ONCOGENES: THE WRONG GENES IN THE WRONG PLACE AT THE WRONG TIME

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Michael S. Brown, M.D .

INTRODUCTION

Anyone who has read a newspaper or looked at a television program or talked to a biological scientist during the past three months has heard of oncogenes. The world is full of them. Everyone is working on them. Every issue of Nature or Science contains two or three articles about oncogenes, in the news dispatches as well as in the scientific sections.

What are oncogenes? Why all the excitement? Is the optimism justified? These are the questions that I will deal with this morning. After an exhaustive review of the literature, and notwithstanding the painfully acquired skepticism of a jaded scientist, I can tell you that I am a believer. The oncogene is one of the great unifying discoveries of modern biology. It unites at least three disparate fields. Until recently some people believed that viruses caused cancer; others believed that chemicals caused cancer; and still others believed that cancer was hereditary. Now all of them believe in oncogenes. Each cause of cancer seems to operate through a mechanism that shares one feature in common - that is, the oncogene. While we don't yet know the precise tune of human cancer, we do know the keyboard on which the tune is played.

DEFINITIONS

Oncogene (onc) - A gene that produces a protein that can single-handedly transform a normal cell into a malignant cell.

V-onc - An oncogene carried by an RNA tumor virus.

c-onc or proto-oncogene - A normal gene of animal cells whose sequence is nearly the same as that of a viral oncogene. **C-onc** genes can transform cells if they are transcribed into mRNA at abnormally high rates. **C-onc** genes are the ancestors of the viral oncogenes.

Retrovirus - A virus that contains RNA as its genetic material instead of DNA.

Promoter - A region of DNA that is immediately proximal to a gene, and dictates the rate at which that gene is transcribed into RNA, and therefore determines whether the gene will be expressed.

Transformation - The conversion of a normal cell into a tumor cell. Transformed cells will grow on top of one another, grow in semi-solid media, and will grow into tumors when implanted into immunodeficient mice.

Acknowledgement: The author thanks Dr. Graham Smith for making available a complete reprint file on oncogenes, and for many helpful discussions during the preparation of these Grand Rounds.

Fig. 2

THE ROUS SARCOMA VIRUS - A PROTOTYPE

1911 - Peyton Rous - Identified a filterable virus from chicken sarcomas that produced tumors when injected into chickens (Rous Sarcoma Virus or **RSV**).

1958 - Temin and Rubin - RSV will transform cells in tissue culture.

1961 - Crawford and Crawford - Genetic material of RSV is **RNA**.

1963 - Temin - RSV replication is blocked by inhibitors that block synthesis of RNA on a **DNA** template. During its life cycle the viral RNA must be converted to **DNA** and then back into RNA.

1970 - Temin and Baltimore - RNA viruses encode a reverse transcriptase, an enzyme that copies RNA into DNA.

Fig. 4

THE SRC ONCOGENE OF RSV

1970 - Vogt, Duesberg - Isolated a mutant RSV that grew normally but lacked the ability to transform cells or cause tumors. They then isolated the segment of RNA that was missing from the mutant virus. This was the **oncogene**.

1970 - Martin - Isolated a **temperature sensitive** mutant of RSV with a mutation in the src gene. This mutant transformed cells at 30°C, but not at 37°C.

1979 - Erikson - Identified protein encoded by **src** gene. Named it pp60. This protein is a protein kinase. It transfers phosphate from ATP to protein. In temperature sensitive mutants the activity of this enzyme is temperature sensitive *in vitro*. Thus, protein kinase is **necessary** for transformation.

1980 - G. Cooper - Pure **src** gene will transform cells in tissue culture. Virus not required.

This morning I will first briefly review the history of oncogenes, and then discuss their applicability to clinical medicine.

Let's start with a few definitions. Fig. 1 lists some of the terms used in this field.

Fig. 2 reviews the early history of the Rous sarcoma virus (RSV), the prototype for all oncogenic viruses (1).

The life cycle of RSV and other retroviruses is shown in Fig. 3 (adapted from ref. 2). The virus has an outer envelope made of lipid. The RNA is contained in the core of the virus, which is known as the capsid. Lipid enveloped viruses enter cells by the fundamental mechanism that was described at this institution - that is, the process of receptor-mediated endocytosis. The virus binds to surface receptors and is then taken up by endocytosis within coated pits on the cell surface. Within the endocytic vacuole the lipid envelope of the virus fuses with the vesicle membrane, allowing the viral RNA to cross the membrane and enter the cell. Part of the RNA encodes the enzyme reverse transcriptase, which can copy DNA strands into RNA strands. Under direction of the viral RNA, the reverse transcriptase is synthesized, and the enzyme then turns around and makes a DNA copy of the viral RNA. The double stranded DNA copy is integrated into the host genome. The DNA is then "transcribed" or copied repeatedly into many copies of RNA which enter the cytoplasm. Some copies of the RNA are packaged into new viral particles. Other copies direct the synthesis of viral proteins.

One of the segments of the RNA of the Rous sarcoma virus is the src gene. When multiple viral RNA copies are made, large amounts of the src gene product are synthesized. This gene product is the agent that actually transforms the cell. Fig. 4 lists the history of the src gene, which was the first oncogene to be discovered (1-3).

Fig. 5 shows a scanning electron micrograph of a normal cell and a transformed cell in tissue culture.

Fig. 6 shows the sequence of reactions by which the src gene was isolated by Bishop and Vermus (2). Fig. 7 shows the general structure of the genome of the retroviruses. The two ends of the virus contain repeated sequences that function as promoters. Fig. 8 lists the properties of the src kinase. Fig. 9 shows the localization of the src kinase at the adhesion points between cells, as envisioned by electron microscopy (2). Fig. 10 shows the localization of the src kinase at adhesion plaques as revealed by immunofluorescence (2).

Other Retroviral Oncogenes

Fig. 11 lists the retroviral onc genes that have been isolated to date (2). I direct your attention to the mouse and rat oncogenes, V-abl from the mouse and V-ras from the rat. The protein encoded by the abl gene is a tyrosine kinase that resembles the tyrosine kinase found in RSV from chickens. The V-ras gene product differs, however. This gene encodes a 21,000 dalton protein that also has kinase activity. However, instead of phosphorylating tyrosine residues on proteins it phosphorylates threonine residues. And instead of using ATP as a substrate it uses GTP. Despite these important differences the V-ras kinase, like the src kinase, is located at the cell membrane. This localization strongly suggests that these kinases may mimic the action of second messengers that are normally released at the cell surface when an extracellular hormone stimulates cells to divide. Another gene to note is the V-myc gene. The protein encoded by this oncogene does not seem to be a kinase. Preliminary evidence suggests that it is a protein that localizes to the cell nucleus. The abl, ras and src genes have all been linked to human cancers (see below).

Fig. 3. Life Cycle of Rous Sarcoma Virus.

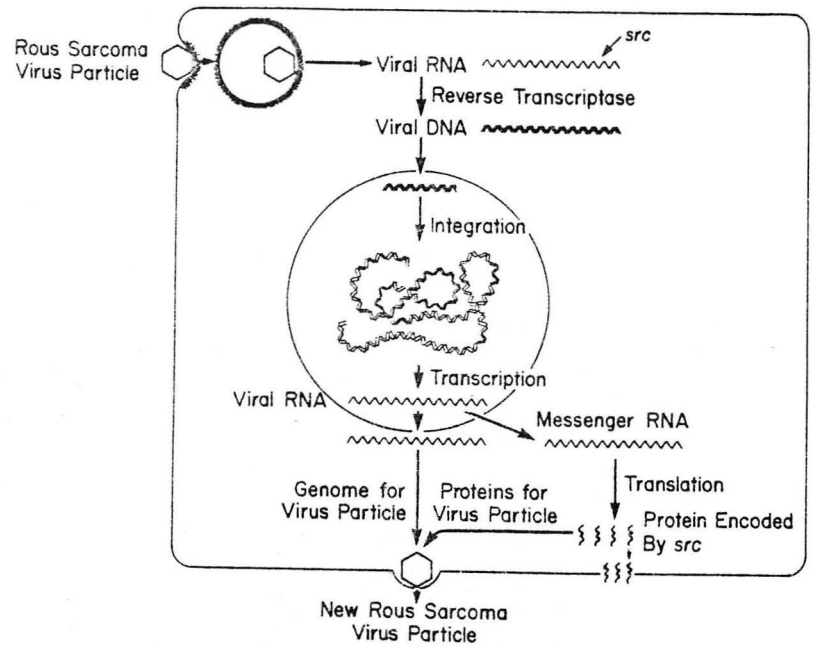


Fig. 5. Scanning electron micrograph of normal cultured fibroblasts (left) and the same cells after transformation by Rous sarcoma virus (right) (2).

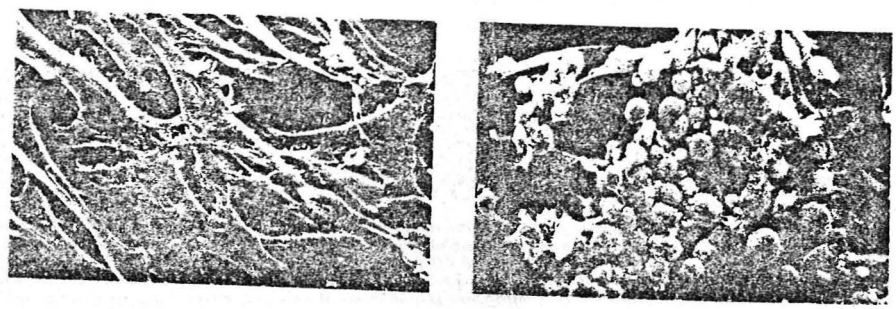
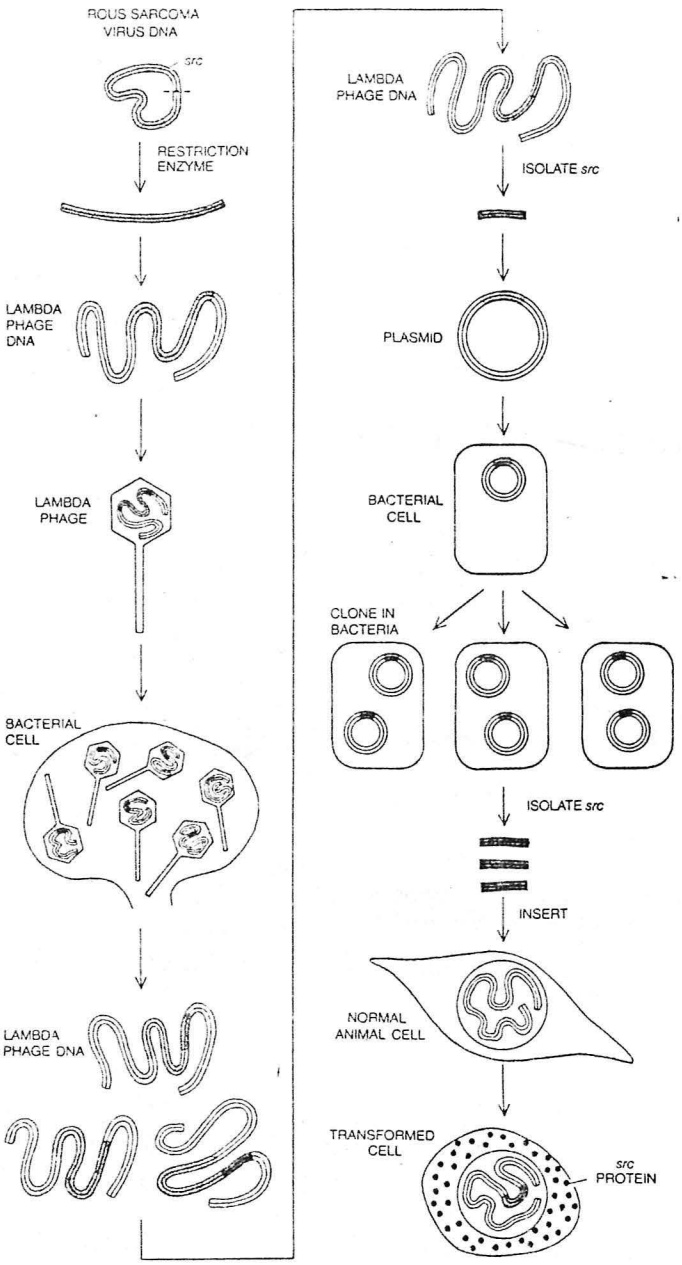


Fig. 6.



VIRAL ONCOGENE is purified, and its capacity to transform cells is tested, by methods of genetic engineering. The circular DNA of the Rous sarcoma virus is isolated from newly infected cells, cleaved with a restriction enzyme and inserted into the DNA of phage lambda, a bacterial virus. The growth of the phage in bacteria makes large quantities of the viral DNA, which is cleaved with a restriction enzyme to yield a fragment bearing only *src* and a bit of flanking DNA. The fragment is inserted into a plasmid (a small circle of bacterial DNA), which is introduced into bacteria for further amplification. Now many copies of the *src* fragment can be excised from the plasmids, purified and introduced into animal cells in culture. Fragment directs synthesis of a viral protein that induces transformation. Experiment was done by William J. DeLorbe and Paul A. Luciw to show that *src*, acting alone, gives rise to cancerous growth.

Fig. 7.

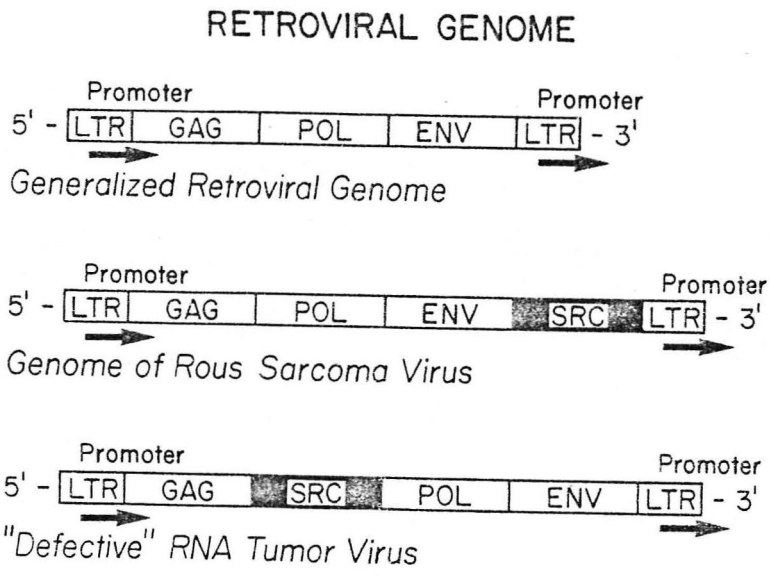

RETROVIRAL GENOME
Promoter
Promoter
5' - LTR GAG POL ENV LTR - 3'
Generalized Retroviral Genome
Promoter
Promoter
5' - LTR GAG POL ENV SRC LTR - 3'
Genome of Rous Sarcoma Virus
Promoter
Promoter
5' - LTR GAG SRC POL ENV LTR - 3'
"Defective" RNA Tumor Virus

Fig. 8

THE SRC PROTEIN KINASE

The pp60 src kinase phosphorylate tyrosine residues on proteins instead of the usual serine and threonine. The kinase is located on the cell membrane at points where the cells join each other, and also on the adhesion plaques where the cells attach to the substrate.

One target of the kinase is vinculin, a protein that is located at adhesion plaques. When virculin is phosphorylated the cells lose attachment to the surface and round up.

The src kinase also phosphorylates other proteins, one of which must be linked to cell division.

Fig. 9. Localization of src kinase protein at junctions between cells as revealed by immuno-electron microscopy (2).

Fig. 10. Localization of src kinase at adhesion plaques as revealed by immuno-fluorescence (2).

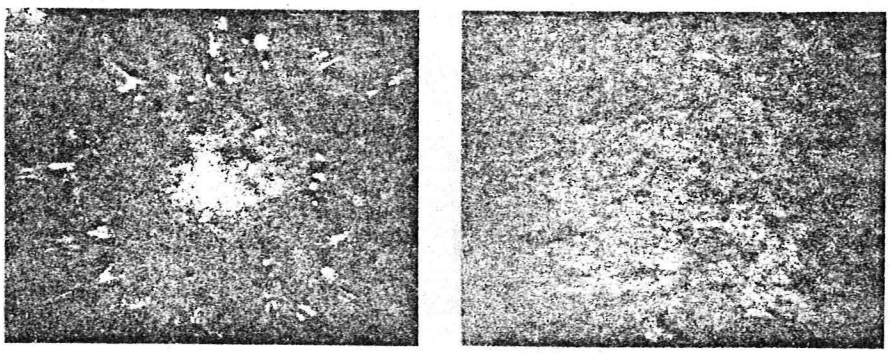


Fig. 11. Retroviral Oncogenes Described to Date (...).

ONCOGENE	SPECIES OF ORIGIN	TYPE OF TUMOR	PROTO-ONCOGENE IN VERTEBRATE DNA	ONCOGENE PRODUCT		
				PROTEIN KINASE	PHOSPHORYLATES TYROSINE	LOCATED ON PLASMA MEMBRANE
v-src	CHICKEN	SARCOMA	YES	YES	YES	YES
v-fps	CHICKEN	SARCOMA	YES	YES	YES	YES
v-yes	CHICKEN	SARCOMA	YES	YES	YES	?
v-ros	CHICKEN	SARCOMA	YES	YES	YES	?
v-myc	CHICKEN	CARCINOMA, SARCOMA, LEUKEMIA	YES	?	?	?
v-erb	CHICKEN	LEUKEMIA, SARCOMA	YES	?	?	?
v-myb	CHICKEN	LEUKEMIA	YES	?	?	?
v-rel	TURKEY	LYMPHOMA	YES	?	?	?
v-mos	MOUSE	SARCOMA	YES	?	?	?
v-bas	MOUSE	SARCOMA	YES	?	?	?
v-abl	MOUSE	LEUKEMIA	YES	YES	YES	YES
v-ras	RAT	SARCOMA, LEUKEMIA	YES	YES	?	YES
v-fes	CAT	SARCOMA	YES	YES	YES	?
v-fms	CAT	SARCOMA	YES	YES	?	?
v-sis	MONKEY	SARCOMA	YES	?	?	?

Fig. 12.

CELLULAR AND VIRAL ONCOGENES are visualized in an electron micrograph made by Richard C. Parker. Viral DNA carrying the *src* gene and chicken DNA carrying the cellular version of the gene were isolated and the double-strand DNA's were denatured. Then the single strands were allowed to hybridize with any closely related strands. In this case, as is shown in the drawing, the viral gene (*black*) and the cellular one (*color*) have formed a heteroduplex. (Extraneous DNA required for cloning the genes is shown in gray.) The loops in the cellular strand are six introns: intervening sequences that interrupt the protein-encoding sequences (exons) in many cellular genes but not in retrovirus genes. Such electron micrographs helped to establish that the oncogenes in cells are native to the cell and were not introduced by viruses.

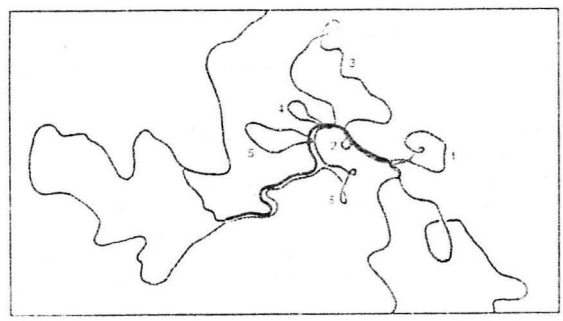
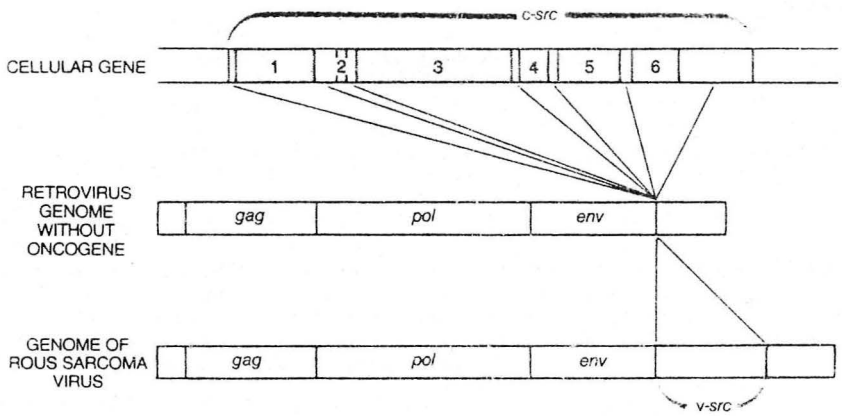


Fig. 12a. Intervening sequences in c-src, but not v-src.



Normal Cellular Homologues of Viral Oncogenes

In the late 1960's Huebner and Todaro (4) advanced the "oncogene hypothesis", which stated that early in evolution retroviruses inserted their oncogenes into the genome of animal cells and these genes have been transmitted through the germ line to all animals ever since. Usually these genes are silent. Occasionally, these inherited viral genes are activated by carcinogens, producing cancer.

Todaro's notion of heritable oncogenes turns out to be correct, but the origin of these genes is opposite to the one he proposed (5). The original oncogenes were normal cellular genes that play a crucial role in cellular development. During evolution these normal genes have been acquired by retroviruses and converted into cancer-causing genes. The first evidence for this sequence came from studies with the src gene of Rous sarcoma virus, as shown in Fig. 12(2). Bishop and Varmus used the enzyme reverse transcriptase to prepare a radioactive DNA that was complementary to the RNA from RSV (5). This "cDNA" was incubated with cellular DNA from normal chickens. The src cDNA hybridized to a gene that was present in the DNA of normal chickens. That means that the DNA of normal chickens does contain a copy of the src gene, as Todaro had proposed.

The next question is: which came first? Is the cellular src gene derived from the viral gene or did the cellular gene come first? The answer came from the crucial observation that the normal gene contains intervening sequences (also known as introns) (Fig. 12a). Most mammalian genes contain introns, which are stretches of nonsense DNA that are inserted at various places in the middle of genes. These introns interrupt the coding sequences. After the gene is transcribed into RNA the RNA corresponding to the introns is removed and the RNA segments that code for protein (the so-called exons) are spliced together. This splicing reaction generates a continuous coding RNA from discontinuous segments.

In contrast to normal cellular genes, retroviral genes contain no intervening sequences. Thus the src gene from RSV contains no intervening sequences. However, the cellular src gene does contain intervening sequences. This means that the src gene must have originated in the cell's genome. If the gene had originated in the virus, and was then transferred to the cellular genome, it could not have acquired intervening sequences later. Therefore, the oncogene must have been a normal cellular gene that was picked up by the virus through the process of recombination.

A current theory for the mechanism by which retroviruses acquire oncogenes is shown in Fig. 13(6). Recall that during the course of infection a DNA copy of the retrovirus RNA is made by reverse transcriptase. This copy is inserted into the cellular genome. The sites at which this integration occurs appear to be totally random. The ancestors of the retroviruses did not contain oncogenes. Rarely, however a retrovirus DNA integrated immediately upstream from a cellular oncogene. A deletion of the intervening genetic material occured so that the genes of the retrovirus became contiguous with the oncogene. Retroviruses contain their own promoters. A promoter is a region of DNA that turns on the transcription of the "downstream"DNA into RNA. Under the direction of this promoter the viral DNA is copied into RNA. After the viral DNA fused with the oncogene, the oncogene was transcribed into the same piece of RNA. The region of the RNA containing the oncogene also contained the signals necessary to remove the intervening sequences. As a result, the cell's own enzymes removed the introns and the RNA was delivered to the cytoplasm as a single copy. This RNA was then packaged into a new virus particle. When the virus infected another cell it brought the oncogene along with it. Thus, a nontransforming virus had acquired the cellular oncogene. From then on it was an acutely transforming virus.

If the oncogene is a normal cellular gene, then why does the viral copy of it cause cancer?

Two theories have been proposed to answer this question: 1) The dosage hypothesis; and 2) the mutational hypothesis. The dosage hypothesis states that the oncogene is transcribed into RNA at a much more rapid rate when it is fused with viral DNA. This is because the viral promoter is very active. Under normal conditions the cellular oncogene is expressed at only low levels because its own promoter is not active. This theory is supported by the finding that normal cells express a very low level of mRNA corresponding to the oncogene, and also a low level of the tyrosine kinase protein that it specifies (5). However, when cells have been transformed by an oncogene-containing retrovirus the amount of mRNA encoding the oncogene increases massively, and the amount of protein also goes up markedly.

Support for the gene dosage hypothesis was provided by the experiments of Scolnick and co-workers (7). They isolated a normal cellular analogue of a rat oncogene (i.e., the cellular analogue corresponding to V-ras). When the cellular gene was injected into normal cells it did not cause transformation, presumably because the gene contained an inactive promoter. However, when these scientists first joined the oncogene to the viral promoter and then injected it into recipient cells, the gene was now transcribed into RNA at much higher rates, and caused transformation of the cells. Thus, a normal cellular gene could cause transformation simply by coupling to an active promoter that caused a high rate of transcription of the gene into messenger RNA.

Until very recently, the gene dosage hypothesis was considered to explain transformation by cellular oncogenes. Recently, however, evidence for the mutation hypothesis has emerged. I will discuss this evidence later in the talk.

What is the normal function of the cellular oncogenes? At the present time at least 12 cellular oncogenes have been described (5). All of them seem to be present in all vertebrate species. Their structures are tightly conserved in species that are widely separated by evolution. This conservation suggests that these genes must perform important functions. All of these oncogenes are expressed at very low levels in the mRNA of most animal cells. However, occasional normal cells express high levels of mRNA's for the oncogenes. Some oncogene mRNA's are expressed early in embryonic life, and then turned off. Other oncogenes continue to be expressed throughout development. Certain oncogenes are expressed only in hematopoetic cells. Observations of this sort have led to the notion that the cellular oncogenes normally function during certain stages of differentiation, perhaps to induce cell division at a critical stage in development. However, once they have acted in this fashion, the normal cellular oncogenes become inactive because their promoters are silenced. The mRNA corresponding to the gene ceases to be made. However, if something happens to stimulate the transcription of the oncogene it recapitulates during later life the same function that it produced during embryonic life, and thereby it stimulates abnormal division of cells.

Slowly Transforming Viruses that do not contain Transforming Genes - The "Promoter Insertion" Hypothesis

All of the studies that I have described were performed with viruses known as "acute transforming viruses." These viruses cause tumors within days to weeks of their injection into animals.

There is another class of RNA viruses, called "slowly transforming viruses" that cause cancer much more slowly. Animals injected with these viruses develop tumors only after many months. These slowly transforming viruses do not cause transformation when they infect cells in tissue culture. Moreover, these slowly transforming viruses do not contain oncogenes.

Fig. 13. Sequence of steps by which viruses acquire oncogenes (6).

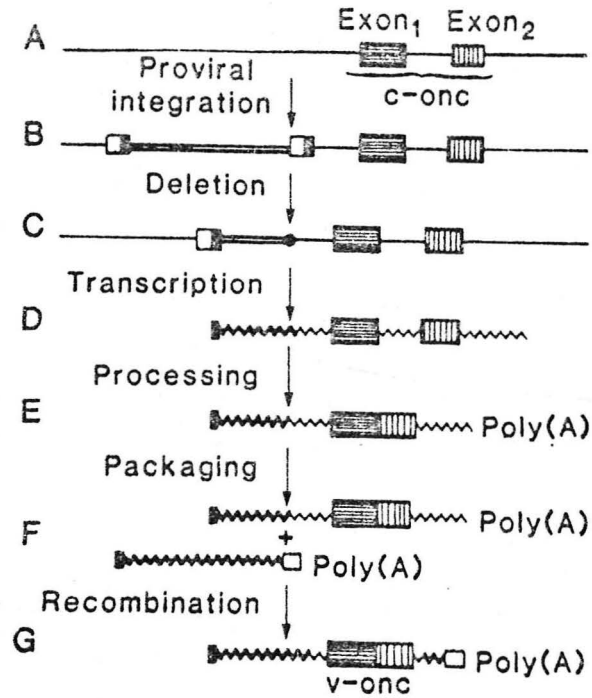
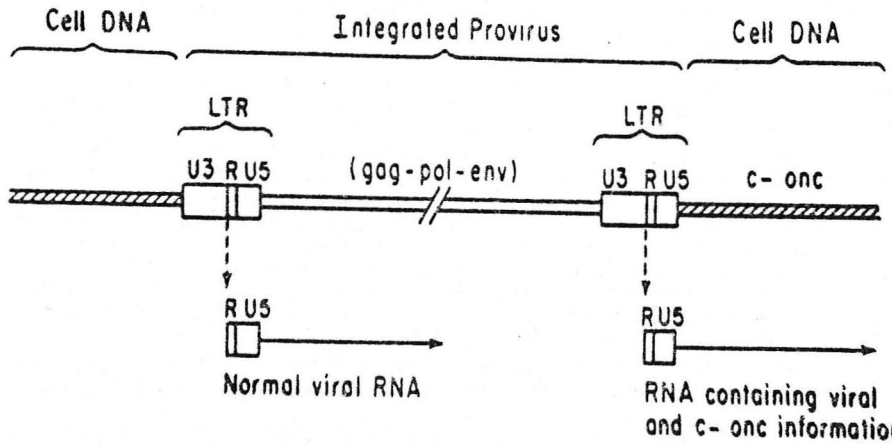


Fig. 14. The promoter insertion mechanism for oncogene activation (9).



The slowly transforming viruses were considered a disturbing exception to the oncogene story of viral tumors. However, over the past few years evidence has accumulated to indicate that these viruses are not really exceptions, but rather they have a different mechanism which produces the same result.

The major breakthrough came from experiments by Hayward and Astrin, who were studying B-cell lymphomas that occurred in the bursa of Fabricius of chickens (8). These tumors are produced by avian leukosis virus (ALV), which is one of the slowly transforming viruses discussed above. Hayward and Astrin found that in most B-cell lymphomas a DNA copy of the retrovirus had integrated into cellular DNA immediately upstream from a cellular oncogene (Fig. 14)(9). The cellular oncogene was myc. Myc was known to be an oncogene because a viral copy of it is carried by one of the rapidly transforming chicken leukemia viruses. However, ALV does not carry myc or any other oncogene. For some reason, ALV has a tendency to integrate into the genome immediately upstream from myc. The LTR at the terminal end of the ALV functions as a promoter. In the tumor cells of the animals infected with ALV, Hayward and Astrin found markedly increased expression of a messenger RNA that started at the promoter region of the viral gene and continued through the cellular myc oncogene. As a result, even though the virus did not carry the myc transforming gene it did cause the cell to start overproducing the myc transforming protein.

These findings led Hayward and Astrin to propose the promoter insertion hypothesis (8). Insertion of viral promoters can turn on cellular oncogenes and produce cancer. So far in mammalian tumors, no one has demonstrated the insertion of a viral promoter adjacent to a cellular oncogene. However, slowly transforming viruses cause cancer in mammals, and it seems likely that they may function by a promoter insertion mechanism in mammals as they do in chickens.

Oncogenes in Non-viral Human Tumors

The next chapter in the oncogene story emerged from the laboratories of Robert Weinberg at MIT (10) and Geoffrey Cooper at the Sidney Farber Cancer Institute in Boston (11). These investigators used a technique called "transfection" to determine whether non-viral tumors contained oncogenes. In the transfection technique (Fig. 15) cellular DNA is extracted from tumor cells and transferred or transfected to non-tumor recipient cells, which take up the DNA and then become transformed. The success of this technique depends on the astounding discovery that normal animal cells will take up foreign DNA and express the genes. So far, only one line of recipient cells has been useful for this purpose. This is a line of cultured mouse embryo fibroblasts called NIH 3T3 cells. These cells were grown from a normal mouse embryo and have been maintained in culture for many years. Although they grow continuously, they are not transformed. That is, they do not show "overgrowth" in tissue culture, and they will not cause tumors when injected into animals. Weinberg and colleagues extracted small bits of DNA from tumor cells and transfected the DNA into the NIH 3T3 cells. The DNA from the tumor converted the cells to the transformed type. The cells showed "overgrowth" and they caused tumors in animals. As a donor for these experiments Weinberg used a line of mouse bladder cells that were treated with the carcinogen 3-methylcholanthrene in tissue culture. The 3-methylcholanthrene converted the normal bladder cells into a malignant bladder tumor. The DNA extracted from this tumor then caused transformation of the NIH 3T3 cells. DNA extracted from normal cells did not cause transformation. Through a series of experiments Weinberg showed that the ability to transform was dependent on a single oncogene that was active in the 3-methylcholanthrene-induced bladder carcinoma, but not in normal cells.

An oncogene from a Human Bladder Carcinoma

Using this transfection assay investigators have begun to study a variety of naturally-occurring human carcinomas to determine whether they contain cellular

Fig. 15.

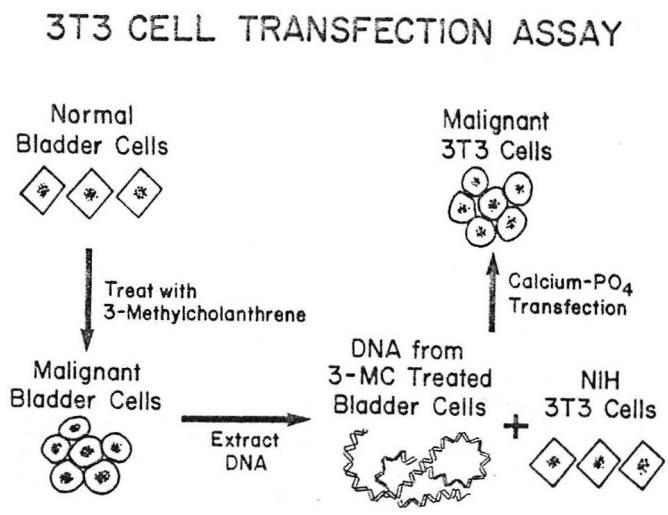


Fig. 16. From Cooper (11).

Transforming genes activated in neoplastic cells.

Cell type	Species	Mode of induction	Positive neoplasms (No.)
Transformed fibroblasts	Mouse	Chemical	5
Bladder carcinoma	Human	Spontaneous	3
	Mouse	Chemical	1
	Rabbit	Chemical	1
Lung carcinoma	Human	Spontaneous	4
	Mouse	Spontaneous	1
Mammary carcinoma	Human	Spontaneous	1
	Mouse	Chemical or virus	6
Colon carcinoma	Human	Spontaneous	2
Promyelocytic leukemia	Human	Spontaneous	1
Nephroblastoma	Chicken	Virus	1
Neuroblastoma	Human	Spontaneous	1
	Rat	Chemical	3
Glioblastoma	Mouse	Chemical	1
Pre-B lymphocyte neoplasm	Human	Spontaneous	4
B cell lymphoma	Chicken	Virus	6
	Human	Spontaneous	6
	Mouse	Spontaneous	3
Plasmacytoma/myeloma	Human	Spontaneous	2
	Mouse	Chemical	2
T cell lymphoma	Human	Spontaneous	1
	Mouse	Spontaneous, chemical, radiation, or virus	6
Mature T helper cell neoplasm	Human	Spontaneous	1
	Mouse	Virus	1
Sarcoma	Human	Spontaneous	1
	Mouse	Chemical	1

oncogenes that will transform the NIH 3T3 cells. The majority of human tumors so far tested have failed to transform the 3T3 cells. However, several human tumors have caused transformation, and these have begun to provide exciting information. The tumors that cause transformation include tumors of the colon, lung, pancreas and gallbladder, as well as the urinary bladder (Fig. 16)(11).

The most extensively studied human tumor is a line of bladder carcinoma cells from a patient who died of a bladder carcinoma. The DNA from these cells transformed the NIH 3T3 cells. Using this transformation as an assay, Weinberg was able to isolate the human oncogene (12). The striking finding was that the human oncogene turned out to be the cellular counterpart of a previously described oncogene from rats (v-ras). This is the gene that encodes the 21,000 dalton protein that is a GTP-dependent protein kinase.

Recall that all cells contain the c-ras gene. Why should the c-ras gene from the bladder carcinoma cells cause transformation of NIH 3T3 cells, when c-ras from normal cells does not cause transformation? The answer emerged simultaneously from several laboratories, each of whom was studying the same bladder carcinoma line (Fig. 17)(12). These studies showed that the c-ras gene in the patient's tumor had undergone a mutation. At one position in the c-ras gene a thymidine was substituted for a guanidine, and this changed one codon from glycine to valine. The proof that this alteration could cause a tumor was obtained in an elegant experiment. The whole c-ras gene is several thousand bases long. A small sequence of 350 base pairs of DNA was excised from the patient's gene and substituted for the corresponding sequence in the normal gene. This 350 base pair sequence contained the substituted base. When the modified normal gene, which contained this altered 350 base pair sequence, was transfected into 3T3 cells, it transformed the cells. Substitutions of other regions of the gene did not cause transformation. Thus, the transforming activity was localized to a tiny 350 base pair region of the c-ras gene.

It appeared therefore that this man's bladder carcinoma was caused by a mutation that altered the amino acid sequence of the c-ras oncogene in the region comprising these 350 base pairs. This alteration did not lead to a higher level of transcription of the mutant gene, as judged by analysis of the level of messenger RNA in the transfected cells. Rather, the mutation produced a condition in which the transcription of this mutant at normal rates leads to cancer. Perhaps this mutant protein, which is a kinase, attacks an abnormal substrate, or it may over-phosphorylate a normal substrate.

Within the past several weeks, a new report has appeared which initially seems to confuse the bladder oncogene issue, but which actually may represent a great advance (13). The report comes from the laboratory of George Khoury at the NIH. Khoury studied another patient, a 59 year old man, with a bladder carcinoma. The tumor cells from this patient also showed a mutation in the c-ras gene. The mutation was the same as the one in the cells from the other bladder carcinoma patient previously studied. However, whereas the first patient had died leaving only his carcinoma cells surviving, the second patient was still alive. Therefore, cells other than bladder cells were available. Studies of these cells showed that the mutation in this patient was not restricted to his bladder cells. Leukocytes from his blood showed the same abnormality. These studies demonstrated that the patient was a heterozygote for this mutation. All of his cells had one mutant c-ras gene and one normal gene from the time of conception. The crucial question is whether he inherited the mutant gene from one of his parents, or whether the mutation occurred de novo during his own embryogenesis. No family data is yet available.

Fig. 17. Localization of mutation in c-ras from bladder carcinoma patient through use of recombinant DNA techniques.

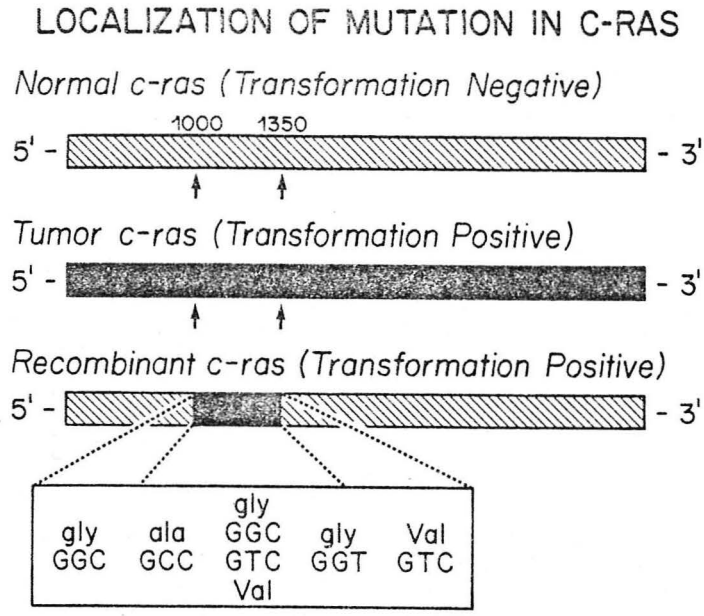
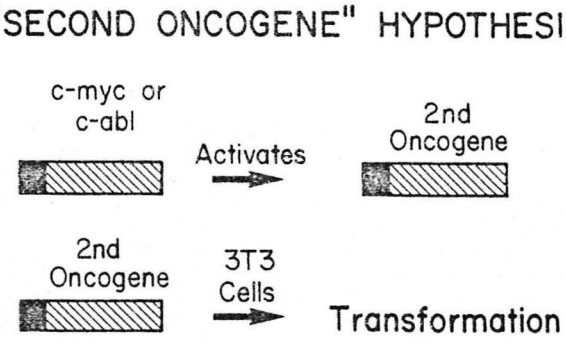


Fig. 18.



The findings of Khoury raise fundamental questions. The patient with the bladder tumor must have had the mutation from the time of conception. Yet he did not develop a bladder carcinoma until age 59. His mutant gene, which is present in all of his cells, is able to transform 3T3 cells to a malignant state. Why did this gene not transform his cells earlier? And why was his bladder transformed, and not some other tissue? Clearly, this mutant gene has the potential to cause cancer in non-bladder cells, such as 3T3 cells. Yet something must keep this gene from causing cancer earlier in this individual's lifetime, and something must keep it from causing cancer in tissues other than the bladder. The most likely explanation is that this mutant gene always had the potential to cause cancer, but normally it was kept suppressed, and so its carcinogenic potential was not realized. At some time during this man's life however, something happened that relieved this suppression, allowing the oncogene to be expressed, and producing his cancer. Whatever happened to increase expression must have been permanent, since the gene retains the ability to cause cancer when given to the 3T3 cells.

Several crucial experiments should be performed immediately. First, we must learn whether the mutant gene that is present in other tissues of this patient will cause cancer when transfected into 3T3 cells. If it does not cause cancer, this would suggest that the gene in the bladder has undergone a second alteration, perhaps a change in the promoter so that it is expressed at higher levels.

The second crucial question is whether this mutant gene is present in any family members, and if so, whether they have a high incidence of cancer, and if so whether these cancers are restricted to the bladder. Is it possible that this same oncogene can predispose other members of the family to different tumors? Is this mutant gene a marker for a generalized genetic susceptibility to cancer? I'm sure that the answer to these questions will be brought forward in the next few months.

Limitations of the 3T3 Cell Transfection Assay - The 2nd Oncogene Hypothesis

The 3T3 cell transfection assay has proved successful in detecting oncogenes from human cancers, as well as from animal cancers. However, many tumors are not able to transform the 3T3 cells. Moreover, the 3T3 cells seem to be essentially unique in their ability to be transformed by tumor DNA. Other cultured cells do not show transformation when transfected with DNA from tumors. These findings suggest that the 3T3 cells may already be "partially transformed", and may therefore detect only those oncogenes that are able to complete this transformation.

Several lines of evidence support this interpretation. First, Cooper and co-workers extracted DNA from chicken lymphomas that were induced by avian leukosis virus (11). These lymphomas expressed high levels of the myc gene mRNA owing to the insertion of the viral promoter upstream from the myc gene, as discussed above. DNA from these tumors caused transformation of the 3T3 cells. However, the gene that transformed the 3T3 cells was not the myc gene. The transformed 3T3 cells did not acquire the myc gene, but they acquired some other unknown oncogene. This suggests that the initial turning on of the myc gene in the tumor cells had led to some secondary alteration in the DNA. The 3T3 cells detected this secondary alteration, and not the myc gene. This is the "second oncogene" hypothesis (Fig. 18).

Graham Smith and Brad Ozanne at this school have made a similar observation. They studied a patient with acute lymphoblastic leukemia whose cells express abnormal sized messenger RNA's corresponding to v-abl, the cellular counterpart of the oncogene from the Abelson leukemia virus. DNA from this patient's cells was able to transform the NIH 3T3 cells. However, the transformed 3T3 cells did not acquire the abl gene, nor did they express the abnormal sized abl mRNA's. It seems likely that the intial activation of the abl gene in the patient's cells caused a

secondary alteration in the DNA, and this secondary alteration is detected in the 3T3 cells.

Chromosome Translocations And Oncogenes - The Unifying Thread.

As described above, oncogenes have united two disparate fields - viral cancer and chemical carcinogenesis. Now the oncogenes have united a third cancer field - chromosome abnormalites.

Many abnormal chromosomes are observed in cancer cells. The vast majority of these changes are secondary and occur only after cells have divided promiscuously for generations. For example, when a patient with chronic myelogenous leukemia develops a blast crisis his cells will usually show a variety of new chromosome abnormalities. This apparent non-specificity for a long time obscured the real significance of chromosome changes in cancer - it gave the whole field an undeserved bad name.

Now, however, that is all changed as a result of two developments - chromosome banding techniques and oncogenes. The new banding methods, developed in the 1970's, allowed multiple regions of each human chromosome to be visualized - up to 5000 regions can now be identified on the 22 pairs of autosomes and the sex chromosomes. Fig. 19 illustrates the nomenclature need to describe the chromosome bands.

The best understood chromosomal abnormalities in human cancer occur in the lymphomas and leukemias. This is not because chromosomal abnormalities are restricted to blood cell tumors, but simply because it is easiest to study the chromosomes of cells from the blood or bone marrow.

Fig. 20 illustrates the most common chromosomal abnormality seen in malignant cells of patients with Burkitt's lymphoma and the related acute nonlymphoblastic B-cell leukemias. This is a reciprocal translocation or exchange of DNA between chromosomes 8 and 14. But the 8:14 translocation is not the only one seen. Other translocations are also observed frequently. All of the translocations involve the distal part of the long arm of chromosome 8 (Fig. 21)(14). In some cases the genetic material from chromosome 8 is transferred to the short arm of chromosome 2, or to the long arm of chromosome 22. The striking feature of these translocations is that all of the chromosomes that receive material from chromosome 8 (that is, chromosomes 2, 14 and 22) contain genes for immunoglobulin chains. Chromosome 14 contains the genes for the heavy chain, chromosome 2 contains the gene for the kappa light chain, and chromosome 22 contains the gene for the lambda light chain. Even more striking, however, is the observation that the precise regions which receive the translocated material are exactly the bands in which the immunoglobulin genes are located. Even more profound, is the observation that the immunoglobulin secreted by each of the B-cell tumors corresponds to the chromosome that receives the translocated material. Thus, malignant B-cells that contain the 8:2 translocation secrete immunoglobulins with kappa light chains B-cell lymphomas that contain the 8:22 translocation secrete immunoglobulins that contain lambda light chains. Cells that contain the 8:14 translocation secrete either kappa or lambda light chains.

This remarkable correlation led to the suggestion that the distal end of the long arm of chromosome 8 contained an oncogene, and that this oncogene became activated when it was translocated to the region of a chromosome that was actively producing immunoglobulin (14a).

This prediction has recently been confirmed - but not exactly as originally proposed. Two laboratories (those of Philip Leder in Boston [15] and Carlo Croce in Philadelphia [16]) have simultaneously shown that the broken region of

Fig. 19. Human chromosome 14 at three increasing levels of resolution with the use of chromosome banding techniques.

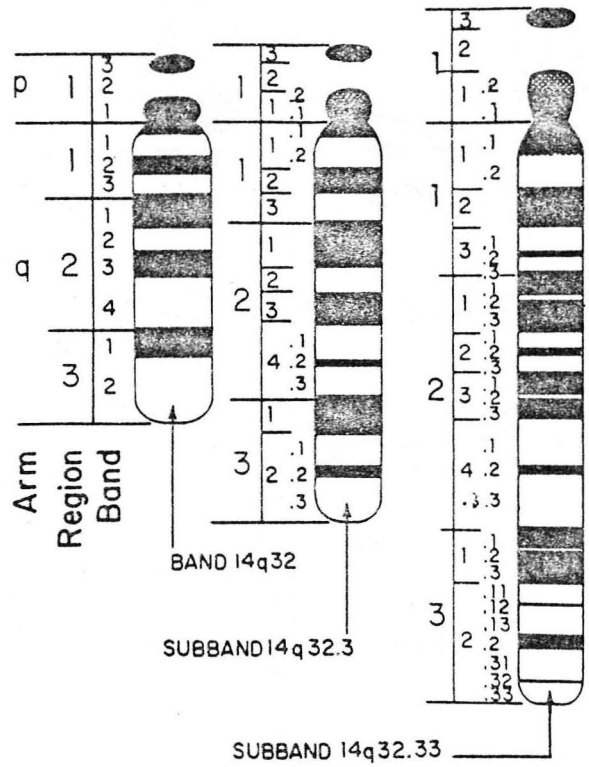


Fig. 20. Reciprocal 8:14 translocation in Burkitt's lymphoma.

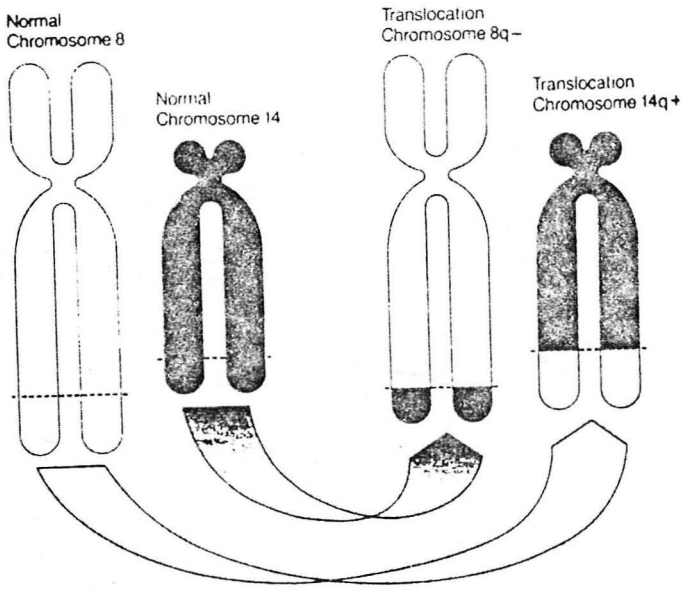
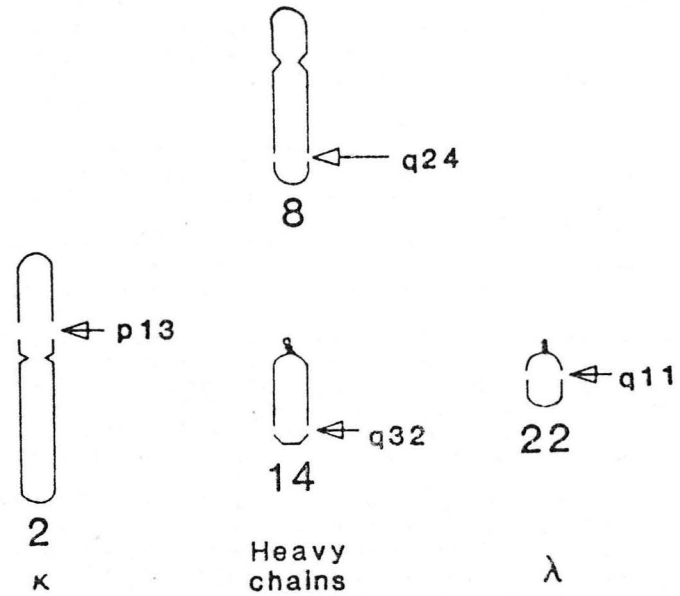


Fig. 21. Sites of translocations among patients with Burkitt's lymphoma: correlation with loci for immunoglobulin chain production.



chromosome 8 is the precise site at which the c-myc gene is located. Recall that c-myc is the normal cellular counterpart of a viral oncogene (v-myc) that produces B-cell lymphomas in birds. The most amazing finding is that in Burkitt's lymphoma the c-myc gene moves with the small piece of chromosome 8 and inserts into the immunoglobulin gene on chromosome 14.

Fig 22 shows the rearrangement detected by Leder's group in a human Burkitt lymphoma cell line (15). Recall that the normal heavy chain of immunoglobulin is assembled by a normal recombination of genes on the 14th chromosome. This normal rearrangement brings the segment of DNA encoding the variable region of the immunoglobulin gene into continuity with the DNA segment encoding the "switch" region and the constant region. In these Burkitt's lymphoma cells the c-myc gene has moved from chromosome 8 to chromosome 14 and has replaced the variable region. The c-myc gene is now in continuity with the "switch" region and the constant region.

A similar translocation has been demonstrated by Croce's group at the level of the whole chromosome (16). The c-myc gene has moved from chromosome 8 to chromosome 14. In turn, the segment of chromosome 14 containing the variable region gene has moved reciprocally to chromosome 8. Thus, the c-myc gene has been placed adjacent to the constant region gene, exactly as shown by Leder.

The finding of a translocated myc gene in Burkitt's lymphoma cannot be mere coindidence. This gene was originally identified because it is carried by a virus that causes lymphomas on birds. Now we know that in human lymphomas the same gene has moved from its normal location and inserted precisely into the gene that specifies the production of immunoglobulin. And not any old immunoglobulin, but precisely the same immunoglobulin that the cell is producing. At first glance these findings would seem to strikingly confirm the promoter insertion by pothesis. Yet a closer look reveals several features that rule out a simple promoter insertion model.

1) The promoter for the heavy chain is connected to the variable region of the gene which is at the 5' end. Although the c-myc gene has been inserted into the heavy chain geneon chromosome 14, the promoter has gone the other way - it is now on chromosome 8, where the oncogene used to be! How could it activate transcription of c-myc?

2) The orientation of c-myc is opposite to that of the heavy chain gene. The c-myc gene must be transcribed in the opposite direction to the heavy chain gene, and this cannot be a simple function of a heavy chain gene promoter.

3) The translocation disrupts the continuity of the heavy chain gene. Thus, although these cells synthesize heavy chains, the synthesis must be taking place on the other 14th chromosome, i.e., the one that is not affected by translocation.

These findings indicate that the single promoter insertion model of Klein (14a) and Hayward (8) cannot explain the active transcription of c-myc in these lymphomas. Nevertheless, transcription of this gene is increased by 10-fold as measured by Croce (16). Therefore, there must be some attribute of the heavy chain gene that allows increased transcription of the translocated c-myc gene, even when the putative promoter has moved to another chromosome.

The power of these observations in humans is amplified by the finding of an identical situation in mice with plasmacytomas induced chemically by injection of mineral oil. All plasmacytomas of mice have a translocation of material from chromosome 15 to either chromosome 12 or chromosome 6. Chromosome 15 is the site of the myc gene in mice. Chromosomes 12 and 6 are the sites of the genes for the

Fig. 22. Rearrangement of c-myc gene in a patient with Burkitt's lymphoma (15).

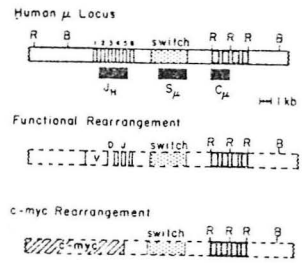


Fig. 23. Reciprocal translocation that moves gene for heavy chain variable region to chromosome 8, and c-myc gene to chromosome 14 in a patient with Burkitt's lymphoma (16).

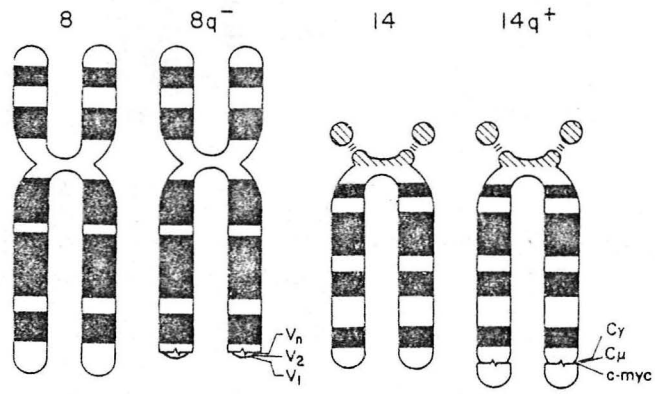
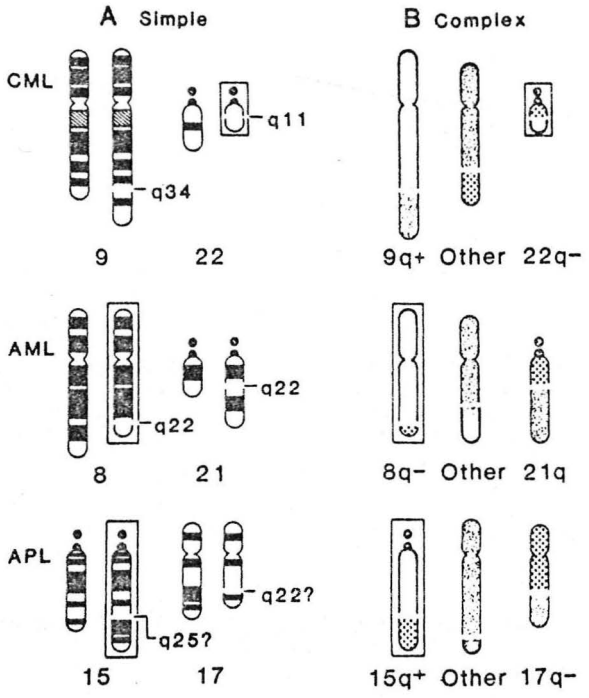


Fig. 24. Translocations in chronic myelogenous leukemia (CML), acute myelocytic leukemia (AML) and acute promyelocytic leukemia (APL) (19).



heavy chain and kappa light chain of the immunoglobulins. Leroy Hood's group has shown that in mouse plasmacytomas the c-myc gene is translocated to the immunoglobulin heavy chain region in mouse plasmacytomas (17). Thus, plasmacytomas in mice and B-cell lymphomas in man have the same pathogenesis - the translocation of the c-myc gene to the site of active immunoglobulin production.

A similarly astounding translocation has now been observed for chronic myologenous leukemia (CML) in man (Fig.24). 96% of these patients have an abnormally short chromosome 22 which is called the Philadelphia chromosome. This short chromosome actually results from a reciprocal translocation. A large part of chromosome 22 has been moved to chromosome 9, and a small part of the distal end of chromosome 9 has been translocated to chromosome 22.

The mind-boggling observation is that chromosome 9 contains the human counterpart of v-abl, the transforming oncogene of the Ableson murine leukemia virus. The c-abl is located at the precise position where the break comes in chromosome 9 in patients with chronic myelogenous leukemia. As a result, the c-abl is moved from chromosome 9 to chromosome 22. We do not yet know the identity of the promoter that activates the c-abl gene when it is moved to chromosome 22. However, the site of insertion is near the locus of the lambda immunoglobulin light chain gene.

Fig. 25 summarizes the human chromosomes which are known to contain c-onc genes as of February, 1983 (19). Nine of these genes have been located, and they are distributed among 8 chromosomes. The arrowhead to the left of each chromosome indicates the band carrying the cellular oncogene. The arrows to the right of the chromosome indicate the specific band involved in consistent translocations or deletions in patients having the disorders listed. Not all of the chromosomal abnormalities are translocations. In some tumors there is a deletion. One of the most interesting involves Wilms' tumor. Many cases of Wilms' tumor are associated with a congenital syndrome in which patients have congenital absence of the irus, or aniridia, associated with the subsequent development of Wilms' tumor. All of the cells in these patients have a deletion on chromosome 11 in the region that corresponds precisely to the location of an oncogene called ras. This is the same oncogene that undergoes mutation in bladder carcinoma. This finding suggests that a deletion of a part of a chromosome containing an oncogene can also lead to a malignant state.

Oncogenes in Familial Tumors

Several syndromes are known in which a dominantly transmitted genetic defect leads to the occurrence of malignant tumors. One of these is hereditary renal cell carcinoma, a relatively rare cause of renal cell carcinoma. As with other hereditary tumors, hereditary renal cell carcinomas tend to occur bilaterally and to develop at an earlier age than nonfamilial renal cell carcinomas.

In 1979 a Boston group reported a striking family in which 10 members had renal cell carcinoma associated with a hereditary balanced chromosomal translocation (20). The karyotype of the proposition is shown in Fig. 26. The translocation involved the transfer of material from the distal end of chromosome 8 to the short arm of chromosome 3. The break point in chromosome 8 is at the precise position in which the myc gene is located. The pedigree of this family is shown in Fig. 27. Six of the family members had bilateral renal carcinomas. All family members with renal cell carcinoma had the same balanced translocation in all cells of their bodies.

It seems likely that the short arm of chromosome 3 harbors some gene that is actively transcribed in the kidney, in analogy to the immunoglobulin genes in B-lymphocytes. For example, it could be the gene for the renal form of Na/K

Fig. 25. Correlation of oncogene locations and known break-points for translocations in humans with various malignancies (19).

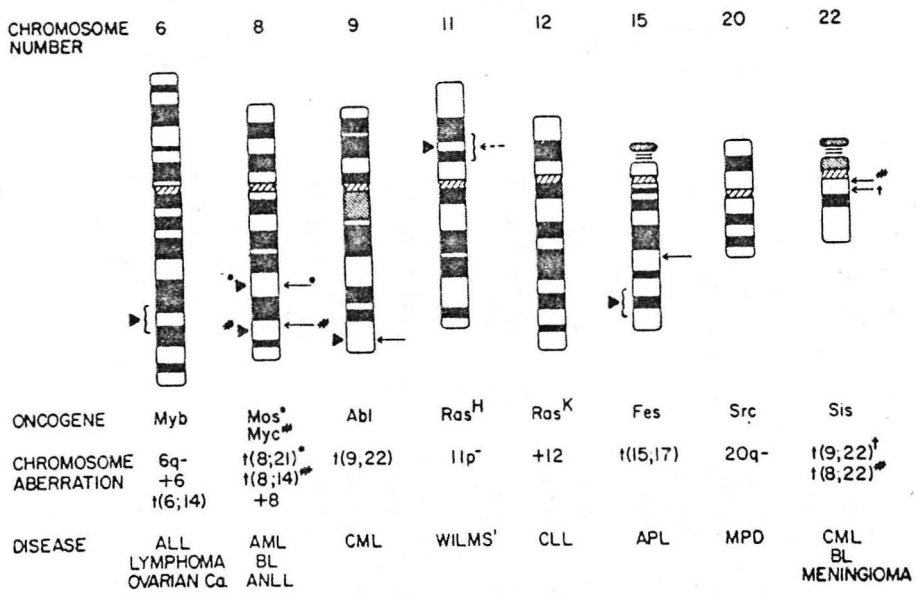


Fig. 26. Pedigree of a family with hereditary renal cell carcinoma (closed figures). T indicates presence of 8:3 translocation. t indicates probably 8:3 translocation by inference (20).

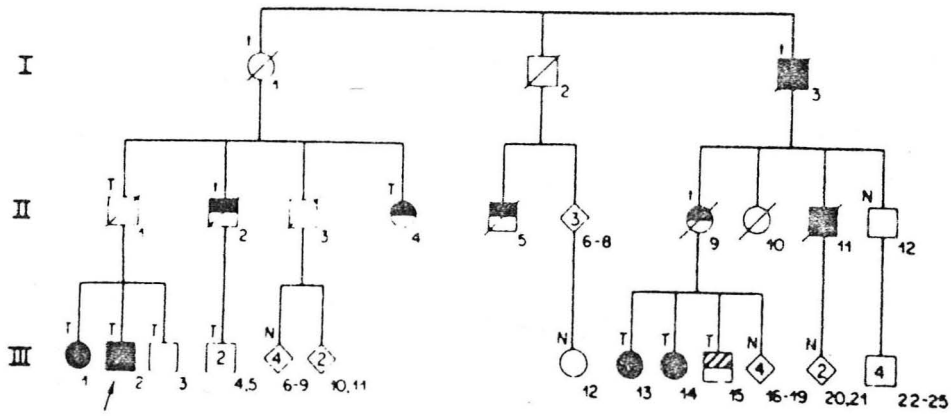
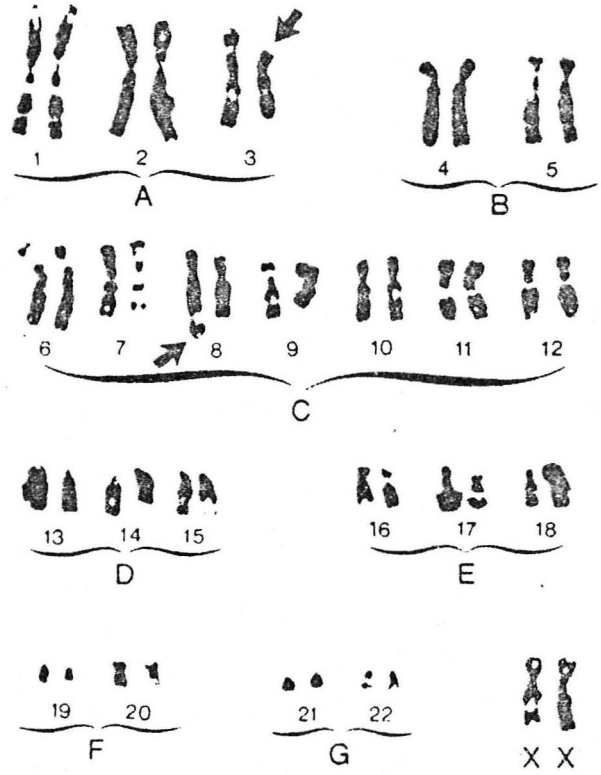


Fig. 27. 3:8 translocation (20).



ATPase. When the c-myc gene moves from chromosome 8 to chromosome 3 it comes under the control of this active promoter and this causes malignant transformation in kidney cells.

These observations raise a crucial question: Since these patients are born with this translocation why does the carcinoma develop only after 30 or 40 years? Is it possible that renal tumors containually develop, but the immune surveillance system keeps disposing of them until by chance, one clone of cells escapes? Or is it possible that the translocation is only one component of the carcinomatos transformation and that the actual development of a tumor requires a second event, such as a somatic cell mutation? The answer, of course, is not yet known. However, our knowledge of the oncogene will certainly help answer this crucial question.

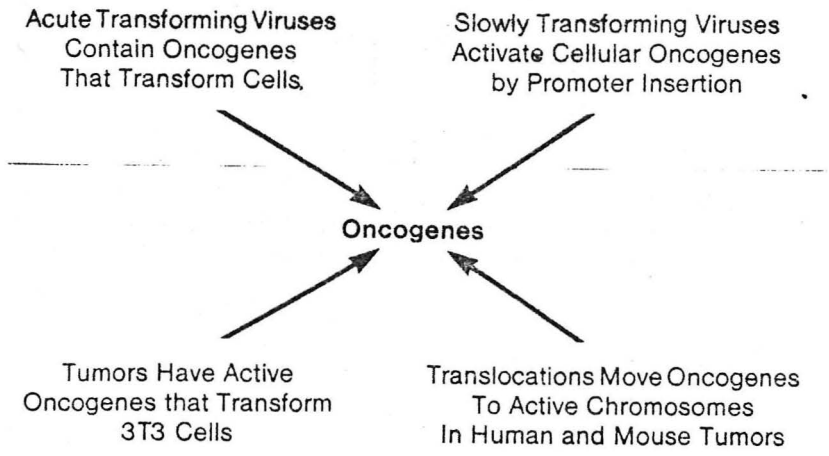
SUMMARY

Cancer is a complex disease. Many cancers in man develop in multiple stages, suggesting that multiple events are necessary to convert a normal cell into a fully malignant one. Tumors initially grow locally, then suddenly metastasize. The cells in a tumor are wildly heterogeneous by visual inspection as well as by chemical analysis. Experimental tumor production is frequently due to a synersistic interaction of turnover inducers and turnover promoters. Clearly, cancer involves scores of alterations. This complexity has led many to dispair of ever learning the fundamental basis of cancer and has led to the devotion of much money and effort to cancer treatment programs in the hope that cancer can be cured empirically before its basis is ever known.

The unravelling of oncogenes has changed all of this. Cancer is still complex, but now we have our hands on one of the elements that must be very close to the source of this disturbance. Since one of the elements, the oncogene, has been defined, it is now possible to use that element to elucidate the other contributory events.

For example, we can now formulate direct questions: What are the substrates of the protein kinases encoded by oncogenes? How do these substrates trigger the uncontrolled cell division and anti-social behavior of tumor cells? How does a switch from glycine to valine activate a protein kinase oncogene? Why are only certain oncogenes detected by the NIH 3T3 cell transfection assay? What triggers the sudden development of cancer late in life in a person who has harbored a translocated oncogene from birth?

Thousands of questions remain to be answered. Scores of seeming paradoxes must be resolved. All of these questions can be answered with existing technology. The answers should go a long way toward giving us a complete understanding of cancer. We seem to be at the same stage as the early bacteriologists were when the disease-causing bacilli were being discovered one-after-another. We have surmounted an enormous energy barrier. Great advances lie ahead.


Acute Transforming Viruses
Contain Oncogenes
That Transform Cells.
Slowly Transforming Viruses
Activate Cellular Oncogenes
by Promoter Insertion
Oncogenes
Tumors Have Active
Oncogenes that Transform
3T3 Cells
Translocations Move Oncogenes
To Active Chromosomes
In Human and Mouse Tumors

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