N.A.S. SYMPOSIUM: NEW EVIDENCE AS THE BASIS FOR INCREASED EFFORTS IN CANCER RESEARCH

The Viral Oncogene Hypothesis: New Evidence

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ABSTRACT Recent studies, primarily with mouse, rat, and chicken cells, have provided evidence to support the concept that vertebrates contain the genetic information for producing a type-C RNA tumor virus in an unexpressed form in their somatic cells as well as in their germ cells. This information, which our associates and we postulated has been part of the genetic makeup of vertebrates since early in evolution, can persist for hundreds of generations in cell culture without overt production of virus. It is proposed that the endogenous virogenes (the genes for the production of type-C viruses) and the oncogenes (that portion of the virogene responsible for transforming a normal cell into a tumor cell) are maintained in an unexpressed form by repressors in normal cells. Various agents, including radiation, chemical carcinogens, and, perhaps, exogenously added viruses, may transform cells by "switching on" the endogenous oncogenic information. Some other implications of the viral oncogene theory are presented.

In 1969 it was proposed that the cells of most or all vertebrate species contain genomes of type-C RNA tumor viruses that are vertically transmitted from parent to offspring (1, 2). Depending on the host genotype and various modifying environmental factors, either virus production or tumor formation, or both, may develop at some time during the life of these animals or in their cells when grown in culture. The evidence for this concept was derived both from cell culture experiments and from various seroepidemiologic studies and was presented as a unifying concept that would be consistent with the facts as they were then known.

In the 2 years that have followed, a great deal more evidence has accumulated to provide support for the hypothesis. One particular prediction that was made was that the genetic information for making an RNA tumor virus, being present in a repressed form in all cells, would potentially be inducible by carcinogenic and/or mutagenic agents (3). Recent evidence from single-cell clones of mouse embryo cells of both the high susceptible strain, AKR (4, 5), and the low susceptible strains, BALB/c, and random-bred Swiss (6), as well as from clones of rat (7, 8) and Chinese hamster cells (Todaro, G. J. *et al.*,

manuscript in preparation), indicates that every rodent-cell clone in culture does contain the information for producing a type-C virus. Infectious virus can be induced from normal as well as transformed cells. A model involving protein repressors of the oncogene and of the virus genetic information is proposed.

The oncogene hypothesis was almost an inescapable conclusion, given the data available from animal, cell culture, and molecular biology studies. A special aspect of the oncogene theory is that it provides a unifying concept to explain a diversity of phenomena. Rather than the effect of agents such as radiation, chemical carcinogens, and the normal aging process being exclusively random and unpredictable, the hypothesis suggested that these agents exert their oncogenic action directly on the oncogenic information (the oncogene) present in all cells and that cancer results from the destruction of the normal repressor systems that keep both the oncogenic and virogenic information in check in the normal adult cell. Since type-C viruses carry oncogenic information as part of their virus genetic information, the most reasonable assumption was that the oncogene is a portion of the virogene (the endogenous genetic information for making a type-C virus) or, alternatively, that the virogene is capable of picking up the oncogenic information with high frequency.

"Repressors" of oncogene activity

In Fig. 1 we show one possible model for the regulation of oncogenic and virus genetic information. This model predicts the existence of repressor proteins in normal cells. A search for repressor proteins that will specifically bind to the viral nucleic acid may help to resolve this question. While this is the simplest model consistent with the present data, control of gene expression and tumor development can operate at various different levels and can be considerably more complex. The point to be emphasized is that while the oncogene is only one portion of the virogene, it is the portion responsible for transforming a normal cell into a tumor cell, presumably because it has the genetic information for making the transforming protein(s), genes that ordinarily are not expressed because

Abbreviation: gs, group specific.

FIG. 1. Model for control of oncogenes and virogenes.

of repressor(s). The repressor(s), we envision, can act either coordinately to control the expression of the whole virogene or independently to allow partial expressions [such as group-specific (gs) antigens] without the production of an intact extracellular particle (1, 2). Indeed, specific host genetic regulation of expressions of gs antigen has been demonstrated in inbred chickens and mice by Payne and Chubb (41) and Taylor *et al.* (56). *In vivo*, those cells that are "spontaneously" transformed (where the oncogene information is functioning) would have a selective advantage and could give rise to tumors. Partial expressions such as the production of gs antigen or even infectious virus with its reverse transcriptase do not in themselves confer oncogenic potential. However, the cell that already contains partial or complete virogene expressions appears to be more susceptible to activation of the endogenous oncogenic information.

It should be emphasized that the basic hypothesis (9, 10) certainly is not altogether new, since vertical transmission of latent RNA tumor viruses has been invoked to explain the transmissible murine leukemia, sarcomas, and lymphomas that were induced by radiation, chemicals, and hormones (11-15). The hypothesis, however, is significantly new and different in specifying that the RNA virus genome responsible for producing the gs antigen, infectious virus, and the tumorigenic functions is part of the inherited genetic material, and is presumably in the DNA of all cells of normal vertebrates. Thus, while certain fully infectious RNA viruses such as the Rous sarcoma virus and various murine leukemia prototypes can be specifically manipulated by virologists to produce specific cancers, it is obvious that horizontal transmissions of such viruses do not explain the natural prevalences of neoplasia in any species, nor can such transmissions explain spontaneous and chemically induced tumors. Evidence presented by Bentvelzen (16) suggests that the genome of mouse mammary tumor virus (type-B), which has been most studied as an infectious (horizontally transmitted) "milk agent," can behave in a similar fashion to the type-C virus genome in being vertically transmitted and genetically controlled in many strains of mice.

Type-C RNA viruses have been isolated from cats, rats, hamsters, and snakes, in addition to mice and chickens (2). There are also reports of type-C viruses isolated from cows (17), pigs (18), monkeys (19), and, perhaps, from man (20, 21). Thus, type-C viruses are known to be expressed naturally in three classes of vertebrates and are found in most mammals, including primates. While type-C viruses in humans have not been unequivocally demonstrated, the fact is that in well-studied animals such as chickens, mice, and cats, these viruses are known to be etiologic agents of naturally occurring cancers; this is perhaps the most important single reason for expecting that type-C virus genome expressions will also be involved in at least certain human cancers.

Virus expression in early embryos

The RNA tumor viruses are different in fundamental respects from classical infectious viruses. In natural circumstances they are transmitted from cell to cell by cell division; thus, since there is no need to become extracellular, we propose that they can be transmitted for hundreds and thousands of generations both in culture and in the animal without overt virus expression. One prediction of the original hypothesis that has since been borne out was that there should be expression of type-C virus genetic information in early embryos. This turned out to be the case in chick embryos (22) and in mouse strains with low incidence of leukemia such as BALB/c, C₃H/Bi, NIH Swiss, and C57BL (see Table 1), as well as in wild (feral) mice (23); all have budding virus particles as well as virus-specific gs antigens detectable in early embryonic life (24). Thus, expressions of RNA tumor viruses in embryos are well regulated, but true oncogenic expression does not occur. Cancer, then, could be viewed as developing from those occasional situations where the regulating system breaks down and the repressed oncogenic and frequently virogenic information is somehow activated. In the context of natural evolutionary biology, information for various expressions of the RNA tumor virus genome in the embryo on the one hand

TABLE 1. *Virus in embryos of mouse strains with low incidence of leukemia*

Strains tested	Specimens	Age (Days)	gs Antigen	Virus (electron microscopy)	Infectious virus
BALB/c	Whole embryo	10-11	+	+	None demonstrated
C57BL					
NIH Swiss	Spleen, thymus	18	+	+	None demonstrated
C ₃ H					
Wild (feral)	Muscle	18	—	—	None demonstrated

* Antigen and budding virus were observed in 70-90% of tissues tested from each strain.

and cancer on the other may have played some essential roles in the development of species and subsequently in the embryonic development of the individual organism.

New methods of virus detection

Serological methods for detecting the gs antigen (25, 26) were critical in developing much of the data on type-C viral expression that led to the viral oncogene hypothesis (2). With the development of sensitive *in vitro* methods for detection of low levels of virus production (27), and more recently, the type-C RNA virus reverse transcriptase (28-30) and an antibody that specifically inhibits this latter reaction (31, 32), the production of as little as 10^2 - 10^3 infectious particles can be readily detected in cell-culture fluids. A special advantage of the reverse transcriptase assay, in addition to its extreme rapidity, is that virus particles can be detected even when a susceptible host is not known. This is particularly important since the "natural" mouse type-C viruses have a much more restricted host range than do the "laboratory" strains, such as Moloney, Friend, Rauscher, and Gross leukemia viruses (33). By these methods, it has been possible to repeat the investigation of the spontaneous production and the induction of virus from several well-characterized clonal lines of mouse cells, in particular the 3T3 and 3T12 cell lines derived from embryo cultures of random-bred Swiss and BALB/c mice (34, 35). These lines have been extensively used for studies of cellular growth control and viral transformation. The 3T3 cell lines are contact-inhibited, continuous lines derived from embryonic mouse cells from strains of mice with a relatively low incidence of spontaneous leukemia.

"Spontaneous" appearance of type-C viruses in normal mouse cell cultures

In previous studies, which led in part to the formulation of the oncogene theory, it had been shown that when cell lines derived from embryos of BALB/c and random-bred Swiss mice were established, the cells were free of detectable virus early in their life *in vitro*. After they had "spontaneously" transformed and acquired malignant properties in culture, some of the lines were found to release type-C virus (36). Since the cells had been frozen by methods that preserved their viability, during the course of their development into established lines, they could be thawed and systematically tested for virus production. The pattern for a given subline was quite reproducible, and virus production developed only in those lines that had lost control of normal cell growth.

Subsequent studies revealed that clonal lines of BALB/3T3 cells, after exposure to high doses of radiation, were virus-negative in tissue culture but became positive for virus after

they had formed tumors in animals (3). These and similar studies with hamster embryo cells (37), however, could not resolve whether the virus came from the inoculated cells or from the animal in which the tumors had developed, or represented complementary information from both sources.

Viruses of the C-type were found to appear "spontaneously" after prolonged cultivation of embryo cells of BALB/c and random-bred Swiss mice in some, but not all, the cell lines that were studied. There were two possible explanations for these results. The first was that the infection was chronic, and that the virus titer was low in the original primary embryo culture, hence the infection could not be detected by the methods available. If this were the case, the virus could have persisted in a carrier state because there were always a few infected cells in the population; the virus would be maintained by "horizontal" spread from cell to neighboring cell. The second explanation was that virus production began spontaneously in previously virus-free cells during the course of establishment of the cell lines. By this model, transmission of viral information could be entirely "vertical," from cell to progeny cells without the need for the virus to ever be extracellular. In the first model, the virus information would be present only in a small fraction of the cells at any one time; the oncogene hypothesis predicts the potential for making type-C virus in each and every cell. It was clear that direct proof of this aspect of the hypothesis would require the specific manipulation in tissue culture of virus-free cell lines derived from *single cell clones*, with the resultant release of complete virus.

Rowe *et al.* (4) have isolated lines of cells from the mouse strain AKR, with high incidence of leukemia. Although most of the cell lines produced high titers of leukemia viruses, two remained free of virus during numerous successive subcultures. Clonal lines derived from them also often remained virus-free for long periods and, then, with a low probability could spontaneously release virus. The probability of virus production could be increased by various experimental procedures, such as infection with SV40 or x-irradiation. Subsequently, bromodeoxyuridine (BrdU) and iododeoxyuridine (IdU) were found by Lowy *et al.* (5) to be exceptionally efficient inducers that activated every clonal line tested. These agents were then tested for their efficiency in inducing type-C virus from cell clones derived from strains with low incidence of leukemia, including BALB/c. By the reverse transcriptase assay and by an antibody that specifically inhibited the viral enzyme, it became possible to search for low titers of virus in tissue culture fluids of strains with a low leukemia incidence. The results showed that each and every BALB/c clone tested can be induced to release type-C viruses (6). These findings provided

TABLE 2. *Time course of appearance of reverse transcriptase in culture fluids of normal and transformed BALB/3T3 clones*

After treatment (days)	Polymerase (pmol/ml of supernatant)		
	Untransformed clone A31	Transformed subclone S2	Transformed subclone R4
0	<0.1	<0.1	<0.1
1	<0.1	<0.1	<0.1
2	0.3	3.7	5.7
3	3.2	12.5	14.5
4	8.5	10.7	8.6
7	1.8	14.8	12.5
14	0.2	35.2	18.6

strong evidence to support the concept that the genetic information for virus production was present in every BALB/c mouse cell and that this information could be transmitted vertically from cell to progeny cells for many hundreds of generations without being expressed. Similar experiments were then performed with cultures of 3T12 cells from random-bred Swiss mice that were virus-negative. Five clones were isolated and each was tested after treatment with 30 μ g per ml of BrdU. The results show that inducible virus was obtained from each of these clones as well (Todaro, G. J. *et al.*, manuscript in preparation). Polymerase was detected 3 days after induction; as in the BALB/c clones, amounts of polymerase were never very high, and were gone by 2 weeks; the polymerase, moreover, was virus-specific since it was inhibited by the antibody to the viral polymerase (31). Virus induced from 3T3, 3T6, or 3T12 cells from random-bred Swiss mice in each case replicated very poorly in the original host cells but replicated well when inoculated in NIH Swiss 3T3 and 3T12 cell clones (38).

The cells from random-bred Swiss mice are type-B cells, as defined by Hartley *et al.* (33) in that they allow the replication of BALB/c-tropic viruses. This failure of the induced virus to grow in cells in which they are induced strengthens the case that in the mass cultures the virus was derived from the endogenous genetic information of the cells rather than from the progeny of some previously undetected external contaminating virus (36).

Virus from "spontaneously" transformed mouse-cell clones

The association between "spontaneous" production of virus and "spontaneous" transformation in culture or "spontaneous" tumor development in the animal must be determined. The 3T12 cells were always maintained at four times the cell density of the 3T3 lines (12×10^5 cells/plate, compared to 3×10^5 cells/plate for 3T3-type cultures); therefore, it could well have been that a spontaneously induced virus would have a greater chance of maintaining itself in the 3T12 transfer culture, where there was continuous, extensive cell-to-cell contact. Another possibility is that transformed cells that have acquired malignant properties, such as the 3T12 cells, are either more inducible or are better able to support the replication of induced viruses than untransformed cells.

When BALB/3T3 clone A31, a highly contact-inhibited cell line that is nontumorigenic, was compared with a "spontaneously" transformed subclone, S2, and a "radiation" trans-

formed subclone, R4 (3), for inducibility, virus was detected in the supernatant fluids of each clone within 2–3 days after a 24-hr exposure to BrdU. However, the virus, as reported (6), tended to disappear from the normal clone, while it not only persisted but actually increased in the transformed cells that were treated with BrdU (Table 2). Further, the induced virus from the transformed S2 cells infected and replicated in other uninduced S2 cells. While the virus induced from BALB/c-embryo cells for the BALB/3T3 cell line replicated very poorly in NIH/3T3 cells, the virus induced from the transformed R4 and S2 subclones grew well in NIH/3T3 cells. It appears, then, that the transformed tumorigenic cells have different properties when compared to the normal cells. The availability of well characterized clonal lines of normal and transformed cells may help to define the cellular controls of endogenous and exogenous virogene and oncogene expressions.

Attempts to induce virus from the widely used Syrian hamster line, BHK 21 clone 13, have so far not been successful. However, Chinese hamster ovary (CHO) cells, a line extensively used for somatic cell genetics and cell hybridization, also have an inducible type-C virus (Table 3). The presence of endogenous virus information that is easily activated may well complicate certain hybridization studies as well as studies on temperature-sensitive and host range mutants of type-C viruses. Klement *et al.* (7) and Aaronson (8) have shown not only that the endogenous type-C virus of the rat can be induced from all clones, but that this virus can rescue the sarcoma genome from virus-transformed nonproducer rat cells. The extent to which endogenous virus information can contribute to the replication and, perhaps, even to the transforming capacity of exogenously introduced virus is as yet unclear. It is interesting to note that IdU, in addition to being an efficient inducer of endogenous type-C virus, also enhances the susceptibility of 3T3 cells to transformation by the DNA tumor virus, SV40, (39) and greatly increases the efficiency of recovery of SV40 from heterokaryons of cells transformed by SV40 and of monkey kidney cells (40). Whether the induced endogenous type-C virus plays a role in fixing SV40 transformation or in facilitating virus recovery remains to be resolved.

In cell culture, at least two factors are involved in the control of virus production. One is the probability of induction of virus information from an individual cell. This we presume depends on the "strength" of the repressor. A cell susceptible to induction would be one with a low level of repressor activity. Various agents we now know can overcome the repressor activity and result in the production of type-C viruses.

TABLE 3. *Rodent continuous cell lines with clones that have been shown to contain inducible type-C virus*

Species	Line designation	Refs.
Mouse (AKR)	Line 26	(4, 5)
	Line 32	(4, 5)
Mouse (BALB/c)	BALB/3T3	(6)
	BALB/3T12	(6)
Mouse (random-bred Swiss)	3T6	(6)
	3T12	(57)
Rat	NRK	(7, 8)
Chinese hamster	CHO	(57)

FIG. 2. Induction of virus from clones of mouse cells.

Whether the culture (or the animal) is at risk as a result of virus induction depends on a second determinant, the ability of the induced virus to infect neighboring cells and spread like a conventional virus. Highly susceptible mice, like the AKR and C58 strains, have cells that are unusually susceptible to infectious expressions of "induced" endogenous virus. Within these animals, once enough virus has developed, it could spread horizontally to adjacent uninduced cells. Strains with low incidence of leukemia, like BALB/c and random-bred Swiss mice, even if they have natural expression of infectious virus, may not be susceptible to spread of infection by the virus induced from a few of their cells to other cells in the body (Fig. 2).

Eventually, with time, mouse leukemia viruses have developed by experimental manipulation that are capable of growing in cells of most mouse strains; these established viruses very likely have been selected for the following properties: their ability to interact with specific cell receptors, high levels of reverse transcriptase, and insensitivity to cellular control systems. Whether the postulated repressor is in the virion itself is not known. The reverse transcriptase may have been important at some point in evolution for the maintenance of the infectious virus, but may well now have little role in the perpetuation of the virus information in noninbred species under natural conditions.

The demonstration that the viruses induced from normal animals or normal cells are, in fact, tumorigenic is still required information for such isolates. An alternate possibility that might be considered is that the type-C viruses do not, or need not, invariably contain oncogenic information. However, the findings that viruses isolated from normal mouse spleens of the BALB/c strain produce leukemia when inoculated into newborn isologous mice (Peters, R. J. *et al.*, manuscript in preparation) suggest that the oncogene function is normally present as a portion of the virogene.

Evidence for virogenes in chicken cells

The likelihood that the generality of inbred chick cells contained endogenous type-C viral genomes came from genetic

studies by Payne and Chubb (41) and from the properties of a "helper factor" that complements defective Rous sarcoma viruses (42, 43). Weiss *et al.* (44) showed the endogenous information in "virus-free" chickens could be activated by ionizing radiation, chemical carcinogens, or various mutagens, and, further, that type-C virus could also be induced from wild Malaysian jungle fowl, the apparent progenitor of the domestic chicken. These observations strongly indicate that the cells of all chickens, whether or not the birds are destined to develop leukemia, have the genetic potential to specify an avian type-C virus.

Another line of evidence comes from molecular hybridization studies by Baluda and Nayak (45) and Rosenthal *et al.* (46), who find that there is virus genetic information for the avian type-C viruses in chicken cells whether or not they are producing virus and whether or not they have detectable titers of gs antigen. In contrast, rodent cells that are transformed by avian type-C viruses show avian virus-specific genetic information in the DNA of the host mammalian cell, whereas uninfected rat cells show no such sequences (47). The conclusion is that the genetic information of exogenously added virus is maintained in the cell in the form of DNA, and that DNA sequences homologous to the type-C virus RNA occur in normal avian cells.

Differences between the RNA virus oncogene theory and the provirus and provirus theories

The virogene may be considered somewhat analogous to the prophage of bacterial cells (48) and to the provirus proposed by Temin (49). However, there are important differences. The first difference is that the provirus implies that, as the result of fairly recent events, the cell of the animal received new information from exogenously added virus that became incorporated into the genetic makeup of the cell. We envision that the virogene and oncogene information are intrinsic parts of the natural genetic fabric of all vertebrate cells, and while it might perhaps at some time in evolution have become a part of the cell as a result of horizontal transmission (infection), we feel the evidence requires that they should be regarded as essentially cellular genes and that the RNA virus information

in uninfected and untransformed cells is unexpressed genetic information. This does not of course exclude occasional horizontal (cell-to-cell) or rare animal-to-animal spread when replicating virus becomes expressed in a susceptible strain.

A second difference is that the provirus theory, modeled on the prophage theory, predicts that the genes for making the virus have become integrated in such a fashion that they are physically located next to one another in the host chromosome. While this may well be the case with exogenously added information of type-C virus, it is not necessary that the endogenous genes responsible for making the virus be linked to each other in the cellular DNA. In fact, for example, the genes for the reverse transcriptase and the genes for the *gs* antigens may well be on separate chromosomes, and there may be multiple copies per cell. The selective loss of chromosomes from somatic-cell hybrids may resolve whether all the endogenous virus information is located on a single chromosome.

The provirus theory is concerned primarily with how viruses get into cells and how they are maintained in the cells that they have infected. It suggests that information is maintained in the form of DNA, and in this fashion, the information becomes part of the cellular genetic machinery. However, if horizontal transmission of tumor-virus information from animals is an infrequent cause of naturally occurring tumors, as we suspect, then the infectious virus itself may not be of primary concern to the cancer researcher studying natural species that have little or no viral expression associated with tumorigenesis. How endogenous virus information is expressed in the cells would further increase our understanding of cancer and suggest ways as to how it might be controlled. We assume that the information is already present in the cells and that the critical events involve decisions as to the expression of the already present virus information. The critical point, then, for keeping cells from becoming transformed would be not by a control of the entry or integration of an exogenous virus into the cell, but by host-cell regulation of the universally present, endogenous genetic information of the virus. This, of course, does not mean that it will be possible to activate a complete virus from every cell of every vertebrate. In some species complete virus expression may in fact be extremely rare either because of alterations or deletions in the virogenes, or because of the "strength" of the repressor systems.

More recently, Temin has proposed the provirus theory (50) that states that there are regions in cellular DNA that serve as templates for the synthesis of RNA. With the reverse transcriptase, this RNA can serve as template for new DNA synthesis. The new DNA can subsequently become integrated into cellular DNA of either the original cell or adjacent cells in the body. This system, then, could play a critical role in gene amplification and in cellular differentiation. Temin postulates that this system could, on a random basis, lead to a rare somatic cell that contains all of the information for production of a type-C virus. This information, the provirus theory states, does not exist in the germ cells but is probably generated in each animal as it develops. The induction of complete type-C virus from each somatic clone of mouse cells, rat cells, hamster cells, and from each chick embryo, as described above, argues strongly against the provirus theory. The provirus theory would have predicted that only a very small fraction of somatic cells, through this process of trans-

mission of RNA from cell to cell and its incorporation by the reverse transcriptase into DNA, would contain all the genes necessary for producing a complete type-C virus*.

SUMMARY AND IMPLICATIONS

To summarize, type-C viruses are found in three orders of vertebrates—reptiles and birds, as well as mammals. The viruses isolated from normal mouse spleens or from chickens are capable of producing leukemia in mice and chickens, respectively, and thus, carry oncogenic information (Peters *et al.*, manuscript in preparation). Although type-C viruses are particularly abundant in certain inbred mouse strains with high incidence of leukemia, they also have been found in random-bred mice and in wild (feral) mice. Virus expression is found in embryos: after birth and in early life such expressions are much diminished; late in life, when the cancer incidence increases, virus expression is often increased, in incidence and titer (2).

Long-term embryo cultures from mouse strains with low incidence of leukemia, spontaneously produce virus. Treatment of cell cultures with carcinogens, mutagens, radiation, and, perhaps, passage of the cells in animals serve to activate endogenous type-C virus information. Clonal lines of mouse, hamster, and rat cells all have inducible type-C virus; the thymidine analogs, BrdU and IdU, have been found by Lowy *et al.* (5) to be highly efficient inducers. With cell clones from mouse strains with low incidence of leukemia, the induced virus is very poorly infectious for the cells from which it was derived. The endogenous virus (endovirus) induced from transformed, tumorigenic cells may, however, have different properties from the virus induced from normal cells. It seems likely that normal cells contain repressors that control the expression of the endogenous virus information; this control system can act to prevent expression of all virogenic information or it could allow partial expression of the endogenous virus information. If that partial expression includes that of the oncogene, the cell has the potential to produce tumors whether or not a complete type-C virus is produced.

The theory predicts that there is a complete copy of the type-C virus information in the genetic material of all somatic cells. Tests by hybridization with cellular nucleic acid and the nucleic acid of the type-C virus derived from the same species may resolve this point. Since the viruses are transmitted as genetic elements, we would expect them to evolve as the species have evolved. This can be tested with species-specific viral proteins, such as *gs* antigens (53) and the reverse transcriptase (54). The extent of relatedness of these proteins

* Evidence for the persistence, in a repressed "switched off" state, of oncogenic DNA virus information is also accumulating. Smith *et al.* have been able to select clones of BALB/3T3 cells that have been abortively, or transiently, transformed by SV40 (51). Clones have been isolated that show no detectable morphologic change or evidence of virus expression. Nevertheless, these abortively transformed clones can be shown to contain copies of the SV40 genetic information (52). Apparently, the virus is capable of integrating and becoming a part of the genetic information of that cell, and yet not be expressed; there is no virus production, SV40-antigen production, or expression of the transforming gene of SV40. Subclones of the original abortively transformed clones show the same properties and also have SV40-specific DNA sequences. The presence of potentially oncogenic viral information in normal-appearing cells may be much more common than was generally thought.

would be a measure of the genetic relatedness of the organisms from which they were derived, in much the same manner that serum albumins can be used as an index of genetic relatedness (55).

Transformed cells and tumor cells, if the oncogene is "switched on," should have common messenger RNA sequences not found in normal cells; these should be present whether the cells were transformed by infectious virus, chemical carcinogens, radiation, or if the transformation occurred "spontaneously." The messenger RNAs, the product(s) of the oncogene, should, in turn, produce the transforming proteins that are directly responsible for the conversion of a normal cell into a neoplastic cell. This perhaps can be best tested in cells in which the virogene, as distinguished from the oncogene, is not expressed. The study of the regulation of endogenous tumor-virus information, in general, and the oncogene, in particular, at the level of control of gene expression in the individual cell in culture offers a promising approach to the understanding of the etiology of cancer.

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