

RETROVIRUSES AND ONCOGENES II

Nobel Lecture, December 8, 1989

by

J. MICHAEL BISHOP

Department of Microbiology and Immunology, Department of Biochemistry and Biophysics, and The G. W. Hooper Research Foundation, University of California at San Francisco, San Francisco, U.S.A.

I am grateful to the family in whose embrace I formed my aspirations; the institutions modest and grand that helped me nurture those aspirations; the many colleagues with whom I have shared the pursuit of nature's secrets; the Nobel Foundation and the Karolinska Institute for glorious hospitality; and Swedish Medicine, which restored my larynx to some semblance of function.

The English critic Cyril Connolly once remarked that: "The true index of a man's character is the health of his wife." My life in science has been rich and rewarding. I have sacrificed very little. But my partner in that life has sacrificed a great deal: Kathryn, my wife of thirty years. I take this moment to speak my gratitude for her forbearance, and to acknowledge that matters are not likely to improve.

If I were asked to choose a biographical theme for today, it would have to be procrastination. Peyton Rous withdrew from medical school for a year to spend time on a ranch in Texas, ostensibly to recover from tuberculosis (1). Once back in medicine, he found himself "unfit to be a real doctor" (in his own words) and turned to Pathology for an entry into research. Harold Varmus dallied with English Literature before entering medical school, as you have heard (2). And I came close to abandoning medicine entirely at an early age.

I entered Harvard Medical School knowing nothing of research. But during my first two years there, I was awakened to research by new-found friends among my classmates, particularly John Menninger (now at the University of Iowa) and Howard Berg (now at Harvard University). I sought summer work in a neurobiology laboratory at Harvard but was rebuffed because of my inexperience. My interest in practicing medicine declined. I became ambivalent about continuing in medical school, yet at a loss for an alternative.

Like Peyton Rous, I was rescued by pathologists. Benjamin Castleman offered me a year of independent study in his department at the Massachusetts General Hospital, and Edgar Taft of that department took me into his research laboratory. There was little hope that I could do any investigation of substance during that year, and I did not. But I was riotously free to read

and think, which lead me to a new passion: molecular biology. I began my efforts to consummate that passion with two novitiates.

Novitiates

I served the first novitiate with Elmer Pfefferkorn and Sindbis Virus. I had no credentials, other than my desire. Yet Elmer took me into his laboratory, and Harvard Medical School excused me from all but one of my fourth year courses so that I could try research unencumbered. Both were enlightened acts, for which I remain grateful beyond measure.

I sought out Elmer and Sindbis Virus because I had perceived that the inner sanctum of molecular biology was closed to me, that I would have to find an outer chamber in which to pursue my passion. Through Elmer, I found animal viruses, ripe for study with the tools of molecular biology, yet still accessible to the innocent.

I was innocent, but I was brash. I resolved to test the ability of the Sindbis RNA genome to serve as mRNA *in vitro*, and to trace the fate of the genome following its entry into the host cell. These were novel ventures in their time (1961). They were also technically foolish. But they sired an abiding interest in how the genomes of RNA viruses commandeer the molecular machinery of the host cell, an interest that led me eventually to retroviruses.

My work with Elmer was sheer joy, teaching me the inebriation of research, the practice of rigor and the art of disappointment. But it produced nothing of substance. Twenty five years later, on the occasion of my fiftieth birthday, Elmer recalled my first novitiate in science with a quote from T. H. Huxley: "There is great practical benefit in making a few failures early in life."

After two years of clinical training at the Massachusetts General Hospital, I began my second novitiate by entering the Research Associates Program at the National Institutes of Health in Bethesda, Maryland, where my mentor was Leon Levintow. My subject was the replication of poliovirus, which had become a test case for the view that the study of animal viruses could tease out the secrets of the vertebrate cell. In my first publishable research, I obtained evidence that the replication of polioviral RNA engendered a multi-stranded intermediate, although my description of that intermediate proved flawed in its details (3).

Midway through my postdoctoral training, Levintow departed for the faculty at the University of California, San Francisco. In his stead came Gebhard Koch, who soon lured me to his home base in Hamburg, Germany, for a year. Together Gebhard and I explored the basis for the infectivity of multi-stranded RNAs (4). I continued this work for several years to come, eventually showing that the double stranded form of polioviral RNA is infectious-because the positive strand of the duplex can be expressed in mammalian cells, as if the duplex RNA might be unraveling within the cell (5). This finding perplexed us and seemed abstruse, but it appears to have been a harbinger of unanticipated enzymatic activities whose existence and functions are coming into view only now (6).

Retroviruses

As my year in Germany drew to a close, I chose to join Levintow as a member of the faculty at the University of California, San Francisco. The decision proved providential beyond all measure. In San Francisco, I found Warren Levinson, who had set up a program to study Rous Sarcoma Virus, an archetype for what we now call retroviruses. At the time, the replication of retroviruses was one of the great puzzles of animal virology. Levinson, Levintow and I joined forces in the hope of solving that puzzle. We had hardly begun before David Baltimore and Howard Temin announced that they had solved the puzzle with the discovery of reverse transcriptase (7,8), work that brought them Nobel Prizes a scant five years later.

The discovery of reverse transcriptase was sobering: a momentous secret of nature, mine for the taking, had eluded me (and others, of course). But I was also exhilarated because the DNA synthesized by reverse transcriptase *in vitro* represented an exquisite probe for viral nucleic acids, a reagent that would give us unprecedented access to the life cycle of retroviruses. To paraphrase a memorable simile from Arthur Kornberg (9), we now had a wedge with which to pry open the infected cell, and the hammer to drive that wedge would be molecular hybridization.

I had become enamored of molecular hybridization while working on poliovirus, because of the exceptional sensitivity and specificity the technique offered in the pursuit of viral nucleic acids. It was a tool made to

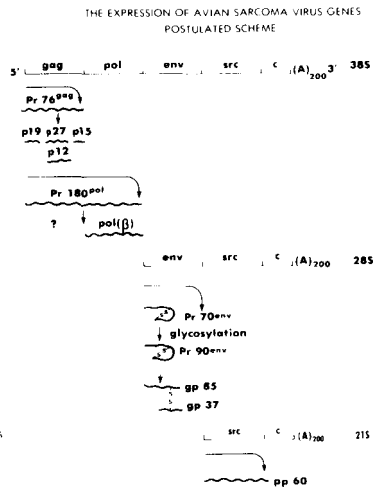


Figure 1. Expressing the genes of Rous Sarcoma Virus.

The diagram was prepared in 1980 to portray how splicing and the processing of proteins facilitate expression of the Rous Sarcoma Virus genome. In the interim, we have learned that frame-shifting during translation is also required to produce the *gag-pol* polyprotein (13). Individual genes are designated by conventional nomenclature, according to the proteins they encode: *gag*, structural proteins of the capsid and nucleocapsid; *pol*, reverse transcriptase; *env*, the glycoproteins of the viral envelope; and *src*, the oncogene of the virus. Other viral functions not illustrated include the protease that mediates maturation of several viral proteins; and integrase, the enzyme that catalyzes integration of viral DNA.

order for the study of retroviral replication, which proceeds in concert with and is obscured by the normal metabolism of the host cell.

Improvising assays as we went, my colleagues and I soon had our first glimpse of viral RNA in cells infected with retroviruses (10). We were to pursue the character and genesis of those RNAs for years to come, laying out the manner of viral gene expression in considerable detail (Fig. 1). The work helped uncover an early example of RNA splicing (11, 12) and set the stage for the much later discovery of frame-shifting in the translation of retroviral mRNAs (13). But our exercises with viral RNA had a larger resonance, as well, because we had constructed the technical stage on which the discovery of retroviral transduction would eventually play out.

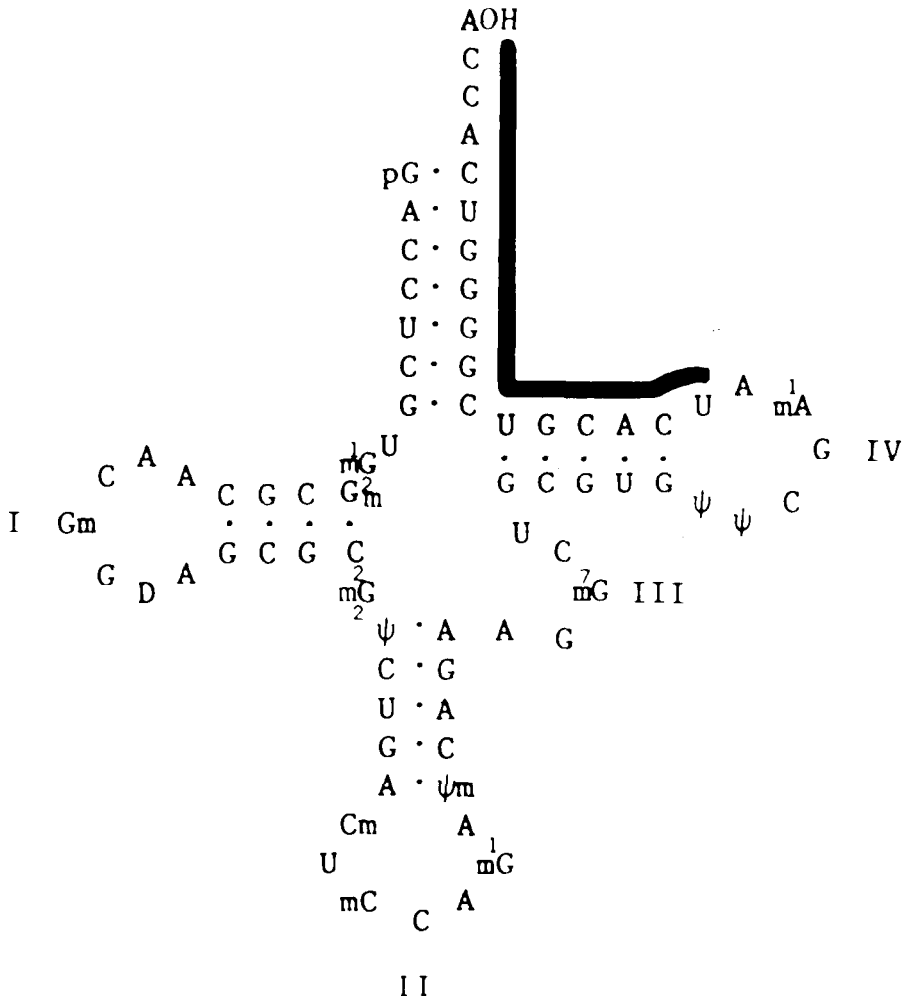


Figure 2. The tRNA primer for reverse transcription in Rous Sarcoma Virus.

The avian tRNA for tryptophan serves as primer for reverse transcription from the genome of Rous Sarcoma Virus. The RNA is displayed here in a conventional representation of secondary structure. The first sixteen nucleotides at the 3' end of the tRNA are paired with the genome by hydrogen-bonded complementarity, as highlighted here.

I also picked up the study of reverse transcriptase itself, perhaps to exorcise my sense of failure at not discovering the enzyme in the first place. We began by working *in vitro* to explore the details of DNA synthesis by the enzyme (14). The most notable outcome was the demonstration that the reverse transcriptase of Rous Sarcoma Virus uses the cellular tRNA for tryptophan as a primer to initiate transcription from the retroviral genome (Fig. 2) (14, 15). The point was soon generalized to other retroviruses and is now a feature that serves as a signature of retrotransposons at large (16).

But what I wanted most to know was the course of events in the infected cell. Could we find the proviral DNA first imagined by Howard Temin and then foretold in substance by the discovery of reverse transcriptase? Where in the cell was this DNA synthesized following infection? What form did it take before and after integration into chromosomal DNA? Harold Varmus arrived to provide the answers (2), and to alter my life and career irrevocably. Within the year, his name became consubstantial with expertise on the synthesis and integration of retroviral DNA. In the process, I had lost a postdoctoral fellow and gained a coequal.

Oncogenes

To this point, we had thought little of cancer. But as the virus of Peyton Rous first lured me away from poliovirus, now it lured us to the study of neoplastic transformation. Peyton Rous received his Nobel Prize two years before my initial encounter with his virus. The award dramatized the great mystery of how Rous Sarcoma Virus might cause cancer. It was a mystery whose solution lay in genetics.

Soon after my arrival in San Francisco, a graduate student and I had conducted a search for temperature-sensitive conditional mutants of poliovirus and Rous Sarcoma Virus, without a particle of success. But where we had failed, others succeeded, and the study of viral tumorigenesis was transformed (17 — 19). The data showed with luminous clarity that a gene within Rous Sarcoma Virus is responsible for cancerous growth of infected cells, that continuous action of the gene is required to sustain cancerous growth, and that the gene probably works by directing the synthesis of a protein. The oncogene *src* had been sighted.

The genetic identification of *src* was reported in the same year as the biochemical discovery of reverse transcriptase. The two became themes that intertwined and nourished one another in the daily life of our laboratory. Much as our deployment of molecular hybridization in the study of viral replication set the stage for the discovery of cellular *src*, so our ensuing success in isolating viral proteins from infected cells (20) emboldened us to seek the protein encoded by *src*.

We were in the midst of efforts to prepare antisera that would recognize the *src* protein when news of success came from Denver (21). Erikson and his colleagues had obtained persuasive evidence that the oncogene encodes a 60 kilodalton protein. Once the product of *src* was a physical reality, the puzzle of its action loomed larger than ever. How could this single protein

elicit the pleiotropic change in cellular phenotype that we call neoplastic transformation?

The answer came quickly. *Src* encodes a protein kinase (22, 23), whose amino acids substrate later proved unexpectedly to be tyrosine (24). By phosphorylating numerous cellular proteins, the enzyme could rapidly change myriad aspects of cellular structure and function; by being the first exemplar of protein-tyrosine kinases, it gave notice of a previously unappreciated regulatory device that we now realize is second to none in the signalling pathways of the cell (25).

The ways in which these answers emerged are illuminating. In Denver, insight came from an inspired guess, based on the pleiotropism of *src* (22): protein phosphorylation ranks among the most versatile agents of change known to biochemists. In our laboratory, enzymological reasoning led the way (23): phosphorylation of the *src* protein in cellular extracts displayed properties suggestive of a unimolecular reaction, as if the protein were phosphorylating itself — which indeed it was (26). And at the Salk Institute, the use of an erroneous buffer led to the fortuitous separation of phosphotyrosine from phosphothreonine for the first time in recorded history, the only example of productive laziness that I have ever seen acknowledged with both candor and gratitude in the biomedical literature (25).

The sighting and subsequent characterization of *src* opened the way to a biological cornucopia. We now know of more than twenty retroviral oncogenes, whose diverse specificities in tumorigenesis provide experimental modes for most forms of cancer that afflict human kind (Table 1). Each of these genes encodes a protein whose biochemical action provides distinctive purchase on the mechanisms of neoplastic growth (27, 28).

Table 1. The oncogenes of retroviruses (1989)

Oncogene	Pathogenicity	Oncogene	Pathogenicity
<i>abl</i>	B-cell tumors and fibrosarcomas	<i>mil/raf</i>	sarcomas and supplemental
<i>akt</i>	thymomas	<i>mos</i>	sarcomas
<i>cbl</i>	B-cell and myeloid tumors	<i>myb</i>	myeloblastosis
<i>crk</i>	sarcomas	<i>myc</i>	carcinomas; myelocytomatosis;
<i>erb-a</i>	supplemental		sarcomas
<i>erb-b</i>	erythroleukemia and fibrosarcomas	<i>ras</i>	sarcomas; erythroleukemia
<i>ets</i>	supplemental	<i>rel</i>	b-cell tumors
<i>fes/fps</i>	sarcomas	<i>ros</i>	sarcomas
<i>fgr</i>	sarcomas	<i>sea</i>	sarcomas; leukemias
<i>fms</i>	sarcomas	<i>sis</i>	sarcomas
<i>fos</i>	osteosarcomas	<i>ski</i>	carcinomas
<i>jun</i>	sarcomas	<i>src</i>	sarcomas
<i>kit</i>	sarcomas	<i>yes</i>	sarcomas

The products of oncogenes are deployed to various reaches of the cell, including the nucleus, the cytoplasm, the plasma membrane, even the exterior beyond the cell (27, 28). And they act in different ways, which for the moment are subsumed by three genre: i) the phosphorylation of proteins, with either serine and threonine, or tyrosine as substrates — the immediate role of the oncogene product may be induction of the phosphorylation (as in the case of growth factors) or catalysis itself (as with the receptors for growth factors) (25); ii) the transmission of signals by GTP-binding proteins, as exemplified by the products of RAS genes — whose exact position in signalling pathways remains unresolved (29); and iii) the control of transcription from DNA (30).

Diversification of this list in the future seems likely, since the functions of many oncogenes have yet to be elucidated. But the list displays an economy of style that may survive because it reflects the need for pleiotropism: nature may have only a limited number of ways to achieve the manifold changes that create the neoplastic phenotype.

Retroviral transduction

At first it seemed that the lessons to be learned from retroviral oncogenes might apply only to the cancers induced by viruses in animals, that the oncogenes of retroviruses might be alley cats of evolution with little importance to human kind. The discovery of cellular *src* and the inference that it gave rise to the oncogene of Rous Sarcoma Virus inspired hope that this narrow view might be wrong (31). If cells contain genes capable of becoming oncogenes by transduction into retroviruses, perhaps the same genes might also become oncogenes within the cell, without ever encountering a virus. By means of accidental molecular piracy, retroviruses may have brought to view the genetic keyboard on which many different causes of cancer can play, a final common pathway to the neoplastic phenotype (Fig. 3).

The hope had its detractors (32). Even transduction itself was challenged. But for us in San Francisco, the reality of transduction seemed inescapable. The cellular homologue of *src* had been conserved through eons of evolution, whereas the other genes of Rous Sarcoma Virus could be found only in chickens and a few close kin (2). The ineluctable conclusion was that the two sorts of genes, an oncogene on the one hand and genes devoted to viral replication on the other, had separate origins.

We would eventually muster more sophisticated arguments, all of which pointed to the same conclusion: the progenitor of *src* was a conserved (and hence vital) cellular gene that found its way into Rous Sarcoma Virus by recombination (2). The images of cellular and viral *src* gained eventually from molecular cloning sustained our argument in a gratifying manner (33), but for me, they were anticlimatic.

Arguing for the cellular origins of *src* provided my first experience with the heuristic force of evolution. "Nothing in biology makes sense except in the light of evolution", to recall a famous aphorism from Dobzhansky (34).

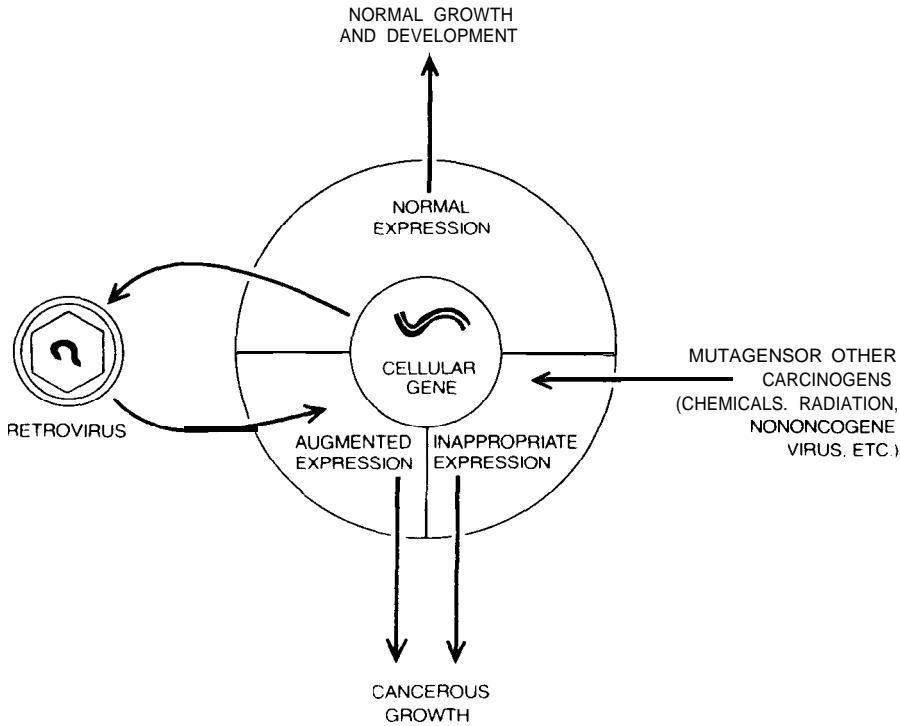


Figure 3. Transduction by retroviruses uncovers cancer genes.

The cartoon was designed in 1982 and exemplifies the now conventional view of proto-oncogenes as both important elements in the governance of normal cellular phenotype and potential substrates for various causes of cancer. The figure is reprinted here with the kind permission of *Scientific American*.

The aphorism embodies a truth that has been dishonored in the United States, where religious zealots continue their efforts to hound the teaching of evolution from public schools, and men of little learning assail the truth of evolution under the fraudulent rubric of "creation science".

The genesis of retroviral oncogenes by recombination with cellular genes had been postulated by several observers. Although it may sound self-serving, I confess my ignorance of those speculations when we began our work on cellular *src*. I was motivated by a desire to test the Virogene-Oncogene Hypothesis of Huebner and Todaro (35) not by an interest in the origins of oncogenes. But in due course, Howard Temin provided a useful inspiration with his suggestion that all retroviruses arose by the cobbling together of disparate genetic elements in the cell, with intermediates that he called proto-viruses (36). The inspiration was for taxonomy, not experiment.

As transduction by retroviruses came into common discourse, the need arose for a generic term to describe the cellular progenitor of *src* and other retroviral oncogenes. The first to find general usage was "cellular oncogene". Although I was a nominal member of the responsible committee on nomenclature, I was uncomfortable with this term because of its unwarrant-

ed implication that the native cellular genes carried intrinsic tumorigenic potential, that they need not be changed to cause trouble. So in playful homage to Howard Temin, I began to use the term "proto-sarc" (37). The generic "proto-oncogene" followed in short order.

In the interim, proto-oncogene has come into general use, as the colloquial counterpoise to oncogene. It has also become an embarrassment, because the precise connotation of the word is that of prototype rather than progenitor, not far removed from the offensive connotation of cellular oncogene. But the intent of the taxonomic invention was clear: numerous investigators (ourselves included) have spent the past decade exploring the sorts of genetic damage that can convert a harmless proto-oncogene into a pathogenic oncogene (38).

The manuscript that announced our discovery of cellular *src* concluded with the speculation that the gene might be involved in "the normal regulation of cell growth and development or in the transformation of cell behavior by physical, chemical or viral agents" (39). These words were pure bravado, particularly because we then had no assurance that cellular *src* was in fact a full-fledged gene. The assurance accrued over the ensuing two years, first in the form of evidence that cellular *src* was transcribed in normal cells (40,41), and then with the identification of the protein encoded by the gene (42,43).

Expanding the repertoire

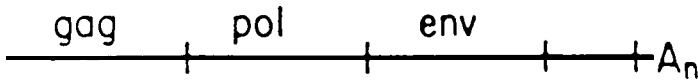
As our confidence in the reality of cellular *src* grew, a new challenge took shape. Could we generalize the principle of transduction? Had the oncogenes of other retroviruses also originated from cellular genes? In order to pursue the generality of transduction, we turned first to a retrovirus known as MC29 (44), which attracted our attention because it offers a model for the induction of carcinomas, the most prevalent of human cancers.

During the search for an oncogene in MC29, the impatience of molecular biologists held sway, the rigor of formal genetics was cast aside. Some investigators (ourselves included) used molecular hybridization to detect nucleotide sequences unique to the genome of MC29 (45,46), others used chemical procedures to identify the same sequences and to map their position on the viral genome (47). Both strategies took liberties that we had not allowed ourselves with *src*. Lacking a deletion mutant that might define the oncogene, we and others made the assumption that the genomes of MC29 and its necessary helper virus were congenic except for the presence or absence of the oncogene (Fig. 4).

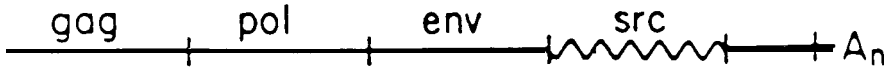
The same assumption had been applied previously in an effort to define the oncogene of a murine sarcoma virus and had led to a molecular quagmire (48). But now the lessons from *src* told us more precisely what we might be seeking. So we forged ahead and soon had a molecular probe that represented nucleotide sequences found in MC29 and other retroviruses with similar tumorigenicities, but not in the related helper viruses (45).

The newly found locus was taken to be the oncogene of MC29 and

LEUKOSIS VIRUS



SARCOMA VIRUS



ACUTE LEUKEMIA VIRUS

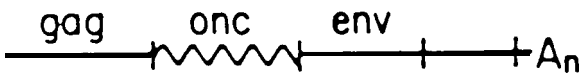


Figure 4. The genomes of Rous Sarcoma Virus and related avian retroviruses.

The genomes of Rous Sarcoma Virus, Avian Leukosis Viruses and Acute Avian Leukemia Viruses appear to have a common origin and, thus, are congenic with the exception of oncogenes, inserted into the genomes by transduction from host cells. The leukosis viruses have no oncogenes and induce malignancy by insertional mutagenesis (2, 38). The viral genes are designated as in Fig. 1, except for the use of *onc*, the generic term for retroviral oncogenes.

eventually designated *myc*, in deference to the form of leukemogenesis (myelocytomatosis) from which the virus acquired its name. Over the next several years, the authenticity of *myc* would be ascertained by molecular cloning (49), nucleotide sequencing (59), and gene-transfer (51). To this day, the gene has never been defined by the strategies of classical genetics, and there is now no need to do so. The new biology is upon us.

The lessons of *src* were powerful. We and others were able to argue that the molecular probes for the oncogene of MC29 were legitimate because they also detected nucleotide sequences in the DNA of normal vertebrates (46, 52), sequences that were transcribed into RNA in normal cells (52, 53) and that diverged among species in rough accord with phylogenetic distances (46, 52, 53). No other portion of the MC29 genome displayed these properties. Similar findings were described in parallel for the avian retroviral oncogenes *erb* (in reality, two oncogenes) and *myb* (46). The example of cellular *src* was not an exotic anomaly; it was an archetype.

In the years that followed, *myc* proved to be a great provider, a vehicle for several seminal discoveries that included the activation of cellular genes by insertional mutagenesis (54), the involvement of proto-oncogenes in chro-

mosomal translocations (55, 56), and the amplification of proto-oncogenes in human tumors (57). These discoveries had exceptional logical force because they involved a gene whose tumorigenic potential was already known from the study of retroviruses.

No sooner had the bounty of transduction become evident than other routes to proto-oncogenes took shape, some serendipitous, others designed (58): the ability of retroviruses to mutate cellular genes, creating oncogenes at their place of residence within the cell; the dissection of chromosomal abnormalities in cancer cells, such as translocations and amplifications; the use of gene-transfer to detect mutant proto-oncogenes by means of their biological activity; and the pursuit of phylogenetic kinships. The definition of proto-oncogene had now become more expansive, subsuming any gene with the potential for conversion to an oncogene — by the hand of nature in the cell, or by the hand of the experimentalist in the test tube.

The normal function of proto-oncogenes

The tally of proto-oncogenes has now reached sixty or more. Most are genes never glimpsed before by any other means. What are these genes in their normal guise? What purposes preserved them through one billion years of evolution? Why do they harbor the potential to wreak cellular mayhem?

We formed our hypotheses from the lessons of transduction. The properties of retroviral oncogenes must echo the functions of proto-oncogenes. Three properties seemed especially telling: the stimulation of cellular proliferation; the specificity of tumorigenesis, as if each gene were designed to work in only certain cells; and the ability of many oncogenes to interrupt or sometimes reverse cellular differentiation (59). Like father, like son: it seemed possible that the actions of viral oncogenes are merely caricatures of what proto-oncogenes are normally intended to do. Retroviruses may have revealed to us not only touchstones of tumorigenesis, but clues to the nerve-center that governs the normal cell cycle and the differentiation of cellular function.

Eager to explore these thoughts in a living organism, we turned to the fruit fly, *Drosophila melanogaster*. Why the fruit fly? First, because the full collection of genes in this creature is within our scope. Second, because we have in hand a rich catalogue of normal and mutant genes from the fruit fly, the products of more than half a century of labor. Third, because the fruit fly is for the moment the only metazoan organism in which we can manipulate genes with reasonable facility, although a soil worm on the one hand and the laboratory mouse on the other are now making strong bids for our favor. Fourth, because when reduced to essentials, the fruit fly and *homo sapiens* are not very different. And fifth, because the fruit fly has a large complement of proto-oncogenes with counterparts in mammals (60).

What we have learned of proto-oncogenes from *Drosophila* indicates at least some of the bravado in our first publication on cellular *src*. Mutant alleles have been identified for the counterparts of six proto-oncogenes in the fruit fly (Table 2). In two instances, the *abl* and *myb* proto-oncogenes,

Table 2. Mutant alleles of proto-oncogenes in *Drosophila melanogaster*

Proto-oncogene	<i>Drosophila</i> counterpart	Biochemical function	Mutant phenotype
<i>abl</i>	<i>abl</i>	tyr kinase	embryonic lethal/ defects in late development
<i>int-1</i>	<i>wingless</i>	? growth factor	segment polarity
<i>raf</i>	<i>pole hole</i>	ser/thr kinase	defective cellular proliferation
<i>rel</i>	<i>dorsal</i>	?	dorsal-ventral polarity
<i>erbB-1</i>	<i>flb/top</i>	tyr kinase	embryonic lethal/ dorsoventral patterning
<i>myb</i>	<i>myb</i>	transcription factor	embryonic and pupal lethality

the search for mutations was deliberate; in the remainder, mutations recognized first by their phenotypes later proved to be in *Drosophila* counterparts of mammalian proto-oncogenes. All of the mutations elicit profound disturbances of development. The work from our own hands has revealed a requirement for activity of the proto-oncogene *myb* during two distinct stages of development, embryogenesis and pupation (unpublished data of A. Katzen and J.M.B.). More precisely, a deficiency in *myb* appears to reduce the number of cell divisions that can occur in certain developmental lineages.

The actions of proto-oncogenes in development have their underpinnings in the elaborate circuitry that governs the behavior of vertebrate cells (Fig. 5). The junction boxes in this circuitry include polypeptide hormones that act on the surface of the cell, receptors for these hormones, proteins that convey signals from the receptors to the deeper recesses of the cell, and nuclear functions that orchestrate the genetic response to afferent commands (typically, by regulating transcription). Diverse lines of enquiry have brought these junction boxes to view, but the study of proto-oncogenes has been among the richest sources. Time and again, the several lines of enquiry have converged on the same junction box. We may have most of the circuitry in view. The cell is not infinitely complex; the cell can be understood.

Seeds of cancer

But what of cancer? Are proto-oncogenes the seeds of this disease in all our cells? Are they a common keyboard for many different players in tumorigenesis? As a physician, I found these questions attractive. As a scientist, I

Cellular Phenotype: A Regulatory Circuit

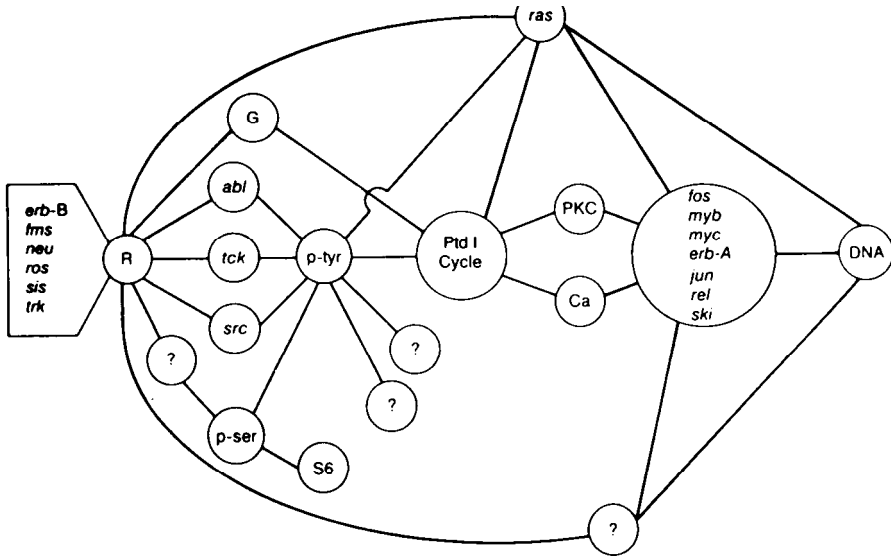


Figure 5. The biochemical circuitry that mediates cellular phenotype.

The diagram illustrates how some of the functions encoded by proto-oncogenes fit into the circuitry that regulates the phenotype of vertebrate cells. The scheme is in part hypothetical and is not intended to be comprehensive. Functions encoded by proto-oncogenes have been designated by the conventional terminology for the genes themselves. Other abbreviations include: G, GTP-binding proteins that transduce signals, from cell-surface receptors; R, generic receptor; p-Ser, phosphorylation of serine in proteins; p-Tyr, phosphorylation of tyrosine in proteins; PtdI, phosphatidylinositol; S6, a ribosomal protein that is phosphorylated in response to diverse mitogenic signals; PKC, protein kinase C. Reprinted here with the kind permission of *Science*.

found them intimidating. Exploration of the cancer cell is akin to archaeology: we must infer the past from its remnants in the present, and the remnants are often cryptic.

But the first remnants to emerge from the proto-oncogenes of human cancer cells told a vivid story. Molecular dissections revealed that chromosomal translocations in human and murine tumors often affect proto-oncogenes already familiar from the study of retroviruses, with the great provider *myc* prominent among them (55, 61). Emboldened by these findings, my colleagues and I mounted a belated excavation for the genetic shards of tumorigenesis. But we chose a neglected terrain in which to dig: amplified DNA.

Focal amplification of domains within chromosomes is a scheduled and purposeful event during the life cycles of diverse organisms (62). In mammals, however, gene amplification is an unscheduled aberration that gives rise to karyotypic abnormalities known as double-minute chromosomes and homogeneously staining regions. When my colleagues and I began our excavations, gene amplification in cancer cells was known principally as a

consequence of selection by chemotherapeutic agents. But the literature also contained occasional examples of double-minute chromosomes and homogeneously staining regions in untreated cancers. When a limited sampling of these were examined by ourselves and others, they proved to involve previously identified proto-oncogenes (yet again, *myc* was prevalent) (57). In due course, it became apparent that gene amplification is relatively common in untreated tumors and that it affects proto-oncogenes.

Intent on a more systematic study, we chose human neuroblastomas, in which gene amplification seemed exceptionally common. We resolved to ask whether the amplified DNA in neuroblastoma cells contained any of the proto-oncogenes then known. When we got to *myc*, we struck rich ore of an unexpected sort: a gene related to *myc*, sighted for the first time in the parallel work on neuroblastoma by ourselves and others, and eventually designated *N-myc* (63 – 65). In due course, it became apparent that *N-myc* is an authentic proto-oncogene, a close kin of *myc* itself that apparently evolved to serve a separate purpose in the normal organism (66 – 68).

As the survey of neublastomas broadened, a fertile correlation became apparent. Amplification of *N-myc* was found only in the more aggressive variants of the tumor, in perhaps one-quarter of all the specimens examined (69, 70). Moreover, within single tumors, *N-myc* was expressed abundantly only in neuroblasts, the least differentiated (and presumably most malignant) cells of the tumor (64). These correlations had two implications: first, that amplification of *N-myc* might embody a step in tumor progression, one of several events that exacerbate the malignancy of neuroblastomas; and second, that we had brought to hand a prognostic tool, a device with which to supplement the conventional staging of the disease.

The passage of time has dealt kindly with these hopes. The New England Journal of Medicine has now provided its imprimatur by arguing that “in neuroblastoma, amplification of the *N-myc* gene is of greater prognostic value than the clinical stage of the disease” (71). Thirty years after deserting the bedside, I have found clinical relevance in my research.

The excavation that unearthed *N-myc* was a pedestrian exercise in both concept and execution. Yet in one fell swoop, it served up an important proto-oncogene without the assistance of a retrovirus, hinted at a major role for gene amplification in tumorigenesis, foreshadowed the molecular dissection of tumor progression, and gave first notice that the emerging knowledge of oncogenes would eventually prove useful at the bedside.

These themes have since found wider resonance. First, we can point to a variety of human malignancies in which damage to one or another proto-oncogene has been found with some consistency (Table 3). The damage takes diverse forms, including translocations, amplifications and point mutations, all of which appear to have dominant effects on gene function. The list of malignancies containing these lesions is impressive: because of the diversity of tumors involved; because of their identities — several can be counted among the principal nemeses of humankind; and because the list has been assembled after only a few years of pursuit, with imperfect tools — there is

Tabel 3. Proto-oncogenes and human tumors: some consistent incriminations

Proto-oncogene	Neoplasm	Lesion
<i>abl</i>	chronic myelogenous leukemia	translocation
<i>erbB-1</i>	squamous cell carcinoma glioblastoma	amplification
<i>erbB-2 (neu)</i>	adenocarcinoma of breast and ovary	amplification
<i>myc</i>	Burkitt's lymphoma small cell carcinoma of lung carcinoma of breast carcinoma of cervix	translocation amplification
<i>lmyc</i>	small cell carcinoma of lung	amplification
<i>nmyc</i>	neuroblastoma small cell carcinoma of lung	amplification
<i>kras</i>	carcinomas of colon, lung and pancreas	point mutation
<i>nras</i>	acute myelogenous and acute lymphoblastic leukemia; carcinoma of thyroid	point mutation
<i>hras</i>	carcinomas of genitourinary tract and thyroid	point mutation

doubtless more to come. Beyond this list, there lies the burgeoning repertoire of recessive lesions in human cancers, whose nature, prevalence and unquestionable importance are just now coming into focus (72).

Second, catalogues of genetic damage within individual tumors are taking shape, showing us how the malfunction of several different genes might combine to produce the malignant phenotype (58): for example, carcinomas of the colon contain no less than five different yet prevalent lesions – some genetically dominant, others recessive; carcinoma of the breast, at least five lesions; carcinoma of the lung, at least four; and neuroblastoma, at least three. Moreover, detection of the lesions is likely to provide information for prognosis, perhaps even for therapeutic management. Examples include neuroblastoma (69 - 71), carcinoma of the breast (73, 74) and ovary (74), and preleukemias (75-77).

For those of us who first studied cancer more than thirty years ago in medical school, then returned to the disease decades later to find it little less a mystery, the contemporary image of the cancer cell is both thrilling and an unexcelled vindication for fundamental research. The image was forged from the vantage point of molecular genetics and with the tools of that discipline. The lines of discovery trace back to minuscule columns of hydroxyapatite from which the molecular probe for *src* first flowed; to *src* itself, which even now has not been persuasively implicated in the genesis of human cancer; and to the chickens in which Peyton Rous first found his tumor. From these humble roots, a great tree of knowledge has grown. And

a great truth has been reiterated: we cannot prejudge the utility of scholarship, we can only ask that it be sound.

Prospects for therapy

What of treatment? Will we acquire new antidotes for cancer from our study of oncogenes? There is little likelihood that we will be able to repair or replace damaged proto-oncogenes in the foreseeable future, particularly in the individual already burdened with countless tumor cells. There is talk of restoring functional copies of recessive oncogenes to tumors in which they are defective (78). But realization of this objective in human subjects presently seems many years distant.

If we focus on the protein handmaidens of genes, however, we can see more cause for hope. Given sufficient information about how these proteins act, the pharmaceutical chemist or the immunotherapist may be able to invent ways to interdict their action, even to exploit the specificity of genetic damage and thus to reverse the effects of oncogenes. We are not close to implementing this strategy, but it is a reasonable hope.

I am eager not to appear naive. No single therapy against an oncogene product is likely to become a panacea. We must deal with a large variety of oncogenes, whose products are deployed to the many reaches of the cell and whose actions present great chemical and enzymological diversity; and we must be prepared to cope with evolving genetic damage within cancer cells that can bring a variety of oncogenes into play sequentially. Nevertheless, the search for genetic damage in cancer cells and the explication of how that damage affects the biochemical functions of genes have become our best hope to understand and thus to thwart the ravages of cancer.

Conclusion

At the beginning of this century, the Austrian engineer and novelist Robert Musil offered a description of progress in science that foreshadowed modern views of epistemology and that now exemplifies the course of contemporary cancer research.

... every few years, ...something that up to then was held to be error suddenly revolutionizes all views, ...an unobtrusive, despised idea becomes the ruler over a new realm..." (79).

Harold Varmus, I and our numerous colleagues have been privileged to assist as a despised idea became the ruler over a new realm. The notion that genetic changes are important in the genesis of cancer has met strenuous resistance over the years. But now that notion has gained ascendancy. In the event, I have learned that there is no single path to creativity: we are constrained not by the necessary discipline of rigor, but by the limits to our imaginations and our intellectual courage. In the words of an American sage: "Dare to be wrong, or you may never be right" (SO).

Discovery takes two forms. The first is mundane, but nevertheless legitimate: we grope our way to reality and then recognize it for what it is. The second is legitimate, but also sublime: we imagine reality as it ought to be

and then find the proof for our imaginings. I have been fortunate to know the first form of discovery and am thankful for the privilege. I have miscarried opportunities to know the second and am diminished by the failure. Redemption lies in more imaginings.

“The real truths are those that can be invented” (81).

ACKNOWLEDGEMENTS

I owe a life-long debt to Harold Varmus, with whom I shared most of the events described here. The whole has been greater than the sum of the two parts, or so I believe. Numerous students and postdoctoral fellows have participated in the discovery and pursuit of proto-oncogenes with Harold and myself. Without their dedicated and talented efforts, I would have no story to tell: Since I could not name them all in the text, I have named none. Many of their contributions are documented in the bibliography to this manuscript. I recall the memory of Richard Parker, for his special impact. I am also grateful to colleagues in the scientific community who have helped me at crucial times in my career, including David Baltimore, Howard Berg, Peter Duesberg, Howard Goodman, Robert Huebner, Malcolm Martin, John Menninger, Aaron Shatkin, Howard Temin and Peter Vogt. I acknowledge my many colleagues at the University of California, San Francisco, whose friendship and support have been vital, particularly Bruce Alberts, Mitzi Best, Herbert Boyer, Lois Fanshier, Julius Krevans, Jean Jackson, Marc Kirschner, Tom Kornberg, Suzanne Ortiz, Nancy Quintrell, Rudi Schmid, Lois Serxner, Holly Smith and Karen Smith. I thank my wife and family for a gift of forbearance that cannot be repaid. My work has been supported by both the National and California Divisions of the American Cancer Society and by the National Institutes of Health. The support of biomedical research in the United States is an act of public altruism unexcelled in history. I dedicate this manuscript to the countless individuals who sustain that support, by voluntary contributions and by the payment of state and federal taxes.

REFERENCES

1. R. Dulbecco, in *Biographical Memoirs*, Vol. XLVIII, National Academy of Sciences, Washington, D.C., pp. 275 -306, 1976.
2. H. E. Varmus, in *Les Prix Nobel*, Almqvist & Wiksell Int'l., Stockholm, p. 187, 1989.
3. J. M. Bishop, D. F. Summers and L. Levintow, *Proc. Natl. Acad. Sci. USA*, **54**, 1273-1281 (1965).
4. J. M. Bishop, G. Koch, B. Evans and M. Merriman, *J. Mol. Biol.* **46**, 235- 249 (1969).

5. M. Best, B. Evans and J. M. Bishop, *Virology*, **47**, 592-603 (1972).
6. B. L. Bass, H. Weintraub, R. Cattaneo and M. A. Billeter, *Cell* **56**, 331 (1989).
7. D. Baltimore, *Nature* **226**, 1209- 1211 (1970).
8. H. M. Temin and S. Mizutani, *Nature* **226**, 1211-1213 (1970).
9. A. Kornberg, *Science*, **131**, 1503- 1508 (1960).
10. J. Leong et al., *J. Virol.* **9**, 891 -902 (1972).
11. S. R. Weiss, H. E. Varmus and J. M. Bishop, *Cell*, **12**, 983 -992 (1977).
12. B. Cordell, S. R. Weiss, H. E. Varmus and J. M. Bishop, *Cell* **15**, 79 -92 (1978).
13. T. Jacks and H. E. Varmus, *Science*, **230**, 1237- 1246 (1985).
14. A. J. Faras, J. M. Taylor, W. Levinson, J. Goodman and J. M. Bishop, *J. Mol. Biol.* **79**, 163-183 (1973).
15. J. E. Dahlberg et al., *J. Virol.* **13**, 1126-1133 (1974).
16. H. E. Varmus, *Sci. Amer.* **257**, 56-66 (1987).
17. G. S. Martin, *Nature*, **227**, 1021 -1023 (1970).
18. S. Kawai and H. Hanafusa, *Virology*, **46**, 470-479 (1971).
19. P. K. Vogt, in *Comprehensive Virology*, Vol. 9 (H. Fraenkel-Conrat and R. R. Wagner, Eds.), Plenum Press, New York and London, pp. 341-455 (1977).
20. H. Oppermann, J. M. Bishop, H. E. Varmus and L. Levintow, *Cell* **12**, 993-1005 (1977).
21. J. S. Brugge and R. L. Erikson, *Nature* **269**, 346-347 (1977).
22. M. S. Collett and R. L. Erikson, *Proc. Natl. Acad. Sci. USA* **75**, 2021 -2024 (1978).
23. A. Levinson, H. Oppermann, L. Levintow, H. E. Varmus and J. M. Bishop, *Cell* **15**, 561-572 (1978).
24. T. Hunter and B. M. Sefton, *Proc. Natl. Acad. Sci. USA* **77**, 1311- 1315 (1980).
25. T. Hunter and J. A. Cooper, *Ann. Rev. Biochem.* **54**, 897-931 (1985).
26. A. D. Levinson, H. Oppermann, H. E. Varmus and J. M. Bishop, *J. Biol. Chem.* **255**, 11973-11980 (1980).
27. J. M. Bishop, *Cell* **42**, 23-38 (1985).
28. T. Hunter, *Sci. Amer.* **251**, 70-80 (1984).
29. M. Barbacid, *Ann. Rev. Biochem.* **56**, 779-827 (1987).
30. P. K. Vogt and R. Tjian, *Oncogene* **3**, 3-9 (1988).
31. J. M. Bishop, *Cell*, **23**, 5-6 (1981).
32. P. H. Duesberg, *Science* **228**, 669 (1985).
33. R. Jove and H. Hanafusa, *Ann. Rev. Cell Biol.* **3**, 31-57 (1987).
34. Theodosius Dobzhansky, as cited in D. J. Futuyma, *Science on Trial: The Case for Evolution*, Pantheon Books, New York, p. 114 (1983).
35. R. J. Huebner and G. J. Todaro, *Proc. Natl. Acad. Sci. USA* **64**, 1087- 1094 (1969).
36. H. Temin, in *Nobel Lectures in Molecular Biology 1933- 1975*, Elsevier-North Holland, Inc., New York, p. 509-529 (1977).
37. J. M. Bishop et al., in *The Molecular Biology of the Mammalian Genetic Apparatus*, Vol. 2 (P. O. P. Ts'o, Ed.), North-Holland Publishing Co., Amsterdam, p. 277-287 (1977).
38. H. E. Varmus, *Ann. Rev. Genet.* **18**, 553-612 (1984).
39. D. Stehelin, H. E. Varmus, J. M. Bishop and P. K. Vogt, *Nature* **260**, 170 - 173 (1976).
40. D. Spector et al., *Cell* **3**, 371-379 (1978).
41. D. Spector, B. Baker, H. E. Varmus and J. M. Bishop, *Cell* **3**, 381-386 (1978).
42. M. S. Collett, E. Erikson, A. F. Purchio, J. S. Brugge and R. L. Erikson, *Proc. Natl. Acad. Sci. USA* **76**, 3159-3163 (1979).
43. H. Oppermann, A. D. Levinson, H. E. Varmus, L. Levintow and J. M. Bishop, *Proc. Natl. Acad. Sci. USA* **76**, 1804 - 1808 (1979).
44. A. J. Langlois, S. Sankaran, P. H.-L. Hsuung and J. W. Beard, *J. Virol.* **1**, 1082 - 1084 (1967).

45. D. Sheiness, K. Bister, L. Fanshier, C. Moscovici and J. M. Bishop, *J. Virol.* **33**, 962-968 (1980).
46. M. Roussel *et al.*, *Nature*, **281**, 452-455 (1979).
47. P. Mellon, A. Pawson, K. Bister, G. S. Martin and P. H. Duesberg, *Proc. Natl. Acad. Sci. USA* **75**, 5874-5878 (1978).
48. R. W. Ellis *et al.*, *J. Virol.* **36**, 408-420 (1980).
49. B. Vennstrom, D. Sheiness, J. Zabielski and J. M. Bishop, *J. Virol.* **42**, 773-779 (1982).
50. K. Alitalo *et al.*, *Proc. Natl. Acad. Sci. USA*, **80**, 100- 104 (1983).
51. B. Vennstrom *et al.*, *EMBO J.* **3**, 3223-3231 (1984).
52. D. Sheiness and J. M. Bishop, *J. Virol.* **3**, 514-521 (1979).
53. D. K. Sheiness, S. H. Hughes, H. E. Varmus, E. Stubblefield and J. M. Bishop, *Virology* **105**, 415-424 (1980).
54. W. S. Hayward, B. G. Neel and S. M. Astrin, *Nature* **290**, 475-480 (1981).
55. G. Klein, *Cell* **32**, 311-315 (1983).
56. M. D. Cole, *Ann. Rev. Genet.* **20**, 361-378 (1986).
57. K. Alitalo and M. Schwab *Adv. in Cancer Res.* **47**, 235-282 (1986).
58. J. M. Bishop, *Science* **235**, 305-311 (1987).
59. H. Beug, M. J. Hayman and T. Graf, in *Cancer Surveys, Vol. J* (M. F. Greaves, Ed.), Oxford University Press, U. K., pp. 205-230 (1982).
60. Shilo, B.-Z., *Trends in Genetics*, 69 - 73 (1987).
61. F. G. Haluska, Y. Tsujimoto and C. M. Croce, *Ann. Rev. Genetics*, **21**, 321-347 (1987).
62. G. R. Stark, M. Debatisse, E. Giulotto and G. M. Wahl, *Cell* **57**, 901-908 (1989).
63. M. Schwab *et al.*, *Nature* **305**, 245-248 (1983).
64. M. Schwab *et al.*, *Proc. Natl. Acad. Sci. USA*, **81**, 4940-4944 (1984).
65. N. E. Kohl *et al.*, *Cell* **35**, 359-367 (1983).
66. L. W. Stanton, M. Schwab and J. M. Bishop, *Mol. Cell. Biol.* **83**, 1772- 1776 (1986).
67. N. E. Kohl *et al.*, *Nature* **319**, 73-77 (1986).
68. K. M. Downs, G. R. Martin and J. M. Bishop, *Gen. Dev.* **3**, 860-869 (1989).
69. G. M. Brodeur *et al.*, *Science* **224**, 1121- 1124 (1984).
70. R. C. Seeger *et al.*, *New Eng. J. Med.* **313**, 1111-1116(1985).
71. D. J. Slamon, *New Eng. J. Med.* **317**, 955-957 (1987).
72. G. Klein, *Science* **238**, 1539 - 1545 (1987).
73. D. J. Slamon *et al.*, *Science* **235**, 177- 182 (1987).
74. D. J. Slamon *et al.*, *Science* **244**, 707-712 (1989).
75. E. Liu, B. Hjelle, R. Morgan, F. Hecht and J. M. Bishop, *Nature* **330**, 186- 189 (1987).
76. H. Y. Hirai *et al.*, *Nature* **327**, 430-432 (1987).
77. R. A. Padua, *Leukemia* **2**, 503-510 (1988).
78. H.-J. S. Huang *et al.*, *Science* **242**, 1563- 1566 (1988).
79. R. Musil, *The Man Without Qualities* - I, (E. Wilkins and E. Kaiser, Trans.), Pan Books Ltd., U. K., p. 41 (1979).
80. Attributed to Fats Waller, on constructing musical chords.
81. K. Kraus, in *Half-Truths & One-and-a-Half Truths* (H. Zohn, Ed. and Trans.), Carcanet Press, Ltd., Manchester, U. K., p. 61, 1986.