What Do Oncogenes Do?

The evidence implicating oncogenes as causes of human cancers, although still circumstantial, has been accumulating rapidly during the past few years

The idea that our own cells contain genes with the potential of causing the cancerous transformation of those cells is not a comfortable one. Nevertheless, some 20 oncogenes (from *onkos*, the Greek word for mass or tumor) have been identified that can, when appropriately activated, produce cancers in animals and cause the malignant transformation of cultured cells.

The evidence implicating oncogenes in the development of human cancers is still largely circumstantial. As J. Michael Bishop of the University of California School of Medicine in San Francisco puts it, "It is guilt by association at the moment, but the association is provocative. One or another of the 15 to 20 oncogenes pops up consistently. I'm not a master statistician, but I know what that means."

Much of the intense research of the past few years has been concentrated on the issue of oncogene activation. Oncogenes are found in all the cells of the body, except mammalian red blood cells which lack nuclei, but the vast majority of cells never become cancerous. One thing that has become clear is that no sweeping generalizations can be made about the manner in which the oncogenic potential of the genes is realized. In some cases, this may be the result of increased synthesis of the protein product of the genes; in others, an altered gene may produce a defective product. Also, more than one oncogene may have to be activated to transform a cell to malignancy.

The way in which the oncogenes might bring about this transformation is still unknown. What their products do is almost a total mystery, although there are a few intriguing clues suggesting that some of the products may reproduce the effects of naturally occurring growth factors. Learning about oncogene function may shed light on the control of normal growth and development, as well as leading to a better understanding of the origins of cancer.

The oncogene story dates back to 1911 when Francis Peyton Rous discovered a virus that causes sarcomas in chickens. Over the years, additional cancer-causing viruses were isolated from tumors of various types and other species. Most of them turned out to have RNA as their genetic material and were classified as "retroviruses" because their life cycles include a step in which the RNA is copied into DNA—the reverse of the direction in which genetic information more commonly flows. Until the early 1970's when reverse transcriptase, the enzyme that copies RNA into DNA, was discovered, it was generally thought that DNA could only be transcribed into RNA.

The history of the oncogene-carrying retroviruses has had its ups and downs. Because they had been isolated from tumors of laboratory animals and did not appear to cause epidemics of cancers in more natural settings, there were questions about whether they were artifacts and not related to how cancer usually develops, especially in humans.

Then, in the mid-1970's, work from several laboratories revealed that the cancer-causing ability of the Rous sarcoma virus could be attributed to just one of the four genes in the viral genome. The production of a single protein with a molecular weight of about 60,000 was sufficient to effect the malignant transformation of cells. At the very least, investigators thought, the viral oncogene, which was designated *src*, could be used to find out what kind of changes in the cell might underlie malignancy.

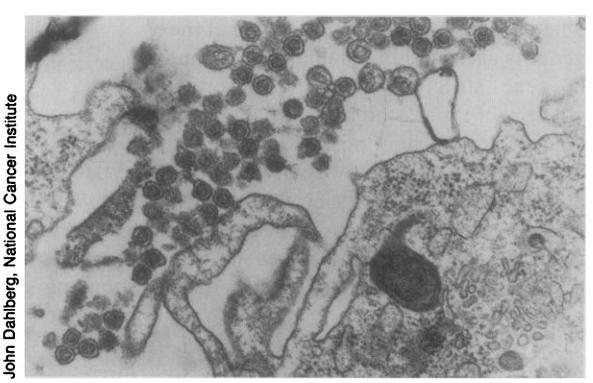
Investigators had also learned that normal cells contain DNA sequences that are very similar to the viral *src* gene. The cellular gene had apparently been picked up by an originally nontransforming virus during the course of infection. In this new situation the oncogenic potential of the gene was revealed.

Similar findings were made with many other cancer-causing retroviruses that proved to carry genes with the capacity to transform cells. All these viral oncogenes contain DNA sequences picked up from cells. Five of them, including *src*, are composed only or almost exclusively of cellular sequences. The oncogenes of the remainder consist of cellular sequences that have been fused with viral sequences, usually a portion of the gene coding for the viral core proteins.

The cellular counterparts of the viral oncogenes have been closely conserved throughout evolution. They are found in species as diverse as fruit flies, fish, and mammals, including humans, and, according to two recent reports, even in yeast. "Oncogenes are but wayward copies of genes found in all metazoan organisms," Bishop says.

The close conservation of the cellular genes suggests that they have essential roles to play, probably in cell differentiation or in the regulation of cell division. The question then is, what makes the genes go wrong and produce the uncontrolled cell division and abnormal differentiation patterns seen in cancer cells? The early work favored the idea that increased production of the gene product, or production at the wrong time in the life of the cell, causes cells to become cancerous. Large amounts of viral gene products are made in infected cells.

Three additional developments supported the theory that it is increased oncogene expression that leads to cancer. A virus that did not carry its own



John Dahlberg, National Cancer Institute

Retroviral particles

Particles of murine leukemia virus, one of the type C transforming viruses.

oncogene was found to cause cancers in birds when a viral sequence called the long terminal repeat (LTR) was inserted near the cellular counterpart of the *myc* oncogene. LTR's are found at the ends of all retroviruses and are effective promoters of gene expression, although the possibility remains that the *myc* gene near the LTR insertion may also undergo structural alterations that could change the activity of its product.

However, attachment of LTR's to otherwise nontransforming cellular *mos* and *ras* genes does confer on them the ability to transform cultured cells. And very recently, researchers have found that some human cancer cells, both from cultured cell lines and from primary tumors, carry many extra copies of certain oncogenes. This gene amplification is associated with increased expression of the genes (*Science*, 6 January, p. 40).

Simply showing increased expression does not prove that this is sufficient, or even necessary, to cause transformation, especially if the cellular genes have undergone structural changes when they were picked up by the viruses or otherwise activated. Trying to determine whether the transforming potential of the genes is activated by qualitative changes in gene structure or by quantitative changes in expression has not been easy. And of course it is possible that there may be more than one way of activating an oncogene. This may be true for the *myc* gene (see box). But recent evidence suggests that structural changes may activate at least some oncogenes in some circumstances.

Advances in recombinant DNA technology have made it relatively easy to clone the viral oncogenes and their cellular counterparts and to compare their nucleotide sequences. Differences, some subtle and others more dramatic, have been found between them. "Wherever you look, wherever there are sequence data available, they are not identical," says Peter Duesberg of the University of California at Berkeley.

But, just as demonstration of increased gene expression does not unequivocally prove that this is the change that caused transformation, neither do the findings of altered structure. The alteration has to be directly correlated with the acquisition of transforming behavior. This has been done for three members of the *ras* gene family.

The *ras* gene work was made possible by the discovery a little over 2 years ago that transforming genes could be detected in some cancer cells by a gene transfer assay. DNA is prepared from the cancer cells and transferred into cultured cells, which may become transformed as a result. Many of the transforming genes thus identified turned out to be identical to viral oncogenes, including the *ras* genes of the Harvey and Kirsten sarcoma viruses, or to be previously unknown genes that are structurally related to the viral oncogenes.

This is one of the recent developments in which oncogenes have "popped up." They were isolated from cancer cells of both human and animal origin, and many of the cancers either arose spontaneously or were induced by chemical carcinogens. The discovery gave impetus to the view that activation of the cellular oncogene counterparts might be involved in the etiology of cancer in general, not just those involving viruses.

About a year ago, three groups of investigators reported that the only functionally significant difference between the nontransforming cellular Harvey *ras* gene and its transforming cellular counterpart from a line of bladder carcinoma cells was a change in just one nucleotide. These experiments involved replacing segments of the normal gene with corresponding sequences of the transforming gene and also performing the reciprocal exchanges. The ability to transform cultured cells could be correlated with that one sequence alteration. The amount of *ras* gene product made by the cells was not critical for transformation.

Oncogene products may be either growth factors or other molecules needed for growth factor activity.

Since then, three groups, those of David Goeddel of Genentech, Inc., in San Francisco, Manuel Perucho of the State University of New York in Stony Brook, and Michael Wigler of Cold Spring Harbor Laboratory have cloned and determined the nucleotide sequences of the coding regions of the normal human Kirsten *ras* gene and its transforming counterpart from lung and colon carcinoma cells. The Wigler group has done the same for the N-*ras* genes, a member of the *ras* gene family that was originally identified in neuroblastoma tumors cells by the gene transfer assay.

Again the transforming activity could be attributed to small, specific changes in nucleotide sequences. "There are at least five sites at which a *ras* gene can be activated," Wigler says. The sites cluster in two regions, affecting the codons for amino acids 12 and 13 and for amino acids 59 to 63.

There are also indications that activation of the *src* gene involves structural alterations. For example, very high expression of the normal cellular gene does not transform cells whereas a somewhat lower expression of the viral gene does, according to Richard Parker of the San Francisco group.

Investigators have focused on the 3′ (right-hand) end of the gene as a possible cause of the difference in transforming abilities between the viral and cellular genes. The product of the *src* gene is a kinase, an enzyme that attaches a phosphate group to the amino acid tyrosine. Most of the other kinases put it on serine residues. The portion of the *src* gene product that has the kinase activity is encoded in the 3′ end of the gene.

There are very few differences between the cellular and viral *src* genes throughout most of their nucleotide sequences, but Hidesaburo Hanafusa and his colleagues at Rockefeller University find a significant variation at the 3′ end. The last 19 amino acids of the cellular gene product have been replaced with a shorter sequence, which contains only 12 amino acids. This change may contribute to the transforming activity of the viral *src* gene. Hanafusa says, "It is very likely that the cellular *src* gene has to undergo some kind of change."

One of the great questions surrounding oncogene research is whether it has anything to do with the natural origin of human cancers, many of which are triggered by chemical carcinogens. One indication that it may comes from Mariano Barbacid and his colleagues at the National Cancer Institute. They detected a mutation in codon 12, one of those frequently found to be altered in activated *ras* genes, in the Harvey *ras* gene from tumor tissue of one of eight human lung cancer patients. They could not detect the mutated gene in blood cells or normal bronchial tissues from the same patient, however. "I think this is the first time the mutated gene has been found in a primary tumor and not in a normal tissue from the same patient," Barbacid says.

The Barbacid group also has evidence implicating *ras* gene activation in chemical carcinogenesis in rats. If rats between the ages of 50 to 60 days are given a single dose of the chemical nitrosomethylurea, almost 100 percent develop mammary cancers within 6 to 12 months. The NCI workers found that tumor cells from each of the nine animals that developed the carcinoma after this treatment contained a transforming Harvey *ras* gene. Sequence analyses showed that

Perusing the *myc* Gene

Tumor cells from human patients with Burkitt's lymphoma consistently show certain chromosomal abnormalities, the most common of which is an exchange of segments between chromosomes 8 and 14. Toward the end of 1982 several groups of investigators found that one consequence of this abnormality is the translocation of the cellular *myc* gene from its normal position on chromosome 8 to a new location on chromosome 14, in the region containing the genes for the heavier of the two chains of which antibody molecules are formed. A similar translocation was also found in cells of an analogous tumor, the mouse plasmacytoma.

The discovery that naturally occurring tumors carry an abnormality of the *myc* gene attracted a great deal of interest because it was still another piece of circumstantial evidence suggesting that an oncogene might be involved in the etiology of cancer. The hope was that studies of the translocated gene might provide some clues to the manner in which its oncogenic potential is activated.

Results obtained during the past year have indicated that the translocation affects *myc* gene regulation, usually causing increased expression of the gene in tumor cells. "The essence of what one sees is that there is some disturbance in *myc* regulation," says Philip Leder of Harvard Medical School. Nevertheless, the situation is still somewhat complicated as there appear to be several different ways of disrupting *myc* gene control.

In the antibody-producing cells from which Burkitt's lymphoma and mouse plasmacytomas are derived, the heavy chain region of the genome is a site of active transcription of DNA into messenger RNA, the first step in gene expression. However, a simple increase in transcription of the *myc* gene into messenger RNA as a result of its coming under the influence of the regulatory sequences that stimulate transcription of heavy chain genes seems unlikely as a general mechanism for activating *myc*. The translocated gene is oriented the wrong way for it to be influenced by one type of regulatory sequence, the promoter, which acts only in one direction.

Enhancer elements, another type of control sequence that stimulates gene expression, work in both directions. Adrian Hayday of Susumu Tonegawa's laboratory at the Massachusetts Institute of Technology has identified a cell line in which the translocated *myc* gene is in a position near the heavy chain enhancer, where it might be turned on. This appears to be an exception, however. Investigators have generally found that, as a result of the chromosomal exchange, the enhancer is moved to the chromosome where the *myc* gene was originally located. Consequently, the known heavy chain enhancer cannot contribute to the activation of the translocated *myc* gene in these lines.

Much attention has focused on changes in the *myc* gene itself as a possible cause of altered control. Early on, investigators, including Michael Cole of Saint Louis University School of Medicine and Kenneth Marcu of the State University of New York at Stony Brook, found that the first exon is lost as a result of the *myc* gene translocation in mouse plasmacytomas. "All the breaks displace the exon," Cole says. "Otherwise they are variable in position."

Determination of the nucleotide sequence of the *myc* gene reveals that the first exon, which is more than 650 base pairs long, cannot code for protein structure. Nevertheless, the sequence of the first exon appears to have changed little during evolutionary history, in contrast to what is usually found for noncoding gene segments, which suggests that the first exon serves some kind of essential function.

The function of the first exon may be control of *myc* gene expression. It contains a segment of about 70 base pairs that is homologous to a segment in the second, coding exon. These segments might combine to form a loop structure that could impede translation of the *myc* messenger RNA into protein. Loss of the first exon might then result in increased production of the *myc* product.

Although loss of the first exon might activate *myc* in the mouse plasmacytomas, it does not ordinarily do so in human lymphoma cells. "In the human rearrangement,' Leder says, "there is usually no decaptitation of the *myc* gene, as is almost always seen in the mouse. The loss of the first exon is not necessary for activation."

Nevertheless, there are indications of altered control of the translocated *myc* gene in human cells. "The translocated and untranslocated *myc* gene are under differential control," says Carlo Croce of the Wistar Institute. "The untranslocated gene is normally silent." One possibility is that the gene is controlled by its own product, a common type of feedback inhibition. The product may be able to suppress the activity of the gene when it is in its normal location but not after it has been moved.

In addition, there is a shift in the preferred site for initiation of transcription, according to the Leder group and also to that of Terence Rabbitts of the MRC Laboratory of Molecular Biology in Cambridge, England. The net effect is to produce a preponderance of somewhat longer transcripts in Burkitt's lymphoma cells than in normal cells. How this change might contribute to transformation is unclear.

Although the expression of the *myc* gene is usually higher in the tumor cells than in normal cells, there are some exceptions in which the transcription is not especially elevated. This has led to suggestions that it is not necessarily an increase in *myc* gene product that contributes to transformation but production at the wrong time in the cell cycle. The Leder group's results indicate that the gene is normally turned on only when cells are primed to divide. In the event that it is not turned off when it should be, uncontrolled division, as in cancer cells, may result.

Despite the emphasis of much *myc* gene research on regulation of expression, the possibility that structural changes might also be required, at least occasionally, for activation of its oncogenic potential has not been ruled out. In fact, the Rabbitts' group has found changes in the nucleotide sequence of the second exon of the translocated gene in one line of Burkitt's lymphoma cells. These might alter the function of the product or they might increase the synthesis of the product if the second exon can no longer form the proposed loop structure with the first exon. Structural alteration of the *myc* gene is not required for transformation, however. According to the Leder and Croce groups, the translocated genes of other cell lines do not show sequence differences.—**J.L.M.**

The viral oncogenes. The names of the viral oncogenes are loosely derived from the names of the viruses in which they were identified or from the types of cancers they cause (*src* from Rous sarcoma virus or *ras* from rat sarcoma, for example). A half dozen or so additional transforming genes, some related to the viral oncogenes and some not, have been identified.

Oncogene	Virus and species of origin	Function of products
abl	Abelson murine leukemia virus (mouse)	Tyrosine kinases
*fes**	ST feline sarcoma virus (cat)	
*fps**	Fujinami sarcoma virus (chicken)	
fgr	Gardner-Rasheed feline sarcoma virus (cat)	
ros	UR II avian sarcoma virus (chicken)	
src	Rous sarcoma virus (chicken)	
yes	Y73 sarcoma virus (chicken)	
*erb*B	Avian erythroblastosis virus (chicken)	Structure of a tyrosine kinase but no kinase activity detected
fms	McDonough feline sarcoma virus (cat)	
*raf**	3611 Murine sarcoma virus (mouse)	
*mil(mht)**	MH2 virus (chicken)	
mos	Avian myeloblastosis virus (chicken)	
sis	Simian sarcoma virus (woolly monkey)	Growth factor
Ha-*ras*	Harvey murine sarcoma virus (rat)	Bind guanosine triphosphate
Ki-*ras*	Kirsten murine sarcoma virus (rat)	
fos	FBJ osteosarcoma virus (mouse)	Nuclear location—bind DNA?
myb	Avian myeloblastosis virus (chicken)	
myc	MC29 myelocytomatosis virus (chicken)	
*erb*A	Avian erythroblastosis virus (chicken)	Cytoplasmic location—function unknown
ets	E26 virus (chicken)	
rel	Reticuloendotheliosis virus (turkey)	
ski	Avian SKV770 virus (chicken)	

**fes* and *fps* are feline and avian versions of the same oncogene; *raf* and *mil(mht)* are murine and avian oncogene counterparts.

one of these transforming genes differed from the normal counterpart by a mutation, again in codon 12. The Barbacid group has preliminary evidence that the other eight transforming genes may also have a mutation in that codon. "I hope that no one thinks that this is the only step [in the development of the cancers]," Barbacid points out. "But in this case we can postulate that it is a necessary step."

Barbacid referred in his comment to another major criticism of the oncogene work. Oncogenes, when introduced into cells by viruses or by gene transfer methods, appear to transform very rapidly, presumably in one step. But a vast amount of experience with human cancers shows that they develop slowly, in many steps and over a period of years.

Recent evidence suggesting that two or more oncogenes must cooperate to transform cells may help to reconcile the differences between the laboratory and clinical observations (*Science*, 11 November 1983, p. 602). The NIH 3T3 cells, which are commonly used as recipients in the gene transfer assay, are not transformed by some oncogenes, of which *myc* is a prominent example. That the cells are transformed so readily by others, such as the *ras* gene, has been a source of concern. There is general agreement that NIH 3T3 cells are already "immortalized," which means that they grow indefinitely in culture. They are partially transformed and not representative of normal cells. As Robert Weinberg of the Massachusetts Institute of Technology points out, "A single oncogene when transferred into a normal cell is unable to transform that cell."

However, Weinberg and his colleagues and also Earl Ruley of Cold Spring Harbor Laboratory have shown that cells, which have not been immortalized, can be completely transformed by a combination of a *ras* gene either with *myc* or with the E1A gene of adenovirus, a DNA-containing virus that also transforms animal cells. These findings, taken in conjunction with additional work on certain DNA-containing cancer viruses, have contributed to the growing view that transformation requires the activation of at least two types of oncogenes. One type, of which *myc* and E1A may be representatives, immortalizes cells and then one or more additional genes, *ras* for example, may act to complete the malignant transformation.

Another interesting instance of possible cooperation between oncogenes was reported near the end of 1983 by Philip Leder's group at Harvard Medical School. They found that the cellular *myc* gene is turned off in normal, nondividing cells, but is turned on by growth factors, including platelet-derived growth factor (PDGF). This past summer two groups of investigators discovered that the *sis* oncogene codes for a product that very closely resembles, if it is not identical to, PDGF. The *myc* gene product is a nuclear protein that may help to regulate other genes needed for cell division.

These findings touch on what may now be the biggest gap in our knowledge of what oncogenes are doing. There is very little information about the functions of the products of the oncogenes themselves or their cellular counterparts. Says Bishop of research in this area, "It's turtle against turtle. It's a slow and vexing business." This is true even for the *src* gene and the other oncogenes that code for tyrosine kinases. Even though it has been more than 5 years since investigators discovered that the *src* gene product can phosphorylate proteins, the identity of the physiologically important target—or targets—is still unclear.

There are some intriguing clues, however, especially in regard to the possibility that oncogene products may either be growth factors that stimulate cell division or be other molecules needed for growth factor activity. The *sis* gene appears to code for one of the two protein chains of which PDGF is composed. The continuous production of such growth-stimulating agents might keep cells in a persistent state of division when they would otherwise not divide.

Moreover, when epidermal growth factor binds to cells, it stimulates a tyrosine kinase with properties similar to those of the *src* gene kinase. This situation is not clear-cut, however, as the cellular proteins phosphorylated by the two kinases are apparently different.

The results relating oncogenes and growth factors suggest a rational mechanism by which the genes may contribute to the uncontrollable growth of cancer cells. Nevertheless, researchers are just beginning to unravel the functions of the genes. Sequencing them is now relatively easy, but with the exception of the *sis* gene, sequence data have produced few insights into function. The next steps in understanding are going to require a progression from molecular biology to the more difficult domain of cellular biology.—**JEAN L. MARX**

Additional Readings

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2. P. H. Duesberg, *Nature (London)* **304**, 219 (1983).
3. H. Land, L. F. Parada, R. A. Weinberg, *Science* **222**, 771 (1983).
4. P. Leder, J. Battey, G. Lenoir, C. Moulding, W. Murphy, H. Potter, T. Stewart, R. Taub, *ibid.*, p. 765.
5. G. F. Vande Woude, A. J. Levine, W. C. Topp, J. D. Watson, Eds., *Cancer Cells*, vol. 2, *Oncogenes and Viral Genes* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., in press). This volume records the proceedings of a meeting held at Cold Spring Harbor in September 1983.