Cell Transformation by Viruses

Two minute viruses are powerful tools for analyzing the mechanism of cancer.

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It has been known for 50 years that viruses can cause neoplasms in animals, but only recently has substantial progress been made in understanding the mechanisms. This has been the result of the application of the methodologies of molecular biology, immunology, and virology and the use of tissue culture. Animal cells in tissue cultures yield themselves both to genetic studies, in which the progeny of single cells are used, and to biochemical studies of large and uniform cell populations.

Transformation and Neoplasm

In tissue culture, oncogenic viruses produce characteristic changes of the cells, which are transmitted hereditarily from a cell to its progeny. This phenomenon is usually called "transformation." The transformed cells give rise to a progressively growing tumor when they are injected into a suitable host, and therefore have properties similar to those of the neoplastic cells that arise in animals under the influence of a variety of agents. A precise relationship between transformed and neoplastic cells, however, cannot be established because the definition of a neoplastic cell is too vague. In fact, the cells of different neoplasms can differ greatly. Undoubtedly, neoplastic cells often have properties which are not shared by cells transformed in vitro; for instance, they tend to infiltrate neighboring tissues to various degrees, whereas cells recently transformed in vitro by polyoma virus usually grow as an expansive mass when transplanted into an animal.

In order to appreciate the significance of these differences, it must be recognized that an established neoplasm is the result of two distinct processes: (i) the generation of the initial cancer cell, and (ii) the growth of this cell into a neoplastic mass. In the second phase, altered cells emerge as the result of a sequel of genetic changes, such as mutations and chromosomal aberrations; also aneuploid cells—cells with an abnormal number of chromosomes—are formed. Cells with different genotypes respond in different ways to the selective forces of their environment. As a result, the best-growing cells are selected for, and the invasiveness of the neoplasm increases—a phenomenon known as progression.

The characteristics of an advanced neoplasm are, to a large extent, determined by the path taken by progression. In the end, the pathological picture may vary drastically, depending not only on the cell type from which the neoplasm arose but also on the organ in which it grows, on whether the growth is primary or metastatic, on whether or not treatment has been employed, and on many other factors. A very important factor in progression is the immunological reaction of the animal against the neoplastic cells, which appears to be always present but varies greatly in different cases. Virus-transformed cells elicit a strong reaction when injected into an animal host, owing to the antigenic changes of their surface. In vitro, many of the selective forces leading to progression in animals (especially the immunological rejection) are lacking, and those that are present can be kept to a minimum through the use of proper experimental techniques.

A reasonable view of the relationship of transformed cells to neoplastic cells is that the transformed cells are those initially present in the neoplasm when it arises, whereas the cells of established neoplasms have been further modified in the progression process. The spectrum of transformed cells observable in vitro may be wider than the spectrum of transformed cells that grow into an established neoplasm in animals, owing to the different selective conditions. It is likely that a large number of transformed cells arising in animals are prevented from growing by the selective conditions to which they are exposed.

Characteristics of Transformed Cells

Transformed cells are distinguished in vitro from normal cells by their growth habits and morphology. The differences in growth habits have to do with the population density obtained in a culture and with the reciprocal relationship of the cells. On a solid substrate and with standard methods of cultivation, normal cells reach a saturation density of about 1 to 5×10^4 cells per square centimeter of culture surface, whereas transformed cells, under identical conditions, can reach a density ten or more times as great. Cultures of normal cells, however, can be made to grow to higher densities, either by perfusion with fresh medium or through continuous supply of a required growth factor. With some cells, such as those of the 3T3 line of mouse fibroblasts (*1*), the saturation level is determined by the amount of blood serum in the culture medium (*2*). The serum contains essential growth factors for these cells (*3*). Transformed derivatives of 3T3 cells have a much smaller requirement for the serum factors. Another difference between transformed and normal cells concerns the reciprocal relationships of cells in culture. These relationships depend on the cell type and are fairly constant in cultures of the same cell type. For instance, normal elongated cells, designated fibroblasts, tend to stick to each other along their longer sides when growing on glass or plastic, and tend to lie parallel to each other, whereas transformed fibroblasts tend to crisscross each other in random fashion. The differences in reciprocal cell orientation probably depend on differences of the cell surface. Indeed, differences have been found in the chemical nature of side groups of the plasma membranes of normal and transformed cells (*4*). There is another difference of practical

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importance: transformed cells of certain lines grow in suspension in agar or Methocel, whereas the corresponding normal cells do not (*5*). These characteristics of transformed cells allow a clear-cut differentiation from normal cells; their relationship to the ability of the cells to form a tumor in a suitable host, however, is not clear. None of these characteristics has been shown to be necessarily associated with tumor-forming ability.

In most of the work on transformation, permanent cell lines, which can be serially propagated through an indefinite number of transfers, are used. The cells of these lines are not normal but retain normal growth habits, so they clearly display the changes produced by transformation. Three permanent lines are referred to in this article: the BHK line of hamster kidney fibroblasts (*6*), the BSC-1 line of African green monkey kidney cells (*7*), and the 3T3 line of mouse-skin fibroblasts mentioned above.

Transforming Viruses

Viruses containing either RNA or DNA are able to cause transformation in vitro and neoplasms in animals. Model systems have been developed for studying the mechanisms of transformation and neoplasm formation. Among the RNA-containing viruses, the leukosis and sarcoma viruses of chickens and mice have been used. The sarcoma viruses, of which the most famous is the Rous sarcoma virus, cause transformation in tissue culture. Among the DNA-containing viruses, polyoma virus and simian virus 40 (SV40), together with the human adenoviruses, have been used in recent years.

The study of transformation caused by the small DNA-containing viruses, polyoma virus and SV 40, that has been carried out in the last few years has clarified certain points concerning the mechanism of transformation and has set the stage for further advances. I devote most of this article to discussion of these results.

General Characteristics of the Viruses

Polyoma virus and SV40 have a ring-shaped DNA of molecular weight 3×10^6, adequate to accommodate six to ten small genes. About one-third of the genetic information specifies proteins present in the viral particles. The purified viral DNA is infectious.

The interaction of these viruses with their host cells leads either to virus multiplication and cell death or to cell transformation, depending on the cell. Thus the cell type influences in a fundamental way the results of infection by a particular virus. Even when transformation occurs, its molecular aspects may not be identical in different cells, for reasons that will become clear later on.

Cells in which viral multiplication takes place are called "permissive." Cells of the 3T3 line are permissive for polyoma virus, and BSC-1 cells are permissive for SV40. Both 3T3 and BHK cells can be transformed by either virus, but the transformed cells obtained from the same line, with different viruses, are often strikingly different. This difference is interesting in two ways: it shows that viral genes play a fundamental role in transformation, and it offers a tool for studying some aspects of the gene control in the cells themselves.

Stable and Abortive Transformation by DNA Viruses

Both polyoma virus and SV40 cause either a *stable* or an *abortive* transformation. In stable transformation a transformed cell gives rise to a line in which all cells are transformed. In abortive transformation the cells generated by the initial transformed cell are again normal after a small number of cell divisions. Abortive transformation is easily observed with BHK cells; growth in Methocel is the method used to identify the transformed cells (*8*).

When BHK cells infected with polyoma virus are seeded in Methocel, many of them begin to multiply and give rise to small colonies. After five or six cell generations, however, most of the colonies stop growing, and only a fraction, made up of stably transformed cells, continue to grow and develop into large colonies. If the early colonies are picked and plated on solid substrate, their cells show at first the growth characteristics of transformed cells, but, on subsequent multiplication, they generate normal cells. These colonies are derived from abortively transformed cells.

The difference between stable and abortive transformation has not been positively established. A hypothesis consistent with all available evidence is that the main difference is the state of the viral DNA in the cells. In stable transformation, the viral DNA becomes integrated in the DNA of the transformed cells, whereas, in abortive transformation, integration may not occur. The latter point, however, has not been established experimentally.

State of the Viral DNA in Transformed Cells

Evidence for the integration of the viral DNA in stable transformation was hard to obtain because a molecule of viral DNA is a very small fraction, about 5×10^{-7}, of the DNA of a cell. This minute proportion of viral DNA could be detected, however, by hybridization with labeled virus-specific RNA (*9*) on membrane filters, in a modified Gillespie-Spiegelman (*10*) procedure. The virus-specific RNA was synthesized in vitro on viral DNA as template, with *Escherichia coli* RNA polymerase. A difficulty in these experiments was the presence of cellular DNA instead of viral DNA in some of the viral particles (pseudovirions) (*11*). However, cellular and viral DNA's can be separated from each other because the viral DNA is ring-shaped and differs from the cellular DNA, which is linear, with respect to many physical properties (for example, sedimentation velocity in solution). Viral DNA was therefore freed from cellular DNA before being used as the template for the RNA.

The labeled RNA hybridized with 100 micrograms of DNA of untransformed cells to the extent of about 10^{-3} of the input. This background hybridization was similar for polyoma-specific and SV40-specific RNA, with DNA of different untransformed cell lines. This background hybridization limited the sensitivity of the assay to recognition of about three viral DNA molecules per cell, as shown by reconstruction experiments. The DNA of cells transformed by either polyoma virus or SV40 was found to hybridize with the corresponding virus-specific RNA to a greater extent than the DNA of untransformed cells, showing that a transformed cell contains several copies of viral DNA (Table 1). Cells transformed by polyoma virus contain a smaller average number of viral DNA

Table 1. Specificity of recognition of viral DNA in transformed cells (lines SV40 and Py) by hybridization with viral RNA. [From Westphal and Dulbecco (*9*)]

DNA	Viral RNA			
	SV40		Py	
	Count/min, total	Count/min, net	Count/min, total	Count/min, net
3T3	1040 ± 87	0	1400 ± 140	0
3T3, + 5 molecules per cell	1390 ± 115	350		
3T3, + 10 molecules per cell			2100 ± 150	700
3T3, + 16 molecules per cell	1670 ± 164	630		
3T3, + 20 molecules per cell			2950 ± 320	1500
SV3T3	1840 ± 115	800	1400 ± 95	0
Py3T3	1135 ± 214	95	1750 ± 130	350

equivalents per cell than cells transformed by SV40, even if derived from the same cell line (Table 2). This difference suggests that the number of copies of viral DNA present in a transformed cell depends on characteristics of the viral genome itself.

The state of the viral DNA in the transformed cells was studied in a line of 3T3 cells transformed by SV40, designated SV3T3 (*12*), which contains an average of 20 viral DNA equivalents per cell. It is not known whether all cells in this line contain approximately the same number of copies of viral DNA. The possibility that they do is supported by the finding that all the cells contain similar amounts of the virus-specific T-antigen, as shown by immunofluorescence with a suitable antiserum.

The objective of the investigations was to establish whether the viral DNA was free in the cells or bound to a cellular structure, possibly the cellular DNA. Again, the viral DNA was identified by hybridization with labeled virus-specific RNA. Two main lines of evidence pointing to an association of the viral with the cellular DNA were obtained. (i) The transformed cells do not contain free viral DNA, which would have been identifiable either by its ring shape or by its characteristic size. (ii) The ability to hybridize the virus-specific RNA remained associated with the cellular DNA in a series of isolation procedures which went from isolation of nuclei to isolation of metaphase chromosomes, and finally to isolation of the cellular DNA itself. Furthermore, the association persisted through zonal and equilibrium sedimentation in alkali gradients, where only denatured, single-stranded DNA exists; the persistence shows that cellular and viral DNA's are held together by covalent bonds.

These results are strengthened by the observation that cells transformed by SV40, which, as I show below, contain a complete viral genome, do not yield infectious viral DNA on extraction (*13*).

So far, integration of the viral DNA in the cellular DNA has been ascertained only for SV3T3 cells. It is not known whether such integration occurs with other transformed lines. It seems, however, that such a striking biological phenomenon should be of a general nature. If this is the case, transformation by DNA virus is an alteration of the genetic material of the cell caused by the insertion of new genes brought in by the virus. The topographical relationship between viral DNA and cellular DNA is similar to that existing in lysogenic bacteria, where integration of the viral DNA into the cellular DNA has been known for several years.

The problem now is to identify the site or sites of insertion of the viral DNA. A first approach to this problem is to determine which cellular chromosome (or chromosomes) carries the viral DNA. Two recent results show that this is a possible task. One result was obtained with cells transformed by SV40; the other, with cells transformed by polyoma virus.

In the first experiment, transformed human cells were fused to untransformed mouse cells (*14*). The fusion resulted in the formation of hybrid cells each of which had a number of chromosomes equivalent to the sum of the chromosomes of the participating cells. In their subsequent multiplication, the hybrid cells spontaneously lost human chromosomes. Sublines were obtained which contained either a small average number of human chromosomes or none at all. The parental hybrid lines and a number of the sublines were tested for the presence of SV40 T-antigen, a virus-specific product present in the nuclei of cells either lytically infected or transformed by SV40. Immunofluorescence studies showed that the antigen was present in the original transformed cells, as well as in the original hybrid cells. In the sublines, the presence of the antigen was correlated with the presence of human chromosomes. The sublines that had lost all human chromosomes had no T-antigen; those that had a small number of human chromosomes contained a mixture of positive and negative cells. These results suggest that the viral DNA is integrated in either one or a small number of the chromosomes of the transformed cells.

In the second experiment, two variant BHK sublines were used, one lacking inosinic acid pyrophosphorylase (IPP) and resistant to 6-thyoguanine, the other lacking thymidine kinase and resistant to 5-bromodeoxyuridine (*15*). By fusion, a hybrid line was obtained which was sensitive to both analogs and had a number of chromosomes equivalent to twice that of BHK cells. The hybrid line was transformed by polyoma virus; by growing the transformed cells in the presence of 6-thyoguanine, an IPP-deficient subline was isolated. The cells of this subline had fewer chromosomes than the original hybrid line, and presumably had acquired resistance to the drug through the loss of chromosomes carrying the IPP gene. The growth habits of these segregant clones were often different from those of the parental transformed line, and some of the clones had a normal phenotype. As in the preceding case, the revertant clones had also lost the ability to synthesize the virus-specific T-antigen. These results suggest that polyoma DNA was integrated in one of the chromosomes lost during the acquisition of resistance to 6-thyoguanine.

Relevant to the specificity and chemical nature of the insertion site are observations showing that the frequency of stable transformation is increased if the cells are exposed to x-rays before infection (*16*). Since x-rays cause breaks in the cellular DNA, the result suggests that integration occurs at the site of preexisting breaks. Whether or not this result is compatible with the existence of a specific site (that is, a site recognized by a homologous region of the viral DNA or by an integration enzyme) depends on the size of the

site. The proportion of transformed cells after irradiation at a dose of 1000 rad is of the order of 1 percent (*17*). With a single-strand breakage rate of 1.1×10^{-6} break per rad for DNA of molecular weight 10^6 (*18*), a break localization in these cells would require a molecular weight of about 10^7 for the site; the size of the site would be further increased by repair of some of the breaks. A site of this size cannot be specific, since it is larger than the viral DNA itself. Integration must then be possible at many places. Even if it occurs at a given chromosome in a specific case, in other cases it may occur at other chromosomes. This point can be clarified by determining which chromosome carries the viral DNA in different cell lines, either by chromosomal segregation in hybrid cells or by chromosome fractionation.

Activation of Virus Multiplication in Transformed Cells

I have so far shown that the viral DNA can become integrated in the cellular DNA in stable transformation. I now consider the symmetrical problem—namely, the detachment of the integrated DNA.

Detachment and activation of virus synthesis occur in many lines of cells transformed by SV40 after fusion with permissive cells (*19*). In our work, SV3T3 cells transformed by SV40 were fused with BSC-1 cells, ultraviolet-irradiated Sendai virus being used as the fusing agent (*20*). Of the heterokaryons formed, about 10 percent produced infectious SV40, although SV3T3 cells alone did not. These results show not only that the integration is reversible but also that complete viral genomes were integrated in the SV3T3 cells.

In contrast, no line of cells transformed by polyoma virus has so far yielded virus by fusion with suitable permissive cells. The reason for this failure is not clear. A possibility is that defective mutants of polyoma virus cause transformation in most cases.

Virus release was obtained, however, by Vogt (*21*), under different circumstances, from a polyoma-transformed line of a special kind. This is a line of 3T3 cells transformed by a temperature-sensitive mutant of polyoma virus, designated Ts-a (*22*). Cells of the 3T3 line are permissive for polyoma virus and therefore are normally lysed by it, although in exceptional cases they are transformed by wild-type virus. The Ts-a mutant virus does not kill the cells at high temperature (38.5°C), and some cells are transformed. The transformed cells, referred to as Ts-a-3T3, are stable at high temperature and release a small amount of virus; if the temperature is lowered to 31°C, however, a proportion of cells lyse and release virus. At 31°C, this proportion is about 10 percent at first and then increases, with time, to about 80 percent.

Table 2. Viral DNA equivalents per cell in different lines of transformed cells. [From Westphal and Dulbecco (*9*)]

Cell line	SV40 DNA	Polyoma virus DNA
SV3T3-47*	20	0
SV3T3-56†	7	0
H-50‡	58	0
Py3T3§	0	5
Py8‖	0	7
SV Py3T3¶	44	10

* 3T3 cells transformed by SV40. † 3T3 cells transformed by nitrous-acid-treated SV40, and containing very small amounts of T-antigen. ‡ Cells derived from SV40-induced hamster tumor. § 3T3 cells transformed by polyoma virus. ‖ BHK cells transformed by polyoma virus. ¶ 3T3 cells transformed by both polyoma virus and SV40.

Whether or not inactivation of Ts-a-3T3 cells by temperature shift and of SV3T3 cells by fusion represent the same phenomenon is not known. A similarity is suggested by studies of the viral DNA synthesized in the two cases during activation. Newly synthesized DNA labeled in Ts-a-3T3 cells exposed to tritiated thymidine 24 hours after temperature shift contains, in addition to the familiar rings found in the viral particles, rings of multiple size—double, triple, or quadruple. The multimers, which are very infrequent in the regular multiplication of the virus, are infectious (*23*). Preliminary observations by Summers on the DNA synthesized after fusion of a line of SV40-transformed hamster cells to BSC-1 cells also suggest the presence of dimers. If the notion that nonpermissive cells do not contain free viral DNA can be extended to this line, the finding would suggest that multimers are formed during detachment of the integrated DNA. If this is the case, the Ts-a-3T3 cells may also contain integrated DNA. Nothing, however, excludes the possibility that the viral DNA in these cells is in an extrachromosomal location.

A puzzling finding is that the relative proportion of monomers and multimers varies in different clones deriving from the Ts-a-3T3 line, and that the more atypical clones contain more multimers (*24*). This observation can be interpreted in two ways. If the viral DNA is integrated, the number of integrated copies would determine the degree of deviation of the cells from normality. The other interpretation, suggested by the finding that mitochondrial DNA in leukemic cells contains a higher proportion of multimeric forms than normal cells do (*25*), is that the viral DNA in Ts-a-3T3 cells is not integrated, and that the mode of replication of nonchromosomal DNA in animal cells is controlled by cellular functions; then the abnormality of the DNA would be a consequence of the abnormality of the cells.

Functional State of the Viral DNA

Having discussed our present understanding of the process of integration and detachment of the viral DNA in the transformed cells, I now discuss briefly the functional state of the DNA. This question has been attacked in several laboratories by studying the viral messenger RNA produced in the cells in hybridization competition experiments (*26*). In our studies my collaborators and I compared the viral RNA synthesized in SV3T3 cells and that synthesized in BSC-1 cells lytically infected by SV40. In lytic infection of BSC-1 cells, about one-third of the viral DNA is transcribed into messenger RNA in the early phase of infection, before replication of the viral DNA begins. In the late phase of infection, after the viral DNA has begun replicating, the whole viral genome is transcribed. In the transformed cells, again about one-third of the viral DNA is transcribed, but the transcribed genes overlap only partly with those active in the early phase of lytic infection. Experiments on reciprocal hybridization competition between early lytic and transformed cell RNA's showed that two-thirds of the RNA's are identical. In contrast to these results, a study of African green monkey kidney cells (AGMK cells) transformed by SV40 (*27*) has shown that 80 percent of the viral DNA is transcribed. These cells differ from 3T3 cells in that they are permissive for SV40 at high multiplicity of the virus, and it is therefore possible that they are transformed by defective viral mutants. This is conceivable because defective DNA molecules are present in some of the viral

particles (*28*) and induce transformation as effectively as the regular virus does (*29*).

The findings on transcription of the viral DNA have several consequences. They show that, at most, one-third of the total viral genome is essential for transformation, corresponding to two or three genes. Since independent evidence suggests that late functions are irrelevant for transformation, the number of essential genes is further reduced by one-third. The scope of the search for the transforming gene is thus considerably limited.

The results are also interesting for understanding the regulation of transcription of the viral DNA. Different mechanisms operate in early lytic infection, and in transformed cells. Regulation in early lytic infection is likely to be of viral origin, because it is a property of all DNA viruses, both animal and bacterial. In contrast, regulation in transformed 3T3 cells may be of cellular origin. In fact, 3T3 cells never undergo lytic infection with SV40. If infected at high multiplicity of the virus, they undergo abortive infection and still express the same limited set of viral genes as transformed cells. Expression of the other viral genes may be impossible. The molecular basis for the limited transcription in transformed cells is not yet known. Extracts of SV40-transformed cells contain a protein that weakly inhibits plaque formation by SV40 on permissive cells, and it has been proposed that this protein may be a repressor acting on the viral DNA (*30*). The results, however, could be explained in other ways, and further work is required before this proposal is accepted.

Studies of transcription of the viral DNA have not yet been made with BHK cells transformed by polyoma virus, and therefore we do not know whether transcription of the viral DNA is limited. This system may be different from the SV403T3 system, and perhaps similar to SV40-AGMK systems, since, in the BHK cells, late viral functions are expressed in a small proportion of the cells (*31*).

In any case, nonpermissiveness of the cells is important for transformation because it prevents the expression of late functions lethal for the cells. Permissive cells presumably can be transformed only by viral mutants, in which the mutation abolishes the expression of these functions.

Functions of Viral Genes in Transformation

I now turn to a different aspect of transformation—namely, the viral genes whose functions are required if transformation is to occur. I have already shown that these genes are few in number, and it may be asked whether any of them, or their functions, have been identified.

The genetic studies of Eckhart (*32*) and of di Mayorca (*33*) have led to the identification of four classes of mutants for polyoma virus. The temperature-sensitive mutants used are unable to form plaques at high temperature but form them at low temperature. Mutations of two classes prevent replication of the viral DNA at high temperatures, and therefore affect early functions, while mutations of the other two classes may affect synthesis of the viral capsid protein, which is a late function.

Mutations of one class, which affect an early function, prevent stable transformation at high temperature. This group includes the Ts-a mutation mentioned above. Mutations of the other groups do not affect transformation.

These observations show, in agreement with results deriving from the study of transcription in SV3T3 cells, that the functions of a large proportion of viral genes are irrelevant for transformation.

The only known gene we must be concerned with now is the one defined by the Ts-a mutation, which I will call the Ts-a gene. Its relationship to transformation cannot be easily defined, despite the considerable amount of information available. In brief, the properties of the Ts-a mutant at high temperature may be summarized as follows. In lytic infection of mouse cells there is no accumulation of viral DNA (*22*). The mutation prevents the stable transformation of BHK cells, but cells transformed by the mutant at low temperature remain transformed when the temperature is raised (*22*). The mutation does not prevent the abortive transformation of BHK cells (*34*), or the stable transformation of 3T3 cells, as discussed above.

These observations are susceptible of several interpretations. One interpretation, based on results in BHK cells, is that the Ts-a gene is required for integration of the viral DNA in the cellular DNA. If it is, stable transformation of 3T3 cells by Ts-a virus at high temperature could be attributed either to leakiness of the mutation in the 3T3 cells (which can be demonstrated) or to absence of integration. A second interpretation is that the Ts-a gene is required for the detachment of the viral DNA, since activation of virus synthesis occurs under conditions that permit a resumption of the function of the gene, such as temperature downshift in Ts-a-3T3 cells and fusion with permissive cells in SV3T3 cells (it being assumed that in these cells the Ts-a gene is not normally transcribed). The third interpretation is that the requirement is indirect. This is suggested by preliminary results obtained in collaboration with P. Bourgaux and D. Bourgaux which show that, in mouse embryo cells infected by Ts-a virus at high temperature, the viral DNA initiates its replication but cannot complete it, and is probably destroyed. Thus the function of the Ts-a gene, both in transformation and in activation, could be simply to maintain the integrity of the viral DNA. The different effect in BHK and 3T3 cells would still be ascribable to some difference between the two cells.

Even if the Ts-a gene plays a role in integration, it is irrelevant to the main consequences of transformation—namely, the characteristic modifications of cellular physiology. Operationally, the genes responsible for these changes can be defined as those responsible for abortive transformation. The genetic studies give no evidence concerning these genes, since none of the mutants studied affects abortive transformation. That such transforming genes exist, however, is suggested by the appearance of several new functions, which can be attributed to viral genes in the infected or transformed cells. Two of these functions are detected as virus-specific antigenic changes in the cells. One of them is the T-antigen (*35*), easily studied by immunofluorescence; the other is a surface antigen (*36*) which can be detected either by its effect on the transplantation of the transformed cells into an isologous host or by the binding of fluorescent antibodies. Both antigens are virus-specific.

Either the T-antigen or the surface antigen has been implicated as the possible determining factor in cell transformation. A continuing role of the T-antigen seems now excluded be-

cause the antigen appears to be absent in 3T3 cells transformed by the Ts-a virus when they are kept at high temperature (*37*). The possible role of the surface antigen in transformation has not been tested directly since mutants affecting it have not yet been isolated. The likelihood that the surface antigen and other concomitant surface alterations play an important role in transformation is high, however, since most other viral functions have been shown to be irrelevant for maintaining the transformed state of the cells. The rationale for the possible role of the surface changes is that they may alter the response of the cells to regulatory influences coming from the outside.

Another new function is induction of the synthesis of cellular DNA and of cellular enzymes involved in DNA synthesis (*38*). The induction is observed in lytically infected cells, during the time preceding lysis of the cells and at the same time as synthesis of viral DNA. It occurs in cells that, before infection, were in a resting stage and had therefore a very low rate of synthesis of DNA and enzymes. The characteristics of induction reveal that it is due to the expression of a viral gene. In fact, it is displayed only by DNA-containing viral particles; it is sensitive to interferon (*39*) and is inactivated if the virus is exposed to ultraviolet light, the sensitivity being lower than that for the inactivation of infectivity (*40*) but comparable to that required for replication of the viral DNA.

It seems likely that the induction of cellular DNA synthesis is related to transformation, because both phenomena derive from a loss of the normal response of the cells to factors that limit cellular multiplication. The fact that this is a property of transforming viruses and not of viruses unable to cause transformation is also striking. The gene that directly or indirectly causes induction may therefore be the main agent of transformation. Conceivably this gene may cause primarily another change, such as the formation of the T-antigen or of the surface antigen.

The relationship between viral genes and the new functions appearing in infected or transformed cells has in no case been established. For instance, despite the isolation of a considerable number of temperature-sensitive mutants of polyoma virus, none has been found to affect the induction function. This failure may be attributable either to the selection technique used for isolation of the mutants, which is based on lack of virus multiplication at 39°C, or to some special feature of the viral function. A hint favoring the latter possibly is given by studies on the induction of thymidine kinase by either polyoma virus or SV40. Under certain conditions, there is a strong increase in enzymatic activity after infection, and the properties of the enzyme are markedly different from those of the enzyme of uninfected cells (*41*), suggesting the formation of a new enzyme, specified by a viral gene. Yet, no thymidine kinase activity appears after infection of mutant cells lacking thymidine kinase (*42*). It seems likely, therefore, that the new enzyme arises through modification of a cellular enzyme. Perhaps several small proteins that function as modifiers of cellular enzymes are specified by viral genes; this device would enable the virus to utilize to the utmost its own very scanty genetic material. The main role of the modifiers may be that of subtracting some cellular enzymes from normal regulatory influences. If these ideas are correct, temperature-sensitive mutations of genes specifying modifiers may be infrequent, because the modifiers may be small and their functions may be rather insensitive to single amino acid substitutions.

Conclusions

The results that I have discussed raise questions of interest both for the regulation of animal cells and for the mechanism of neoplasms. An interesting question concerns the basis of nonpermissiveness, which is, to a large extent, a species-specific property. It may derive from a block of transcription by cellular repressors which happen also to repress viral genes, or from the inability of RNA polymerases and their cofactors to transcribe certain viral genes. It should be possible to answer these questions and thus to probe, through the use of viruses, into the nature of cellular specificity.

Another interesting question is the biological meaning of integration. It is unlikely that it represents mainly the result of selection for virus' sake, since the virus does not benefit appreciably from it. In fact, the integrated viral DNA only exceptionally initiates a viral replication cycle. An alternative interpretation is that we are witnessing, in these viruses, a more primitive evolutionary stage than the integration of prophage (where the selective value is clear). The basic meaning of the integration could be that DNA recombination is intimately connected to DNA replication, and that enzymes necessary for replication can also perform recombination, although at low frequency and under restricted circumstances. Thus the study of integration may be relevant to an understanding of the mechanism of DNA recombination.

The transforming action of these viruses is probably the consequence of the influence of viral products on crucial steps determining the growth rate of the cells, probably at the level of DNA synthesis. The same steps are apparently involved in the normal regulation of the growth rate of the cells. The virus seems only to cause a breakdown of the regulatory mechanisms, without affecting the efficiency of the growth process itself; the result is unregulated growth. There is a suggestion that this result is caused by virus-specified modifiers, which change the properties of cellular enzymes. The effect on cell regulation may be mediated by the surface changes of the cells. These changes may reflect changes either in the availability of some important intercellular transducer molecule or in some other cellular membrane, perhaps the nuclear membrane, which is likely to play a role in the basic processes of cellular growth.

The results obtained with these viruses throw light on one specific form of transformation, but they may also help explain other examples of neoplasms. The oncogenic action of RNA viruses, for instance, may also be explainable through their action on normal cellular processes, including the function of the surface membrane of the cell. However, many aspects of the replicative processes of viruses of the two classes are very different, and most gene functions of oncogenic RNA viruses are not known. There may be a relationship between these studies and the mechanism by which neoplasms are induced by physical and chemical agents, since these neoplasms might be the consequence of changes at the same step at which alteration by DNA viruses occurs, but through mutations of cellular genes. If this is the case, an

understanding of the mechanism of neoplasm induction by DNA viruses may clarify the mechanism of neoplasia in a general way.

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Mechanical Harvesting of Food

Machine harvesting systems can be developed only as a result of scientific research.

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Scientific research has made American agriculture the envy of the world. Last year less than 5 million U.S. farm workers produced food and fiber for over 200 million Americans and approximately 30 million people in other countries. This is a ratio of 1 to 46 and means that the remaining members of the working force could devote their time to creating the goods and performing the services that make up the high standard of living enjoyed in this country.

The high agricultural productivity is a result of research by many disciplines combined with the willingness of progressive growers to adopt these results. Yields per acre have been vastly increased by introduction of new plant varieties, by use of fertilizers, and by management of water. Losses from insects and disease have been decreased through proper use of chemicals and through methods of biological control. Agricultural engineers have contributed greatly by developing machines to do much of the hard work, so that one man can do the work formerly done by many. Many billions of dollars' worth of farm equipment is used in the United States. It is estimated that more than $2½ billion of farm equipment was sold in the United States last year; of this amount, harvesting machines accounted for approximately $550 million (*1*).

Harvesting requires more labor than any other operation in the production and marketing of most food and fiber crops. Fortunately, machines have been developed for harvesting almost all food crops, with the exception of most fruits and vegetables. For example, in the United States most grain (wheat, rice, oats, and so on) and beans are harvested with large machines called combines. A combine which harvests a swath 12 feet (3½ meters) wide costs approximately $8000. Such a machine enables one man to harvest a crop 100 times as fast as a worker using animal power and 1000 times as fast as a worker using hand tools. Animal and hand power are still used in many countries of the world.

In the last 5 or 6 years it has become difficult to find workers for harvesting fruit and vegetable crops. The only practical answer to the labor problems that are facing the fruit and vegetable industries is mechanization (*2*). The Agricultural Research Service of the United States Department of Agriculture and many state agricultural experiment stations are now conducting research on the problem of mechanizing the harvesting of fruits and vegetables.

This problem involves much more than making a machine to perform a

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