or rendered inactive during the evolution of the tumor-cell clone. This tissue specificity (the association of the loss of certain genes with specific tumor types) suggests that each of
these genes is normally involved in constraining the growth of only a narrow range of cell types in the body.

At first it seemed that the range of action of the Rb gene would also be limited narrowly to tumorigenesis in the retina. However, careful clinical follow-up of children afflicted early with familial retinoblastoma has revealed that later in life they incur a greatly increased incidence of tumors that originate in connective-tissue cells—notably osteosarcomas, which arise in bone-forming cells. In other words, mutant copies of the "retinoblastoma gene" predispose to more than just retinoblastoma. Indeed, recent evidence suggests that observable changes in the Rb gene are as frequent in osteosarcomas as they are in retinoblastomas—even in osteosarcomas from individuals with no history of retinoblastoma. In many osteosarcoma patients the tumor seems to arise solely through somatic genetic accidents affecting the Rb gene.

This introduces yet another puzzle, since the retina and the tissues that yield these other tumors have little in common in either evolution or embryonic development. Perhaps sometime during the evolution of the early multicellular ancestors of human beings, a billion and more years ago, a precursor of the gene we call Rb came to be involved in controlling the growth of two quite different cell types and associated tissues.

The notion that certain tumor cells may be abnormal because they have lost critical bits of genetic information has been encouraged by a totally independent kind of...
experimentation, much of it initiated by Henry Harris of the University of Oxford. He applied techniques that enable one to make a hybrid cell by fusing two genetically distinct cells. This is achieved with an agent that causes the outer membranes of neighboring cells to unite, forming one large membrane that envelops the nuclei of both partners. The two complements of chromosomes then merge into one large array carrying twice the usual amount of genetic information. The motive in these forced marriages is to observe how the traits of the two partners blend following their union. Often the genes of one or the other parent cell may dominate in determining the behavior of the hybrid.

Over the past two decades such experiments have led to the surprising observation that hybrids formed between malignant tumor cells and normal cells often behave like their normal parents, that is, they do not form tumors. This runs counter to intuition, which suggests that virulent tumor cells have traits far more potent than those of their normal neighbors.

An explanation comes once again from the insight that a tumor cell often becomes aberrant through the loss of a critical growth-suppressing gene or genes. In undergoing fusion with a normal cell, the tumor cell regains a growth-regulating gene it lost early in its evolution toward malignancy; the restored gene can reimpulse growth control on a cell that has long lacked such control.

A dramatic demonstration of such reassessment of control was reported recently by Eric J. Stanbridge of the University of California College of Medicine at Irvine. His group worked with a number of tumors, among them Wilms' tumor. Fusion of a Wilms' cell to a normal cell yields a nontumorigenic hybrid.

Stanbridge's group managed to introduce a single normal human chromosome 11 into Wilms' tumor cells. The genetically enhanced cells then reverted to normal: they lost the ability to form tumors. This showed, even more directly than cell-fusion work, that the malignant growth of these tumor cells depends on the absence of a gene or genes normally present on chromosome 11.

The hybrid-cell findings and Stanbridge's result strongly reinforce the impression that a loss of genetic information may be as important for tumor formation as the creation of hyperactive growth-promoting oncogenes. In the long run this realization may open a path toward a therapy based on the introduction of genes into tumor cells that lack them.

SEARCH FOR RB GENE began when Dryja tested fragments of the DNA of the normal chromosome 13 to see if any of them were missing in retinoblastoma DNA's and so were likely to be part of the gene whose deletion leads to tumorigenesis. This electrophoresis gel shows a critical result. Fragments H2-42 and 7D2 have hybridized with, or bound to, matching fragments of the DNA from each of 13 tumors; having been tagged with a radioactive label, they are visible on the gel as dark bands. The third fragment, H3-8, has similarly hybridized with the DNA of 12 of the tumors—but it has failed to find a match in the DNA of tumor No. 5; the segment of chromosome 13 represented by probe H3-8 is deleted from the DNA of this tumor.

In spite of these major advances, until recently the RB gene was only a theoretical entity, its existence deduced solely from the genetic phenomena I have described. An ultimate goal for cell biologists and biochemists is to understand at a molecular level just how a gene such as RB acts to restrain or inhibit cell growth. The clearest path to such understanding is through the isolation of the gene by molecular cloning. The isolation of RB was a particularly challenging task because the gene's most obvious effects are manifested only when it is absent. It is therefore difficult to devise assays that show directly whether one or another candidate DNA fragment is indeed the sought-after gene.

Thaddeus P. Dryja of the Massachusetts Eye and Ear Infirmary undertook the long-shot quest in 1983, concentrating on human chromosome 13. Marc Lalande and Samuel A. Latt of the Children's Hospital in Boston had created a collection of DNA clones, each derived from a fragment of one or another randomly chosen region of the normal chromosome 13. Dryja sifted through this collection, gambling that one of the fragments might be allied with the chromosomal DNA sequence constituting the RB gene. It was a daunting undertaking; the RB gene is now known to account for only a thousandth of the total DNA of chromosome 13.

Each of the chromosome-13 fragments served as a "probe" in a DNA hybridization test. The test showed whether a given probe could find a matching segment in the DNA isolated from one or another of a battery of retinoblastoma DNA's, or, alternatively, was not able to find such a matching segment because the probe derived from a part of the chromosome that had been deleted during the formation of a tumor.

Dryja's gamble paid off when one cloned probe was found to come from a chromosomal segment that was fully deleted in two retinoblastoma DNA's out of the 50 or so he examined. This was far from proving that the cloned fragment was even part of the RB gene. It did suggest at the very least that the fragment represented a sequence that lay near RB on the chromosome that had undergone deletion along with RB in the course of the genetic accidents that had triggered these two retinoblastomas.

It remained for Dryja's group, with Stephen H. Friend and others in my laboratory at the Whitehead Institute for Biomedical Research, to establish the precise relation between the cloned fragment and the ostensibly linked RB gene. First we discovered that the cloned fragment was closely related to a messenger-RNA molecule found in normal retinal cells. Meas-
messenger RNA (mRNA) is the nucleic acid that transfers information from active genes in a cell's nucleus to the cytoplasm, where the information is read out by the machinery responsible for making proteins. The discovery of a retinal-cell mRNA related to the cloned DNA fragment meant the fragment was (by a remarkable stroke of luck) part of a gene that is actively expressed in normal retinal cells. Significantly, we were not able to detect mRNA related to the fragment in any of several retinoblastomas. That meant the gene from which the mRNA derived was inactive or absent in the tumor cells—behavior consistent with that of the Rb gene.

The possibility that the gene was Rb remained to be confirmed. Friend made a DNA copy of the mRNA by the process called reverse transcription. The reverse-transcribed “cDNA” served as a probe with which to survey and map out the entire region on the normal chromosome that was responsible for generating the mRNA—in other words, the gene that might be Rb—and to clone it. The cloned gene turned out to be very large, encompassing 200,000 DNA bases. Again by means of nucleic acid hybridization, we studied the configuration of this large chromosomal domain in the DNA's of 60 retinoblastomas and osteosarcomas. In about 30 percent of the DNA samples we found evidence that one copy or both copies of the cloned gene had suffered substantial changes in structure through deletion. (Subtler changes in DNA structure, which can be equally effective in inactivating a gene, would have escaped detection in this analysis.)

We now had evidence that the cloned DNA gene was frequently knocked out in various, independently arising retinoblastomas. But did this cloned gene represent the Rb gene itself? The convincing evidence ultimately came from examining the precise configuration of the various deletions affecting the cloned segment (see lower illustration at left). The goal was to show that the cloned gene encompassed the Rb gene, and not an irrelevant gene to the right or left of it along the chromosome.

Certain of the mapped deletions caused loss of the entire cloned gene. These were uninformative, since they did not address the critical issue at hand: the cloned gene might just have happened to be deleted along with some other part of the region that included the “intended” target, the Rb gene. Three deletions that seemed to involve both the right-hand end of the gene and adjacent, rightward-lying DNA were more meaningful. They indicated that the target for deletion either lay to the right of the cloned gene or was the cloned gene itself. Yet other mutations caused loss of the left end of the cloned gene and other leftward-lying sequences. It seemed increasingly likely that the cloned gene was indeed the common target of these randomly occurring deletions.

The critical evidence came from the discovery of two deletions that began and ended entirely within the confines of the cloned gene. These showed unequivocally that the random deletions leading to retinoblastoma converged on the cloned gene and not on neighboring DNA segments.

Our work has since been replicated and extended by Wen-Kwa Lee of the University of California School of Medicine at San Diego and by Yuen-Kai Fung and William F. Benedict of the University of Southern California.
**RB GENE ENCODES** a protein that is found in the nucleus of normal retinal cells (left) but not in retinoblastoma cells (middle). The EIA oncoprotein of an adenovirus transforms infected cells by binding to a host-cell target protein that turns out to be identical with the RB protein (right). The fact that the same protein whose absence in retinal cells gives rise to a tumor is also involved in transformation by the adenovirus suggests the protein may be a central growth regulator.

School of Medicine. The aggregate results provide evidence that the cloned gene indeed is the normal RB gene. Most persuasive are the repeated findings that this particular segment of DNA is damaged by major deletions in many different, independently arising tumors.

The ultimate proof of the identity of the cloned gene will have to come from a functional test in which DNA clonese carrying intact versions of the putative RB gene are inserted into tumor cells that have no intact RB gene. If the cells thereupon revert at least partially to normalcy, this will argue strongly that the cloned DNA is indeed providing the critical genetic information whose loss triggered tumor formation. Such experiments are currently under way in several laboratories.

Many experimental avenues can be followed once growth suppressors such as RB have been isolated by cloning. Both applied clinical applications and basic research problems come to mind. The clinical utility stems from the ability to use cloned DNA segments to analyze the structure of related sequences in a variety of normal and tumor samples to detect altered versions of such genes as RB. The reader will recall that mutant RB alleles can be passed from an affected parent to half of the offspring, on the average. A cloned probe could in principle detect defective RB alleles even in the early fetus, providing predictive data on the likelihood of tumor onset later in life.

Beyond the clinical applications loom a number of problems in basic biology. RB is the first growth-suppressing cancer gene to be isolated. The lessons learned from it are likely to prove instructive for an array of genes that act in a similar way, each responsible for constraining the growth of one or another cell type. The array of these genes already known by virtue of their deletion in certain tumors may represent only the tip of the iceberg. The oncogenes already number upward of 50 genes; the size of the repertoire of these countervailing negative regulatory genes cannot yet even be guessed.

The most intriguing question is: What are the mechanisms through which these genes act to limit or shut down normal growth? Some months ago Lee's group reported that the RB gene specifies a protein with a molecular weight of 105,000 that is found in the nucleus of the cell. Nuclear proteins are often involved in regulating the expression of genes.

A strong indication that RB does indeed specify a regulatory protein has come just recently from unrelated work designed to find out how tumor viruses transform the cells they infect. One such virus, an adenovirus, is known to carry an oncogene designated EIA into susceptible target cells. The oncogene specifies a protein that reprograms the metabolism of the host cell, eliciting malignant behavior. How does the oncoprotein act?

Workers in Ed Harlow's group at the Cold Spring Harbor Laboratory in New York and in Philip E. Branton's group at the McMaster University School of Medicine in Ontario had found that in a virally transformed cell the viral oncoprotein is expressed with certain host-cell proteins. Presumably the viral protein alters these targets and thus trips the cellular switches that lead to transformation.

One of the targets with which the viral oncoprotein complexes is a host-cell protein whose molecular weight is 105,000. Harlow, Peter Whyte and Karen Buchkovich noted that this protein has properties similar to those of the RB protein. Together with Jonas than Horowitz in my laboratory, they went on to show that the two proteins are in fact identical. The adenovirus EIA protein, in other words, makes cells malignant by completing with (and perhaps thereby inactivating) the same protein that is encoded by the RB gene and is missing from retinoblastoma cells. Since the EIA protein is a direct regulator of gene expression, the RB protein too must be directly involved in the modulation of gene expression.

It is clear that detailed understanding is only several years away. When it comes, we shall finally see both sides of the coin: how cell growth is turned on and how it is turned off. This will come new insights into the origins of cancer and into the still obscure mechanisms that allow fertilized eggs to develop into complex organisms such as ourselves.

**FURTHER READING**


