Oncogenes in Retroviruses and Cells

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Oncogenes are genes that cause cancer. Retroviruses contain oncogenes and cause cancer in animals and, perhaps, in man. The viruses have appropriated their oncogenes from *normal* cellular DNA by genetic recombination. Correspondingly, uninfected vertebrate cells contain a family of evolutionary conserved cellular oncogenes. Retrovirus infection, introducing additional viral oncogenes into the cells, as well as carcinogen-mediated activation of cellular oncogenes may both lead to increased synthesis of oncogene encoded transforming proteins which convert normal cells to tumor cells. Unique retroviruses of human origin have recently been identified. They may, on occasion, directly cause tumors in man. However, the general significance of retroviruses may better be illustrated by their remarkable genetic composition which allows them to promote tumor growth by a variety of genetic mechanisms.

Retroviruses are RNA-containing tumor viruses that have long been fascinating to virologists not only because they cause tumors in animals and possibly also in man, but also because they are useful reagents for studying molecular events in eukaryotic gene expression and because they have served to identify oncogenes, i.e. genes that cause cancer ([1], Fig. 1).

Individual retrovirus strains are species-specific, i.e. in nature detectable only in a single species. Strains from one species are, in general, biochemically and immunologically highly related. Retroviruses can further be classified into two broad categories.

The *exogenous* strains are horizontally spread between individual members of the host species in the manner characteristic for most virus groups investigated to date. These strains can be oncogenic depending on their genetic composition and the circumstances of infection, e.g., dose, site, age and health of the infected individual.

In contrast, *endogenous* virus strains are characterized by their chromosomal integration in all cells of all members of a given species. They comprise the only known group of viruses that can phylogenetically survive as chromosomally integrated "proviruses", as they are vertically transmitted through the germ line. These strains are usually prevented from replication by largely unknown (for exceptions, see [2]) host control mechanisms and are poorly oncogenic in outbred animal populations. Despite the common absence of overt endogenous particle production, individual virus proteins are often synthesized by various organs of adult animals, notably those involved in hematopoiesis and reproduction [1, 3].

Cells taken into culture can normally be induced to initiate endogenous particle production by adding to the tissue culture medium chemicals like iododeoxyuridine or 5′-azacytidine that interfere with regulation of cellular gene expression [4].

Endogenous retroviruses have been highly conserved during evolution in the sense that strains harboured by related species are much more homologous in nucleic acid and protein structure than when compared to strains from evolutionary more distant species.

The pronounced conservation implies that evolutionary pressure may have helped to preserve the structural integrity of endogenous proviruses. The consistent endogenous virus protein synthesis in certain organs in vivo and the evolutionary conservation may both be taken as suggestive evidence that endogenous retroviruses play a physiological role in normal cellular processes. This notion, however, is disputable, as long as species or at least individual animals [5] exist where endogenous viruses could not yet be detected.

Retroviral Replication Involves Chromosomal Integration

Retroviruses are distinguished from other virus groups by their unusual mode of replication. During cell infection, the plus-strand single-stranded RNA is transcribed into double-stranded DNA which becomes integrated into the host genome (for reviews, see [1, 6–8]). This proviral copy will subsequently be transmitted to all daughter cells and will serve as template for both viral messenger and viral progeny RNA synthesis. The molecular processes leading to virus assembly and involved in characteristic budding at the plasma membrane (Fig. 1) are little understood [1].

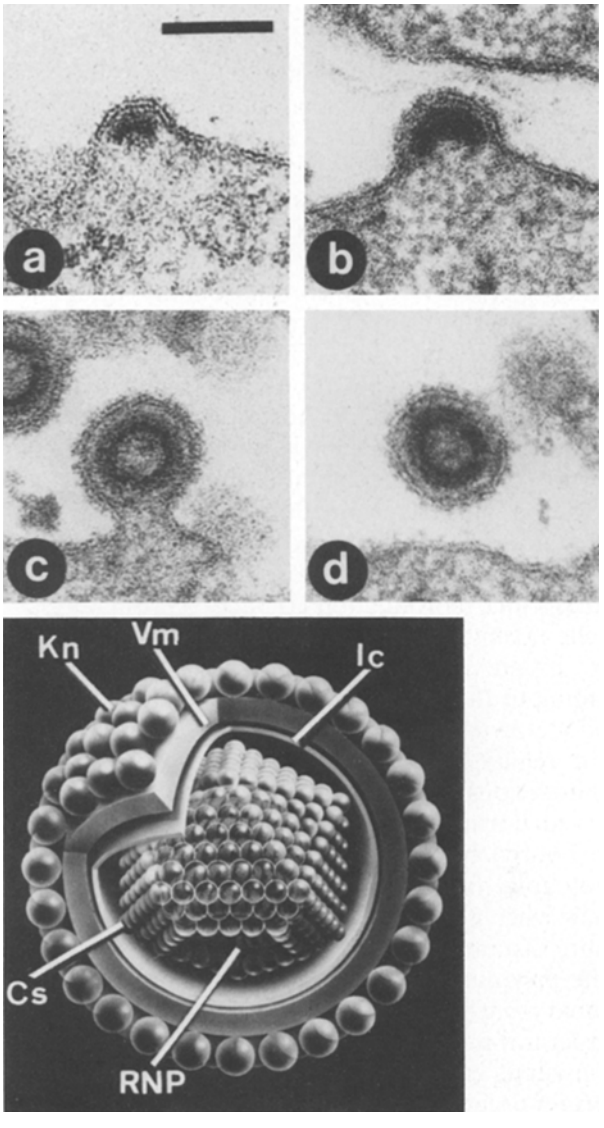

Fig. 1. Electron micrograph of Friend murine leukemia virus and schematic model of retroviruses; a–d) denote the characteristic budding sequence of retroviruses from the plasma membrane of infected cells (bar: 100 nm). Model: *kn* knobs of outer envelope; *Vm* viral membrane; *Ic* inner core; *Cs* core shell; *RNP* ribonucleoprotein. (Photo courtesy of Dr. H. Frank and K. Boller, Tübingen)

The RNA genomes of all replication-competent retroviruses, regardless of their pathogenic potential, or whether they are of endogenous or exogenous origin, contain three genes named *gag*, *pol* and *env*. *Gag*, originally for group-specific *a*ntigens, encodes virus core proteins; *pol* codes for the RNA-dependent DNA-polymerase (reverse transcriptase) and *env* for the virus envelope glycoproteins (Fig. 2B; [1]). All three genes are required for virus replication. The avian Rous sarcoma virus (RSV) strain is an exception in that it contains a fourth gene, *src* (Fig. 2A), responsible for malignant fibroblast transformation [1, 2, 8].

Another large group of exogenous retroviruses are the replication-defective acute leukemia and sarcoma viruses. At first sight, their RNA genome seems to be very simple as it usually encodes only a single protein, which by definition must be the transforming protein of these tumor viruses (Fig. 2C).

Replication-defective retroviruses have been isolated from double-infected tumor cells in association with non-defective strains which provide polymerase activity and structural proteins for progeny formation.

The RNA genomes of both replication-competent and -defective strains possess distinct, unique sequences U_5 and U_3 which flank the coding domains at the 5′ and 3′ ends. U_5 and U_3, in turn, are linked to short terminal repeats, R (Fig. 2).

Retroviruses contain two probably identical RNA subunits packed into each particle. During the course of viral transcription into double-stranded proviral DNA, U_3 and U_5 are duplicated at the 5′ and 3′ ends, respectively, forming two long terminal repeats (LTRs) U_3-R-U_5 (Fig. 3). In contrast to DNA-containing viruses (herpes-, adeno-, papovaviruses; [9]), retrovirus integration into the host genome seems to be a highly ordered event with conservation of the entire coding capacity of the viral genome, suggesting evolutionary forces have been involved in maintaining this integrative mechanism.

Nucleotide sequencing, in vitro transcription and, more recently, DNA transfection experiments strongly suggest that proviral LTRs do not code for proteins but provide regulatory signals fundamental to the expression of eukaryotic genes, namely for promotion, cap formation and polyadenylation of RNA transcripts (for reviews, see [1, 2, 6, 10]). It is not known what factors ensure the preferential use of the left-hand 5′LTR to

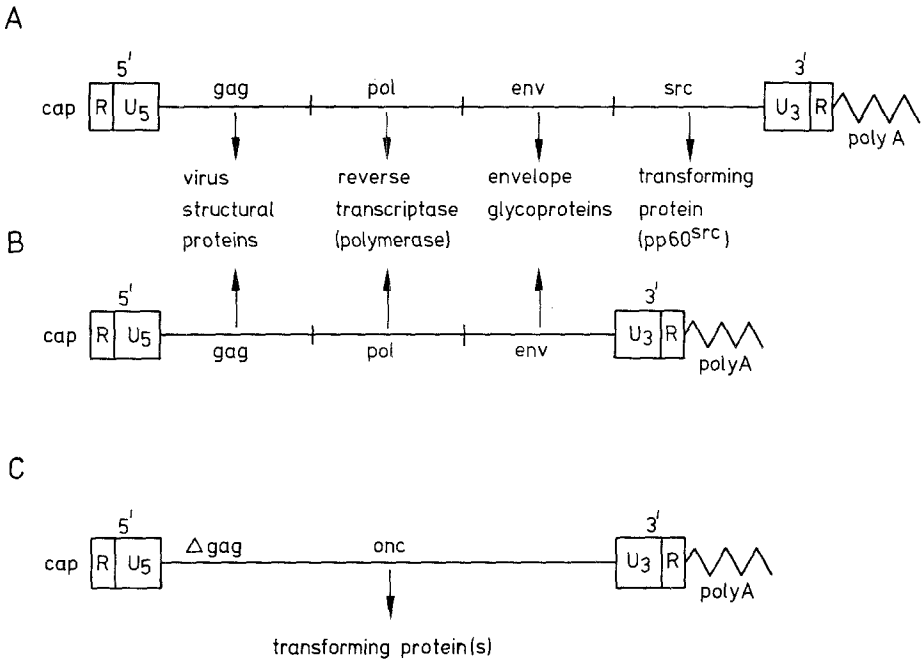

Fig. 2. Schematic representation of retroviral RNA genome structure. A) Replication-competent avian Rous sarcoma virus, the only retrovirus strain that possesses four genes (*gag*, *pol*, *env*, *src*). B) General genome structure of replication-competent retrovirus strains. C) Replication-defective leukemia or sarcoma virus strains: All or parts of the replicative genes are replaced by the *onc* gene. Usually, only segments of *gag* remain linked to *onc*, but the genomes of individual virus strains may also contain segments of *pol* or *env* (Table 1). The coding domain of the RNAs are flanked by two unique sequences U_3 and U_5 and two short terminal repeats, R. A 5′ cap is added to the first encoded nucleotide and a tract of polyadenylic acid is linked to the 3′ end. Scheme is not drawn to scale

promote viral transcription and whether the 3′LTR is normally involved in the transcription of adjacent host cell DNA (Fig. 3). For the consideration of the molecular mechanisms of retrovirus-mediated oncogenesis, it is essential to keep in mind that the proviral DNA introduces regulatory signals that may alter cellular gene expression.

Origins of Retroviral Genes

Early nucleic acid hybridization data suggested that normal cells contain DNA sequences highly related or even identical to retrovirus genes. Today we know that indeed sequences homologous to retrovirus genes are present in normal cellular DNA. Two distinct sets of genes can be distinguished: those that are the cellular equivalents of the viral genes needed for replication (*gag*, *pol* and *env*) and those that are related to viral transforming genes, generally termed *onc* for oncogenes (Fig. 2C) or *src* for sarcoma in case of Rous sarcoma virus (Fig. 2A).

As mentioned before, *gag*, *pol* and *env* can be found as *endogenous* proviruses in the chromosomes of uninfected cells. These proviruses are sim-

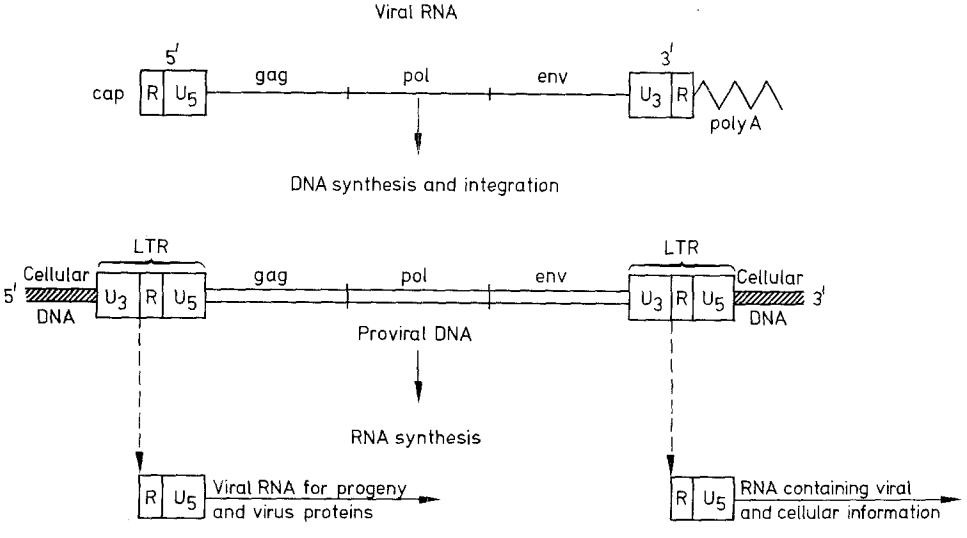

Fig. 3. Schematic representation of retrovirus RNA replication. A sophisticated mode of transcription of the infecting RNA subunits leads to provirus formation and integration with the unique sequences being duplicated at both ends, thus forming long terminal repeats (LTRs; for details, see [1, 6, 48]), which contain all necessary signals for subsequent messenger or progeny viral RNA transcription. Initiation of transcription from the right-hand (3′) LTR is likely to involve adjacent cellular DNA sequences

ilarly structured as their exogenous viral counterparts, i.e. the three replicative genes are linked without interruption by intervening sequences and are flanked by two LTRs (Fig. 3). It appears likely that endogenous retroviruses are the result of infrequent germ-line infections which occurred either prior or subsequent to speciation [1, 3, 11, 12] or even within recent periods of observation [13]. This process could be reproduced in the laboratory by the experimental injection of exogenous murine leukemia viruses into blastula cells of mice, where the viruses became chromosomally integrated. They were subsequently inherited like endogenous virus strains [14].

Like proviruses acquired by horizontal infection or in vitro, endogenous proviruses can be found in many sites in the host genome, even on multiple chromosomes and, therefore, do not seem to prefer specific integration sites. Additional loci containing defective, i.e. deleted proviruses, are also not uncommon. Proviruses behave like stable genetic markers, segregate like cellular genes according to Mendelian laws and are thus uniformly distributed uted among all members of inbred animal populations.

The transforming genes or oncogenes of retroviruses (*v-onc*) are also detectable in normal cellular DNA (*c-onc* or proto-oncogenes). In contrast to the replicative genes, each *c-onc* is normally detected only as single chromosomal locus (or found only in very few copies) not linked to the replicative genes. The structure of *c-oncs* is also different from endogenous provirus genes and reflects the organization of cellular genes: they possess intervening sequences (introns) and no adjacent LTRs. Cellular oncogenes exhibit a very high degree of evolutionary conservation, as related DNA sequences for *all c-oncs* have been detected in *all* vertebrate species, including man. In other words, *c-onc* homologues of a given *v-onc* are not only detectable in the cells of the host species of the corresponding virus strain, but also in the cells of all other vertebrate species.

Generation of Rapidly Transforming Retroviruses

Acute leukemia or sarcoma virus strains are very probably generated by recombination of oncogene-negative retroviruses (Fig. 2B) with cellular oncogenes. With the sole known exception of Rous sarcoma virus (Fig. 2A), such recombination occurs at the expense of variable amounts of virus structural genes, especially of *env* and *pol*, which are replaced either completely or in part by the cellular *onc* sequences (Fig. 2C), yielding replication-defective tumor viruses. The number of cellular oncogenes is unknown but probably limited (less than fifty?), as viruses tend to pick up either the same *c-onc* from a given species or related *c-oncs* from different species (e.g., *fps* and *fes*, Table 1).

Retroviruses may therefore have served to identify cellular transforming sequences which may comprise a family of functionally related genes, comparable to the histocompatibility or immunoglobulin gene families.

Table 1. Examples of cellular oncogenic sequences identified in the genomes of transforming retroviruses. At least seventeen distinguishable oncogenes have been described so far [65]. Examples of the host species from which the oncogenes have apparently been tranduced and of the corresponding viral transforming gene products are given

onc gene	Virus isolates (No.)	Virus strains (examples)	Probable animal origin	Transforming gene products (examples)
src	4	Rous sarcoma	chicken	$pp60^{src}$ [a]
fps	3	Fujinami sarcoma	chicken	$p140^{gag\text{-}fps}$
		PRC II sarcoma	chicken	$p105^{gag\text{-}fps}$
		PRC IV sarcoma	chicken	$p170^{gag\text{-}fps}$
myc	4	Avian myelocytomatosis MC29	chicken	$p110^{gag\text{-}myc}$
		Avian myelocytomatosis OK10	chicken	$p200^{gag\text{-}pol\text{-}myc}$
myb	2	Avian myeloblastosis BAI-A	chicken	n.d.[b]
		Avian leukemia E26	chicken	n.d.
mos	2	Moloney sarcoma	mouse	$p37^{gag\text{-}pol\text{-}env\text{-}mos}$
		Gazdar sarcoma	mouse	n.d.
abl	1	Abelson leukemia	mouse	$p120^{gag\text{-}abl}$
ras	3	Kirsten sarcoma	rat	$p21^{ras}$
		Harvey sarcoma	rat	$p21^{ras}$
		Rasheed sarcoma	rat	$p29^{gag\text{-}ras}$
fes	2	Snyder-Theilen sarcoma	cat	$p85^{gag\text{-}fes}$
		Gardner-Arnstein sarcoma	cat	$p110^{gag\text{-}fes}$
fms	1	McDonough sarcoma	cat	$p170^{gag\text{-}fms}$
sis	1	Simian sarcoma	wolly monkey	n.d.

[a] $pp60^{src}$ = protein of M_r 60000
[b] Not determined or disputed

Viral Oncogenes in Human Cells

Despite intensive investigations, there is virtually no evidence that acutely transforming *onc*-gene containing human retroviruses exist. The question remained whether cellular sequences related to ani-

mal virus transforming genes would be detectable in DNA from normal human cells, as was the case with all tested vertebrate DNA from animal cells. The likely affirmative answer was obtained by Southern blot hybridization analysis employing initially radioactively labelled DNA prepared from purified *v-onc* RNA and subsequently by using labelled viral DNA from molecularly cloned retroviruses [15–17]. All sufficiently purified viral DNA probes detected homologous cellular sequences in normal human DNA. As previously shown with animal species, *c-onc* genes were usually present as single copy or only in very few copies per haploid genome [15–19]. The question then arose to what extent, if any, these genes are expressed. Nick-translated DNA probes of defined *onc*-containing segments from molecularly cloned viral genomes were hybridized to poly(A)-containing RNAs using RNA gel blotting techniques. The data demonstrated that *onc* genes of different transforming retroviruses of animal origin are not only present in normal human DNA, but are also actively transcribed [20, 21]. In animals, preliminary evidence suggests that *c-onc* sequences of individual virus strains may be expressed specifically in certain normal tissues [22–24], but the physiological significance of these tissue-specific expressions are unknown. The data on *c-onc* RNA in normal human tissues are still too limited [20, 21] to justify extensive speculation in this regard.

An attractive working hypothesis suggested that qualitatively or quantitatively altered expression of *c-onc* genes may play a role in the development of non-viral tumors. Furthermore, specific and consistent expression of a given *c-onc* gene in a histologically defined type of tumor would have suggested an etiological role of this gene for this tumor. However, analysis of *c-onc* RNA in spontaneously arising animal and human tumors demonstrated that expression of each tested *c-onc* follows a variable and so far seemingly inconsistent and unpredictable pattern [20, 21]. In other words, the analogues of animal retrovirus *onc* genes are frequently expressed in human tumors and vary in mRNA quantity and size, with yet no recognizable correlation to tumor histology.

These conclusions, however, have to be qualified. Both, the number of human tumor tissues and cell lines investigated so far as well as the number of available cloned viral DNA probes are limited. Furthermore, even with the limited number of presently available viral DNA probes *multiple* expression of several *c-onc* genes in an individual tumor can frequently be observed. It is therefore difficult to determine the individual contribution, if any, of each *c-onc* gene product to the neoplastic process. Finally, attempts to associate specific *c-onc* expression with histologically defined tumors will require precise histopathological classification of tumor biopsies. Studies with tumor cell lines may also not necessarily yield the same pattern of *c-onc* expression as corresponding fresh tumor material.

One probably has to wait for additional data with preferably all known viral oncogenes before a decision can be made whether patterns of *c-onc* expression are associated with certain malignant processes.

Distinct Cellular Oncogenes of Non-viral Nature

A large body of earlier work in chemical carcinogenesis has shown that carcinogens tend to be mutagens [25], implying that damaged DNA may play a role in the establishment of the cancer phenotype. The targets for carcinogenic activation, however, remained undefined as they probably represent only minute elements in the complex DNA of eukaryotic cells. Therefore, mere *structural* analysis of chromosomal DNA offers little prospect for understanding the regulatory events in DNA expression that accompany carcinogenesis.

Functional analysis of tumor cell DNA offers more promise with respect to defining the gene(s) involved in establishing cancer traits. The use of DNA- and RNA-containing tumor viruses has already established the dominant expression of definable segments of their viral genomes initiating transformation. In general, tumor viruses carrying *onc* genes will express this gene and transform the target cell upon infection. There are, however, notable exceptions where the expression of viral transforming genes is under efficient genetic control of the host organism (reviewed in [1, 2]).

The question was whether genes or DNA sequences could be identified that are responsible for the transformed phenotype of non-viral tumors. The functional elimination of a gene leading to reversion of the transformed phenotype represents the direct approach to answer the question, but is of limited value as the corresponding gene would be difficult to identify. An alternative and apparently successful approach was based on the DNA tranfection technique of Graham and van der Eb [26]. DNAs from cell lines from chemically induced or spontaneous animal or human tumors were found to confer oncogenic transformation to NIH 3T3 mouse fibroblast monolayers [27–40]. The DNAs from the recipient transformed

3T3 cells were successfully used in second-cycle transfers to new 3T3 cells, implicating that the oncogenic activity is encoded by a single uninterrupted DNA segment acting dominantly. As the DNA transfection technique leads to extensive DNA breakage and unpredictable uptake of DNA by recipient cells, the very efficient second-cycle transfer of the transformed phenotype would seem impossible if two or more unlinked, cooperating genes would have to be conveyed from donor to recipient cells. This was subsequently confirmed by the demonstration of individual segments of tumor cell DNA in second-cycle-transformed 3T3 recipient cells, using nucleic acid hybridization procedures [40, 41].

The initial successful transfection experiments raised new questions, e.g., as to the number of cellular genes available for oncogenic conversion and their alterations, if any, when compared to their normal allelic counterparts. The number of different activated genes can be estimated by treating transforming DNA with restriction enzymes prior to tranfer. Inactivation of transforming activity will indicate the presence of known enzyme recognition sites. The probability of different genes yielding the same pattern of sensitivity to restriction endonucleases can be estimated by the Poisson distribution from the average cleavage frequency of these enzymes in total cellular DNA in relation to the known (or estimated) size of the transferred gene. Perhaps somewhat surprisingly, DNA from four 3-methylcholanthrene(3-MC)-transformed mouse fibrosarcoma cell lines analyzed in this manner exhibited an identical pattern of sensitivity [29]. The probability of four average-size unrelated genes exhibiting such identical sensitivity is less than 10^{-4}. More recently, the transforming activities of six mouse mammary carcinomas and the one tested human mammary carcinoma cell line showed identical sensitivity patterns to seven restriction enzymes (probability for unrelated genes: 10^{-10} to $4 \cdot 10^{-14}$, depending on assumed gene size; [35]), implying again that a limited number of specifically activated oncogenes mediated mammary carcinomas.

These results led to the hypothesis that carcinogenesis of certain tissues may repeatedly result in activation of identical oncogenes (Fig. 4). The fibroblast oncogene activated by 3-MC is different from the oncogene activated in mammary carcinomas and both are in turn distinguishable from a more recently described oncogene commonly transferred from a number of different human colon carcinomas (cit. from [42]).

In addition, DNAs of 20 human and murine T- and B-cell-derived lymphomas were found to contain rescuable oncogenes representative of specific stages of differentiation within these cell lineages [27]. Common transforming genes were activated in tumors of the same differentiated cell type, whereas different oncogenes were activated in tumors of different pathways of differentiation.

It is known that tumors of a given organ may vary considerably in histopathology. It thus remains to be seen to what extent the histology of individual tumors derived from the same organ can be correlated with the activation of an organ- or differentiation-specific oncogene, which will also have implications for the number of available oncogenes. Regardless of the final number, it is an intriguing puzzle that individual transforming events, occurring in a multistep process over long time periods, result in the activation of tissue-specific oncogenes, even though the tumor cell contains a number of such potentially activatable oncogenes. It is conceivable that the target oncogene already plays a physiological role in a given tissue, whereas the remaining oncogenes may be silent. Thus, a physiologically expressed oncogene may have assumed a unique conformation, i.e. specific configuration of chromatin or DNA demethylation, making it particularly vulnerable for altered regulatory mechanisms for expression.

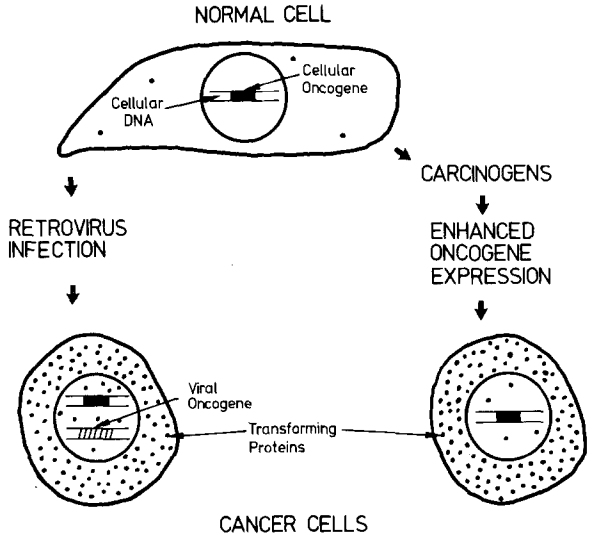

Fig. 4. Dosage hypothesis: Potentially transforming proteins are synthesized in normal cells where they may perform a physiological function. Retrovirus infection leads to additional oncogene integration, whereby the gene is under the control of an efficient viral promoter, leading to overproduction of the transforming protein and cancerous growth. The same result is seen after carcinogen-mediated augmentation of cellular oncogene expression. Carcinogens (chemicals, radiation, viruses without transforming genes, etc.) may act by altering the regulatory control of cellular oncogene expression

The detection of cellular oncogenes in non-viral tumors represents an intellectually fascinating advent in tumor research. One has to remember, however, that all successful transfer experiments published so far were performed using the same recipient semi-normal NIH 3T3 mouse fibroblast cell line. It is not known why other normal cell lines can by far not as easily be transfected. Use of a single recipient cell line may limit the number of detectable oncogenes, as it is unknown whether recipient cell lines can exert control over the expression of transferred genes. It is thus not surprising that virtually all laboratories involved in transfer experiments have reported that a sizeable number of tumors were resistant to this type of transfer analysis, i.e. NIH 3T3 cells could not be transformed successfully with the corresponding tumor DNAs.

For the better understanding of the tranfection assays, one should resort to virus-induced tumors. For example, individual retrovirus strains are known to have a consistent pattern of pathogenicity, i.e. infect and transform in vivo cells from defined target organs. Use of such tumors will reveal whether the chromosomally integrated proviral DNA copy of the infecting virus can be demonstrated to be the transfected oncogene in the recipient NIH 3T3 cell. Use of available virus mutants temperature-sensitive for malignant cell transformation will further refine the assay, as the temperature-sensitive trait should be exhibited in the recipient cell.

Transfer of Oncogenes from Normal Cells

Positive transfection was initially obtained with DNA from non-viral tumors, into which no new genetic information was introduced from outside. The question then arose whether even DNA from normal cells contains oncogenic sequences that can be defined by transfection. The limited data available [28, 30] suggest that DNA fragments from normal cells can transform, albeit with an efficiency (per μg transferred DNA) which is much lower than seen with DNA from tumor cells. The presumable normal cell genes with potential transforming activity can subsequently be transmitted at high efficiency in secondary transfection assays, which indicates that, once isolated, oncogenes from normal cells possess comparable transforming potential as their activated counterparts in tumor cells, provided they have escaped physiological control mechanisms (Fig. 5).

Additional support for the presence of potentially transforming cellular oncogenes in normal cells

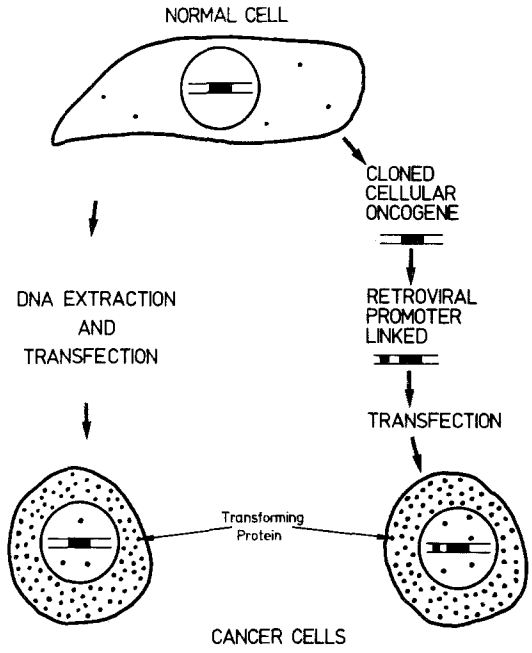

Fig. 5. Demonstration of oncogenes in normal cells by either transfection or recombinant DNA technology. Transferred cellular oncogenes may have escaped accurate transcriptional control, resulting in enhanced synthesis of transforming proteins. This mechanism was directly proven by molecular cloning of cellular oncogenes, providing them with an active retroviral promoter and transferring both to recipient cells [28, 43–45]

was obtained employing recombinant DNA technology [30, 43–45]. Cellular oncogenes cloned from normal mouse or rat cell DNA were shown to be oncogenic in transfection assays, especially after prior in vitro linkage to retroviral promoters which enhanced transcriptional activity (Fig. 5).

Two Classes of Oncogenes

Two classes of dominant transforming genes have now been defined in tumor cells. One class is represented by cellular oncogenes (*c-onc*) which have been appropriated from the cellular genome by retroviruses and used to form chimeric viral-host genomes (*v-onc*). The other class comprises cellular oncogenes demonstrable by transfection assays. Most strikingly, both classes represent genes originating as benign genetic elements likely to be involved in cellular processes concerning growth control and differentiation. Are these two classes structurally and functionally related or are they distinct sets of genetic elements? This question can now be approached by using virus-derived complementary DNA *onc* probes to survey the DNAs of cells which have acquired, via transfection, copies of cellular oncogenes. Conversely, transfected cellular oncogenes can now be molecularly cloned

[33, 34] and be used for hybridization with *v-onc* DNA probes. First results are already at hand. A human bladder carcinoma oncogene has been found to be homologous to the Harvey murine sarcoma virus oncogene (termed *ras*, Table 1; [32]). This gene was found to be activated also in other human bladder carcinoma cell lines [33]. Furthermore, four cellular genes sharing homology to *v-ras* have recently been cloned from normal *human* DNA [30]. One of these cloned genes was ligated to a long terminal repeat from a murine or feline retrovirus to enhance transcription and was found to induce transformation of NIH 3T3 cells with concomitant synthesis of high levels of a p21 protein similar to that specified by the rat gene from where it was originally transduced by Harvey sarcoma virus.

There is no need to postulate that all cellular oncogenes possess viral counterparts. The intensive search for new retrovirus strains has resulted in only a limited number of different *v-onc* genes transduced from cellular genes. Most likely, cellular oncogenes will be found that have never been appropriated by infecting retroviruses. The availability of molecularly cloned *v-oncs* and *c-oncs* from various species and from normal and malignant, histologically defined tissues will allow future detailed analysis of their fine structure, possibly enabling the identification of new genetic elements involved in controlling expression of oncogenes.

Oncogenesis by Downstream Promotion

It has long been an intriguing observation that a group of lymphoma and leukosis retrovirus strains carry only replicative genes (*gag*, *pol* and *env* in Fig. 2B) and no *onc* gene but are still oncogenic. Much of our understanding of the transforming events initiated by these viruses came from studies with avian leukosis virus-induced tumors, which are usually lymphomas but may also include sarcomas, nephroblastomas and erythroblastosis. ALV-induced lymphomas arise after typically long latency periods and are composed of clonal cell populations carrying an integrated ALV provirus. In many cases, the provirus has been partially deleted preventing viral replication or even suppressing the expression of all three virus genes.

It remained an enigma how unexpressed (and anyway only replicative) genes could mediate oncogenesis.

However, all clonal tumor cell populations possessed at least one viral LTR detectable by nucleic acid hybridization. As LTRs carry all necessary signals for initiation of RNA transcription, the predicted transcripts from the 3′-LTRs should contain viral R-U_5 linked to cellular genetic information (Fig. 3). These transcripts were indeed detected by hybridization to a viral U_5 probe in both ALV-induced lymphomas [66] and RSV-transformed mammalian cells [67]. The majority of truncated ALV proviruses were inserted within or adjacent to *c-myc*, the cellular homolog of the transforming gene (*v-myc*) of several acutely transforming retroviruses (Table 1). In these tumor cells, elevated levels of *c-myc* RNA were also demonstrable [66], implying that integration of viral LTR may lead to downstream promotion of adjacent *c-myc*, a mechanism also termed "oncogenesis by promoter insertion". However, the number, structure and function of LTR-activated *c-onc* genes remain to be defined and it cannot be excluded at present that long-distance effects of integrated LTRs may (also) be required for cellular oncogene activation.

The latter hypothesis is somewhat supported by transfection experiments involving DNA from ALV-lymphomas [39]. Surprisingly, neither viral nor cellular *myc* sequences and not even viral LTRs could be demonstrated in the transformed recipient NIH 3T3 cell clones. It thus appears possible that tumor induction by slowly transforming leukosis and lymphoma viruses requires two or several steps: the initial activation of a *v-onc* homolog and the activation of additional cellular oncogenes.

Upstream provision of transcriptional signals does not seem to be the only possible mechanism required for the activation of *c-onc* and possibly other cellular oncogenes. The situation around the activated *c-myc* locus became more difficult to interpret when additional ALV-induced lymphomas were investigated [46]. Tumors were detected with only one copy of viral LTRs positioned *downstream* of *c-myc* or arranged in the transcriptional orientation opposite to that of *c-myc* on the 5′ side. The molecular mechanisms by which LTRs may enhance gene expression other than by providing a strong promoter are still unclear [6, 46].

Retroviruses as Transposable Elements

The elucidation of the genetic structure of retroviruses has triggered speculations as to the similarity between proviruses and transposable elements [1, 6, 47]. Proviruses can be inserted at many sites in the host genome, are terminally redundant (the LTRs) and bear short inverted repeats flanking

the LTRs (not shown in Fig. 3) which in turn are flanked by host sequences duplicated during virus integration.
Proviruses act as insertion mutagens, excise by recombination between terminal repeats, may undergo deletion mutation and acquire genes, not only *c-onc* genes, from host genomes. Most of these characteristics are shared with transposable elements of maize, bacteria, yeast and *Drosophila*. It would go beyond the scope of this review to discuss these similarities in more detail (for reviews, see [1, 6]). However, there is as yet no strict evidence that transpositions as seen with bacterial elements also occur in vertebrate cells. The "transpositions" observed with integrated proviruses even under conditions when superinfection from outside is prevented are probably due to synthesis and integration of new viral DNA from an RNA template [47].

Mechanisms of Malignant Cell Conversion: Transforming Proteins

A situation in which a causal therapy of cancer becomes a real possibility requires the understanding of the molecular mechanisms involved in the establishment of neoplastic cells. As chemical carcinogenesis is even more obscured by the complexity of eukaryotic gene expression, viral carcinogenesis may slowly give insight into the early steps in neoplastic conversion.
Detailed investigation with, for example, the replication-competent (and therefore easy to propagate) Rous sarcoma virus have revealed during the past decade that a functional transforming gene (the *src* gene in Fig. 2A) is a prerequisite for the conversion of fibroblasts into fibrosarcoma cells. Furthermore, the use of transformation-defective deletion mutants or of virus mutants which were temperature-sensitive for transformation has shown that the continuous expression of a functional transforming gene is required for the maintenance of the transformed state of the cell. These observations were quickly extended to other avian and mammalian retroviruses that contain *v-onc* genes and that rapidly induce tumors in vivo and transform tissue culture cells in vitro (for access to the detailed literature, see [1, 7, 48]).

Functions of Transforming Proteins

Knowing about the central role of functional transforming genes, the identification of the corresponding transforming gene products moved into the center of tumor virologists' interest. In a series of elegant experiments, Ray Erikson and his coworkers at the University of Colorado were the first to identify a transforming protein of an RNA tumor virus, namely the *src* gene product of RSV [49]. It is a M_R 60000 phosphoprotein named $pp60^{src}$ which was initially demonstrable by immunoprecipitation from RSV-transformed cells employing antisera from tumor-bearing animals and by in vitro translation of RSV RNA [50, 51]. Comparable experimental approaches with retroviruses and cells from other species subsequently revealed some common features for *v-onc* gene products: The majority of these transforming proteins seem to be phosphorylated and some possess a cAMP-independent ATP: protein phosphotransferase activity. In other words, transforming proteins with protein kinase activity transfer (e.g., radioactively labeled) phosphate groups from ATP to exogenous, phosphate-accepting substrates, e.g. casein or phosvitin. This reaction was initially detected by Erikson et al. when they added ^{32}P-γ-ATP to immunoprecipitates of $pp60^{src}$ which resulted in the phosphorylation of the precipitating antibody [52].
A number of transforming virus proteins with associated kinase activity preferably phosphorylate proteins in tyrosine. In contrast, the ubiquitous *cellular* protein kinases, regardless of whether or not they are cyclic-nucleotide-dependent, transfer phosphate to serine- or threonine residues.
The protein kinase activity is probably not simply due to a virus-associated contaminating, co-purifying cellular enzyme, but probably represents an intrinsic property of the transforming proteins. This has at least been proven for the Rous sarcoma virus $pp60^{src}$ and the Abelson murine leukemia virus p120 (Table 1). The *v-onc* genes were cloned in plasmids and expressed in *E. coli* [53, 19]. Bacterially produced, transforming proteins had still the capacity to phosphorylate in tyrosine residues, suggesting that they encode protein kinases.
The above-mentioned in vitro phosphorylation of precipitating antibody is, of course, a highly artificial (but easy to perform) assay revealing nothing about corresponding in vivo phosphorylation reactions that may be instrumental in the initiation of malignant cell transformation. The search for the cellular targets of the transforming proteins has met with limited success so far. Investigations involving immunofluorescent and immunoelectronmicroscopical techniques revealed sub- and/or intramembranous localization of $pp60^{src}$. In case of the transforming gene product of Abelson murine leukemia virus a portion of the protein

seems to extend through the plasma membrane to the cell surface. These results might be interpreted that transforming proteins exert their functions by phosphorylating cytoskeletal or other proteins of the plasma membrane [55, 56]. On the other hand, the avian myelocytomatosis virus MC 29 coded p110 was recently shown to accumulate in the cell nucleus and was able to bind to double-stranded DNA, suggesting a role in, e.g., DNA replication or alteration in cellular gene expression [57]. These data are not necessarily mutually exclusive. Not only, because we still know much too little about the functions of virus-encoded transforming proteins, but also because several independent mechanisms leading to malignant cell conversion can be envisaged (see also below). It is probably wise to wait for the purification, intracellular localization and structural analysis of the known *v-onc* products before generalizations in this respect can be made [55, 58, 59].

Immunoprecipitation analysis employing sera from tumor-bearing animals have revealed two cellular proteins apparently bound to and co-precipitating with transforming proteins [59, 60]. In experiments employing cells infected with temperature-sensitive virus mutants it was observed that up to approximately ten cellular proteins became rapidly (within minutes) phosphorylated in tyrosine upon shift of the cell cultures to incubation temperatures permissive for transformation [58]. Most of these cellular proteins remain to be characterized, but among them vinculin [56] and a M_R 38000 protein associated with malate dehydrogenase activity [61] have already been identified.

In spite of the first candidate target proteins for the action of transforming proteins, the question remains how the multitude of phenotypic alterations characteristic for tumor cells, for example those concerning morphology and growth behaviour, can be caused by the action of a single transforming protein. A presently easily acceptable hypothesis would predict pleiotropic functions for transforming proteins that initiate a cascade of metabolic and structural alterations which in turn may alter additional cellular regulatory mechanisms to yield the complex phenotypic changes seen in tumor cells.

Dosage Hypothesis: Malignant Conversion May Depend on the Intracellular Concentration of Transforming Proteins

As mentioned above, *v-onc* represent cellular sequences apparently acquired by recombination from cellular DNA. The corresponding *c-onc* genes have also been found to be expressed, i.e. transcribed and translated in uninfected target cells, albeit in very low amounts [22, 23, 62–64].

Retrovirus transformation leads to an up to 100-fold increase in the intracellular concentration of the transforming proteins. It has, therefore, been argued that retrovirus transformation may be a consequence of overproduction of a cellular protein required for normal cellular function (Fig. 4). The remarkable target cell specificity of individual retrovirus strains in vivo may be taken as suggestive evidence that the deleterious effect of dosage is restricted to those cells in which the cellular oncogene is expressed and performs an essential function. It has also been proposed that transforming proteins are differentiation proteins characteristic for the developmental stage of the cell [54]. Overproduction of such proteins was assumed to prevent further differentiation and thereby to arrest the cells at an early immature and replication-competent stage.

One has to keep in mind, however, that transforming proteins encoded by *v-onc* and by *c-onc* are usually not structurally identical. The *v-onc* gene products contain strain-dependent, variable amounts of replicative gene products (Table 1), usually segments of *gag* and, more rarely, of *pol* or *env* (Fig. 2C). On the other hand, corresponding *c-onc*-derived proteins have been found to contain unique cellular peptides in addition to the common *onc*-derived peptides. These structural differences may result in functionally distinct properties or different substrate specificities for *v-* resp. *c-onc* gene products, including their capacity to induce malignant cell transformation.

The transforming protein of RSV, $pp60^{src}$, is not linked to virus structural proteins as it is derived from the distinct *src* gene (Fig. 2A). The corresponding *c-onc* gene also encodes a 60000 M_R phosphorylated protein, designated $pp60^{c\text{-}src}$. Both products have been found to be structurally virtually identical and to be able to phosphorylate in tyrosine residues, suggesting that at least in the case of RSV both viral and cellular *onc* gene products are structurally and functionally indistinguishable.

Retroviruses of Human Origin

It would go beyond the scope of this article to discuss recent developments concerning the identification of human retroviruses (for reviews, see [8, 68]). Suffice it to mention that retrovirus-like

particles can regularly be observed budding from human and animal placental trophoblasts [69] and from human teratocarcinoma cells cultured in vitro [70]. More recently, Gallo and co-workers [71] and subsequently Japanese colleagues [72] detected unique retroviruses in malignant cells of a new disease entity classified as adult T-cell leukemia/lymphoma (ATLL; [73]). Presently available data suggest that the human T-cell lymphoma viruses (HTLV), although they do not possess a *v-onc* gene, may nevertheless play a causative role in the development of ATLL.

A Common Pathway in Malignant Cell Transformation

Retroviruses have served to identify oncogenes (Fig. 6). These genes are normal, benign genetic elements probably indispensable for yet unknown functions in cellular growth control or differentiation. Increased expression of oncogenes may occur in a variety of different mechanisms. Retroviruses may transform not only by introducing additional viral oncogenes into infected cells but also by providing regulatory signals that enhance expression of cellular oncogenes. Chemical or physical carcinogens may act similarly by disturbing control mechanisms that regulate the physiological expression of cellular oncogenes. Thus, quite different transforming events developing over long time periods may finally lead to the activation of a common molecular pathway that transforms a normal cell into its malignant counterpart.

If the molecular mechanisms that establish cancerous cells are qualitatively not different in kind from mechanisms at work in normal cells, design of rational therapeutic regiments to interfere with these mechanisms will remain a formidable task.

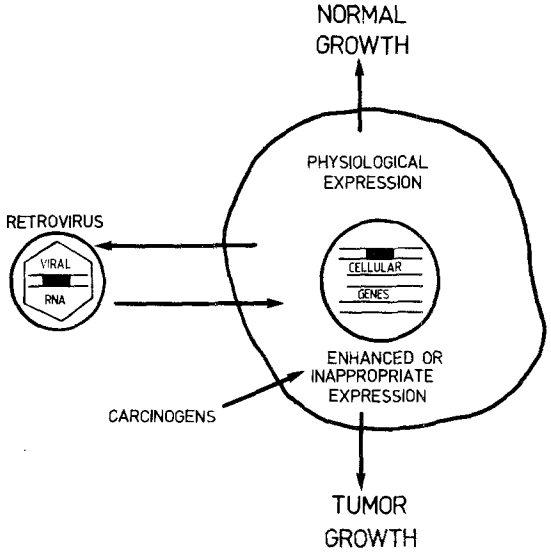

Fig. 6. Cancer gene concept. A family of cellular genes essential for normal growth and development can mediate carcinogenesis either when transduced by retroviruses or when augmented in expression by carcinogens that affect cellular gene control mechanisms (modified from [74])

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1. Weiss, R.A., et al. (eds.): The Molecular Biology of Tumor Viruses, Part III. Cold Spring Harbor Laboratory 1982
2. Lilly, F., Mayer, A., in: Viral Oncology, p. 89 (G. Klein, ed.). New York: Raven 1980
3. Todaro, G.J., et al.: Proc. Roy. Soc. London *210*, 367 (1980)
4. Rowe, W.P., et al.: Virology *46*, 866 (1971)
5. Astrin, S., Buss, E.G., Hayward, W.S.: Nature *282*, 339 (1979)
6. Varmus, H.E.: Science *216*, 812 (1982)
7. Bishop, J.M.: Ann. Rev. Biochem. *47*, 35 (1978)
8. Kurth, R.: Behring Inst. Mitt. *69*, 65 (1980)
9. Weinberg, R.A.: Ann. Rev. Biochem. *49*, 197 (1980)
10. Temin, H.M.: Cell *21*, 599 (1980)
11. Todaro, G.J., Huebner, R.J.: Proc. Nat. Acad. Sci. USA *69*, 1009 (1972)
12. Weinberg, R.A.: Cell *22*, 643 (1980)
13. Rowe, W.P., Kozak, C.A.: Proc. Nat. Acad. Sci. USA *79*, 4871 (1980)
14. Jaenisch, R.: ibid. *73*, 1260 (1976)
15. Duesberg, P.H.: Cold Spring Harbor Symp. Quant. Biol. *44*, 13 (1980)
16. Dalla-Favera, R., et al.: Nature *292*, 31 (1981)
17. Wong-Staal, F., et al.: Science *213*, 226 (1981)
18. Spector, D.H., Varmus, H.E., Bishop, J.M.: Proc. Nat. Acad. Sci. USA *75*, 410 (1978)
19. Goff, S.E., et al.: Cell *22*, 777 (1980)
20. Eva, A., et al.: Nature *295*, 116 (1982)
21. Westin, E.H., et al.: Proc. Nat. Acad. Sci. USA *79*, 2490 (1982)
22. Shibuya, M., Hanafusa, H., Balduzzi, P.C.: J. Virol. *42*, 143 (1982)
23. Chen, J.H.: ibid. *36*, 162 (1980)
24. Witte, O.N., Rosenberg, N.E., Baltimore, D.: Nature *281*, 396 (1979)
25. Mc Cann, J., Ames, B.N.: Proc. Nat. Acad. Sci. USA *73*, 950 (1976)
26. Graham, F.L., van der Eb, A.J.: Virology *52*, 456 (1973)
27. Lane, M.-A., Sainten, A., Cooper, G.M.: Cell *28*, 873 (1982)
28. Cooper, G.M., Okenquist, S., Silverman, L.: Nature *284*, 418 (1980)
29. Shilo, B., Weinberg, R.A.: ibid. *289*, 607 (1981)
30. Chang, E.H., et al.: ibid. *297*, 479 (1982)
31. Murray, M.J., et al.: Cell *25*, 355 (1981)
32. Parada, L.F., et al.: Nature *297*, 474 (1982)
33. Goldfarb, M., et al.: ibid. *296*, 404 (1982)

34. Pulciani, S., et al.: Proc. Nat. Acad. Sci. USA *79*, 2845 (1982)
35. Lane, M.-A., Sainten, A., Cooper, G.M.: ibid. *78*, 5185 (1981)
36. Hopkins, N., et al.: ibid. *78*, 7555 (1981)
37. Padhy, L.C., et al.: Cell *28*, 865 (1982)
38. Krontiris, T.G., Cooper, G.M.: Proc. Nat. Acad. Sci. USA *78* 1181 (1981)
39. Cooper, G.M., Neiman, P.E.: Nature *287*, 656 (1980)
40. Shih, C., et al.: ibid. *78*, 5714 (1981)
41. Krontiris, T.G., Cooper, G.M.: ibid. *78*, 5185 (1981)
42. Weinberg, R.A.: Trends Biol. Sci., April 1982, p. 135
43. Blair, D.G., et al.: Science *212*, 941 (1981)
44. Oskarsson, M., et al.: ibid. *207*, 1222 (1980)
45. De Feo, D., et al.: Proc. Nat. Acad. Sci. USA *78*, 3328 (1981)
46. Payne, G.S., Bishop, J.M., Varmus, H.E.: Nature *295*, 209 (1982)
47. Varmus, H.E., Shank, P.R.: J. Virol. *18*, 567 (1976)
48. Klein, G. (ed.): Viral Oncology. New York: Raven 1980
49. Brugge, J.S., Erikson, R.L.: Nature *269*, 346 (1977)
50. Purchio, A.F., Erikson, E., Erikson, R.L.: Proc. Nat. Acad. Sci. USA *74*, 4661 (1977)
51. Purchio, A.F., et al.: ibid. *75*, 1567 (1978)
52. Collett, M.S., Erikson, R.L.: ibid. *75*, 2021 (1978)
53. Gilmer, T.M., Erikson, R.L.: Nature *294*, 771 (1981)
54. Graf, T., Beug, H.: Biochim. Biophys. Acta *516*, 269 (1978)
55. Hynes, R.O.: Cell *21*, 601 (1980)
56. David-Pfenty, T., Singer, S.J.: Proc. Nat. Acad. Sci. USA *77*, 6687 (1980)
57. Donner, P., Greiser-Wilke, I., Moelling, K.: Nature *296*, 262 (1982)
58. Hunter, T.: Cell *22*, 647 (1980)
59. Kurth, R., et al.: Adv. Cycl. Nucl. Res. *14*, 443 (1981)
60. Brugge, J.S., Darrow, D.: Nature *295*, 250 (1982)
61. Rübsamen, H., et al.: Proc. Nat. Acad. Sci. USA *79*, 228 (1982)
62. Collett, M.S., et al.: ibid. *76*, 3159 (1979)
63. Huesgen, A., et al.: J. Gen. Virol. *48*, 401 (1980)
64. Oppermann, H., et al.: Proc. Nat. Acad. Sci. USA *76*, 1804 (1979)
65. Coffin, J.M., et al.: J. Virol. *40*, 953 (1981)
66. Hayward, W.S., Neel, B.G., Astrin, S.M.: Nature *290*, 475 (1980)
67. Quintrell, N., et al.: J. Mol. Biol. *42*, 363 (1980)
68. Pimentel, E.: Biochim. Biophys. Acta *560*, 169 (1979); Hehlmann, R.: Curr. Top. Microbiol. Immunol. *73*, 141 (1976)
69. Dalton, A.J., et al.: J. Nat. Cancer Inst. *52*, 1379 (1974); Immamura, M., Phillips, P.E., Mellors, R.C.: Am. J. Pathol. *83*, 383 (1976)
70. Bronson, D.L., et al.: J. Nat. Cancer Inst. *60*, 1305 (1978); Bronson, D.L., et al.: ibid. *63*, 337 (1979); Kurth, R., et al.: Cold Spring Harbor Conf. Cell Prolif. *7*, 835 (1980); Löwer, J., et al.: Haematol. Blood Transfus. *26*, 541 (1981); Kurth, R., et al.: Proc. Nat. Acad. Sci. USA *74*, 1237 (1977); Kurth, R., Mikschy, U.: ibid. *75*, 5692 (1978); Barbacid, M., Bolognesi, D.P., Aaronson, S.A.: ibid. *77*, 1617 (1980); Snyder, H.W. Jr., Fleissner, E.: ibid. *77*, 1622 (1980); Löwer, J., et al.: Virology *109*, 409 (1981); Löwer, R., et al.: submitted; Boller, K., et al.: submitted
71. Morgan, D.A., Ruscetti, F.W., Gallo, R.C.: Science *193*, 1007 (1976); Ruscetti, F.W., Gallo, R.C.: Blood *57*, 379 (1981); Poiesz, B.J., et al.: Proc. Nat. Acad. Sci. USA *77*, 7415 (1980); Poiesz, B.J., et al.: Nature *294*, 268 (1981); Catovsky, D., et al.: Lancet *1982*-I, 639; Rho, H.M., et al.: Virology *112*, 355 (1981); Kalyanaraman, V.S., et al.: J. Virol. *38*, 906 (1981); Reitz, M.S. Jr., et al.: Proc. Nat. Acad. Sci. USA *78*, 1887 (1981); Popovic, M., et al.: Nature (submitted); Oroszlan, S., et al.: Proc. Nat. Acad. Sci. USA *79*, 1291 (1982); Gallo, R.C., et al.: ibid. *79* (1982); Posner, E.E., et al.: J. Exp. Med. *154*, 333 (1981); Kalyanaraman, V.S., et al.: Nature *294*, 271 (1981); Robert-Guroff, M., et al.: Science *215*, 975 (1982)
72. Hinuma, Y., et al.: Proc. Nat. Acad. Sci. USA *78*, 6476 (1981); Miyoshi, I., et al.: Nature *294*, 770 (1981); Yoshida, M., Miyoshi, I., Hinuma, Y.: Proc. Nat. Acad. Sci. USA *79*, 2031 (1982); Hinuma, Y., et al.: Int. J. Cancer *29*, 631 (1982)
73. Lukes, R.J., Collins, R.D.: Cancer Treat. Rep. *61*, 971 (1977); Lennert, K., Stein, H.: Recent Res. Cancer Res. *64*, 31 (1978); Uchiyama, T., et al.: Blood *50*, 481 (1977); Takatsuki, K., et al.: Jpn. J. Clin. Oncol. *9*, 317 (1979); Matsumoto, M., et al.: ibid. *9*, 325 (1979)
74. Bishop, J.M.: Sci. Am. *246*, 69 (1982)

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