From the Molecular Biology of Oncogenic DNA Viruses to Cancer

Renato Dulbecco

Oncogenic viruses, able to elicit tumor formation in animals, have been on the scientific scene for many years. After the early discovery of Ellerman and Bang at the beginning of this century, Peyton Rous opened up the field in the next decade and in prophetic words gave a good hint of things to come. However, these discoveries were soon forgotten, and only after a long eclipse was interest in oncogenic viruses revived in the 1950's. My involvement in this field began at that time, when Rubin and Temin worked in my laboratory with the Rous sarcoma virus. When polyoma virus, a new oncogenic virus with different properties, was isolated in 1958, I jumped at the new opportunity and started working with it. Within a short time polyoma virus became the main interest of my laboratory, to be joined, a few years later, by SV40, another papovavirus. It became clear fairly soon that the molecular biology of these viruses could be worked out, and I set out to find the molecular basis of cancer induction. The results that I and a number of brilliant young collaborators have obtained during the following 15 years have brought us close to that goal. I will review the most interesting steps of our work and will then ask some questions concerning the nature of cancer and about perspectives for prevention and treatment. I stress the relevance of my work for cancer research because I believe that science must be useful to man.

 The author is deputy director for research at the Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London, W.C.2, and a fellow of the Salk Institute, San Diego, California. This article is the lecture he delivered in Stockholm, Sweden on 12 December 1975, when he received the Nobel Prize in Physiology or Medicine, a prize he shared with David Baltimore and Howard M. Temin. The article is published here with the permission of the Nobel Foundation and will also be included in the complete volume of *Les Prix Nobel en 1975* as well as in the series *Nobel Lectures* (in English) published by the Elsevier Publishing Company, Amsterdam and New York. The lectures by Baltimore and Temin will appear in later issues.

Integration: The Provirus

Let me start with a brief review of our work on the molecular events in transformation. The first results, crucial for future developments, showed that polyoma virus could be assayed in certain cell cultures (*1*), which we call permissive, and could induce a cancer-like state in other cultures (*2*) in which the virus does not grow, which we call nonpermissive. The induction of the cancer-like state in vitro was called transformation. We were able to show that the virus contains DNA (*3*), and within a few years we gave the first evidence of its cyclic, or circular, shape (*4*), which is important for two critical biological events: DNA replication and integration. In integration, which we discovered a few years later with the virus SV40 (*5*), the viral DNA becomes a provirus, that is, it establishes permanent, covalent bonds with the cellular DNA. The cyclic configuration explains how a complete molecule of the SV40 DNA can be integrated without losses.

Integration is one of the key events in virus-induced cell transformation. It explained the persistence of the transformed state in the cell clone deriving from a transformed cell, since the provirus replicates with the cellular DNA. It also permitted us to resolve one of the main questions about the role of viruses in transformation. It was known at the time that papovaviruses leave their footprints in the cells of the cancers they induce and those they transform in vitro, in the form of characteristic antigens. However, it was not known whether the antigens were expressed by viral genes or by derepressed cellular genes. Hence, it was uncertain whether cells were transformed by the expression of viral genes persisting in the cells or, alternatively, whether the virus altered the cells by a hit-and-run mechanism, changing the expression of cellular genes and then leaving. The demonstration that viral DNA is integrated in the cells, in conjunction with the finding that the provirus is transcribed into messenger RNA (*6*) hundreds of generations after the establishment of a transformed clone, made the hit-and-run hypothesis unlikely and supported a continuing role of viral gene functions in determining transformation. This possibility was later supported by observations with abortively transformed cells, which behave as transformed only for several generations after infection, but then return to normal (*7*). When they are back to normal these cells no longer contain the viral DNA (*8*).

The viral genes that remain unexpressed in the transformed cells, such as those for capsid protein in SV40-transformed cells, were also interesting, although in a different way. In fact their expression could be renewed in heterokaryons formed by fusing transformed cells with permissive cells (*9*), a result that gave the first evidence that the viral functions are under the control of cellular functions. The provirus thus became a tool for studying regulation of DNA transcription in animal cells. Subsequently, the presence of giant RNA's containing viral sequences in the nucleus of transformed or lytically infected cells (*10*) raised the question of the initiation and termination signals for transcription in animal cells, as well as the question of processing of nuclear RNA precursors of messenger RNA, questions that are still largely unresolved.

Viral Functions in Transformation

In the meantime efforts were directed at identifying the viral genes transcribed in the transformed cells. It was established that in lytic infection with SV40 the whole viral DNA is transcribed in two nearly equal parts—one early, before the inception of replication of the viral DNA, the other late, after DNA replication has begun—and that the early RNA is also present in transformed cells (*6*). Subsequently, the early and the late messengers were found to be transcribed from different DNA strands (*11*), an observation that facilitated further characterization of the viral transcripts. Later work in other laboratories with specific fragments produced by restriction endonucleases confirmed and refined these findings, and the results were extended to adenoviruses by showing that a segment of the early part of that DNA is always present and transcribed in transformed cells (*12*).

These facts suggested that some early viral function is essential for maintaining the transformed state but they could also be interpreted differently: for instance, transformation might be caused by the mere presence of the viral DNA in the cellular DNA, the persistent viral functions being perhaps required for establishing and maintaining integration.

Attempts were made to solve the dilemma by isolating temperature-sensitive mutants affecting either initiation or maintenance of transformation. Many transformation mutants were found, with mutations all clustered in a segment of the early region of the viral DNA, designated as the A gene, but they were all initiation mutants (*13*). These mutations prevent the onset of transformation at high but not at low temperature, and cells transformed at low temperature remain transformed at high temperature. It was not possible to find clear-cut maintenance mutants, that is, mutants capable of causing a complete reversion of the phenotype when cells transformed at low temperature were shifted to high temperature. However, careful observation later showed that the initiation mutants were also partial maintenance mutants, since the cells they transform undergo a partial reversion of phenotype at high temperature (*14*). This result shows that the viral genes play a continuing role in transformation; however, the failure to obtain complete maintenance mutants suggests that the relation between viral gene expression and cell phenotype is complex.

Search for the Viral Transforming Protein

Further progress in this subject has been achieved by studying the proteins specified by the early region of the viral DNA. This work has centered around the so-called T antigen (*15*) present in the nucleus of cells infected or transformed by SV40; the synthesis and properties of this antigen are affected by mutations of the A gene (*16*). In nonpermissive transformed cells the antigen is a protein with molecular weight of about 94,000 daltons (*17*), which binds firmly to double-stranded DNA but without much specificity (*18*). That the T antigen is specified by the viral DNA is strongly suggested by its in vitro synthesis by a wheat germ extract primed with various messengers (*19*), especially since the size of the product depends on the nature of the messenger. Thus, when the messenger was viral RNA made in vitro by transcribing SV40 DNA with *Escherichia coli* RNA polymerase, an antigenic protein of about 62,000 daltons was synthesized; but when messenger RNA extracted from infected cells was used, the protein synthesized was, like the T antigen of transformed cells, of about 94,000 daltons. The discrepancy of the two molecular weights makes it very unlikely that the T antigen is a cellular protein modified by a viral function, because two different proteins would have to be modified in the same extract depending on the messenger used. In contrast, the synthesis of a shorter polypeptide chain with the artificial messenger may be justified by the absence of accessory signals, such as the special nucleotide sequence present at the 5′-end, known as "cap," polyadenylate at the 3′-end, and possibly other modifications. Further definition of these findings awaits peptide maps of the various products.

Since the early, transforming, part of the SV40 genome can specify proteins of a molecular weight of about 100,000 daltons altogether, the T antigen is likely to be its sole product and, therefore, to be the transforming protein. However, the same protein must also initiate viral DNA replication, which cannot begin at high temperature in cells infected by mutants of the A gene. The different functions in transformation and lytic infection could be performed by different domains of the same protein, or could result from modifications (such as phosphorylation and glycosylation) or from processing. Processing of SV40 T antigen seems to occur in lytically infected cells which contain a smaller T antigen of about 84,000 daltons; this smaller size contrasts with the regular size (94,000 daltons) of the antigen specified in vitro by messenger RNA extracted from the same cells (*17*). Whether the two forms of the antigen have different roles in transformation and DNA replication remains to be established.

Since the transforming protein should control both initiation and maintenance of transformation, the partial reversion of the phenotype of cells transformed by A mutants when shifted to high temperature may be explained by a decreased requirement for the transforming protein once transformation has taken place, which in turn could result from a positive feedback stabilizing the transformed state. For instance, unstable protein monomers specified by the mutated gene might form self-stabilizing oligomers (*20*), or the transforming protein might generate changes that tend to favor the transformed state. An example of the latter model is the β-galactosidase induction in *E. coli* which is maintained by inducer concentrations much smaller than that required for initiating induction, because inducer is pumped into the cells by the induced permease (*21*). I wonder whether a certain degree of self-stabilization of the state of gene expression is a general property of animal cells which has developed for maintaining differentiation.

Cellular Events in Transformation

I now turn to cellular events participating in transformation, which will be the main problem after the remaining questions on the role of the virus have been answered. Among the cellular events are functional changes and mutations. Some functional changes, which affect many cellular properties, are associated with the shift of resting cells to a growing state after infection with polyoma virus or SV40 (*22*); other changes observed in transformed cells and in cancer cells in general consist of the reexpression of cellular genes normally expressed in a preceding state of differentiation, in fetal life (*23*). These functional changes might be caused by the binding of transforming proteins to DNA; if so, they may be mediated by an alteration of transcription of the cellular DNA. However, we do not know whether the transcription pattern changes, because experiments based on competition hybridization have given ambiguous results. Perhaps the methodology is not good enough. Cloning of cellular DNA fragments in phages or plasmids may afford the necessary probes for carrying out significant experiments.

In order to understand further how the virus deregulates cellular growth we would need detailed knowledge of the mechanisms of growth regulation in animal cells, which is now lacking. However, certain useful ideas about growth regulation are now available, and can be used to draw inferences about the action of the virus. Thus it seems clear that with a given cell type, growth regulation involves a complex chain of events, beginning with extracellular regulators of many kinds, probably interacting with the cell plasma membrane. Cytoplasmic mediators then appear to transmit regulatory signals from the plasma membrane to the nucleus, where they perhaps control DNA-binding proteins similar to the transforming protein of papovaviruses. The complexity of growth regulation increases markedly when different cell

types are considered, since they seem to recognize different sets of extracellular regulators and may have different mediators and DNA-binding proteins.

Proceeding from this general picture it would be tempting to propose that the viral transforming protein replaces one of the normal nuclear regulatory proteins of the cell and, being unaffected by the mediators that control the normal protein, keeps growth-related transcription going, bypassing the signals of the plasma membrane. If so, however, the transformed state should be dominant over the normal state in cell hybrids, whereas the contrary is usually true (*24*). On the other hand, the dominance of the normal state could be explained if the transformed cells had a changed surface, unable to respond to regulatory signals. Such a change could result from the reexpression of fetal functions to make the transformed cells anachronistic, that is, belonging to a stage of differentiation inappropriate to that of the organism which contains them. The cells with an anachronistic surface, being insensitive to the growth regulators which operate on adult cells in the adult organisms, would grow without control. A striking support of the role of cell anachronism in cancer has been obtained with teratoma, a tumor originating when cells from an early embryo are transplanted to an adult environment. When, after many transplants, cells of this tumor are introduced back into a blastocyst (an early embryo), they return to normal (*25*), presumably because the internal growth control of the cells becomes again matched by the environmental regulators of the recipient embryo. In this model a hybrid cell formed by fusing a transformed and a normal cell may be untransformed if the normal partner contributes normal surface components which respond to the normal extracellular regulators. For this result to be possible, anachronistic transcription after cell fusion should not be initiated on the DNA deriving from the normal parent. The virological studies suggest that this may well be the case, since the initiation of transformation seems to require much more transforming protein than its maintenance.

It would be important to recognize the developmental period in which the anachronistic genes of transformed or cancer cells are normally expressed, not only for understanding but possibly also for controlling cancer. In fact, if the growth regulators specific for the periods expressed in cancer cells could be identified, they could be used for halting the growth of the cancer cells.

Role of Cellular Mutations

I will now consider the other cellular events important in viral transformation: cellular mutations. Several results suggest that cellular mutations may be needed for obtaining the full state of transformation with papovaviruses. Thus, after infection primary cultures generate clones with various degrees of transformation, some of which appear to undergo full transformation in steps (*26*) that may correspond to the occurrence of cellular mutations. Cells that achieve full transformation immediately, as is common with permanent lines, may have already undergone similar mutations before infection. Some cellular mutations occurring in transformed cells may even be virus-induced, because in the early stages of transformation by papovaviruses cells of primary cultures have frequent chromatid breaks (*26*). Conversely, cells fully transformed by SV40 can revert to a relatively normal phenotype although they still contain normal viral DNA and T antigen (*27*). It is conceivable that these mutations are reversions of mutations of the former kind, which enhance the transformed state of the cells. Stepwise transformation may occur not only with viruses. Thus I have observed it in primary cultures exposed to a chemical carcinogen. In this experiment fully transformed cells evolved from the normal cells, which have limited life. The normal cells first generate cells with unlimited life but unable to form colonies in agar, then cells with progressively increasing colony-forming efficiency in agar, and finally cells that reach 100 percent efficiency.

All these observations show the important role of cellular mutations in cell transformation induced by different agents. This conclusion is reinforced and generalized by additional findings, such as (i) the experimental enhancement of the transforming activity of viruses by mutagenic agents (*28*); (ii) the elevated cancer frequency in some genetic diseases; and (iii) the evidence that most carcinogens are promutagens, that is, generate mutagenic substances when acted upon by normal metabolism (*29*). Most of the carcinogens themselves must be activated by metabolism in a similar way in order to induce cancer.

Prospects for Cancer Prevention

I now turn to some general deductions concerning the etiology and possible prevention of human cancer which derive from the various points I have discussed so far. One deduction, deriving from the persistence of the viral DNA in the cells, is that we can test whether a given DNA virus is a possible agent of human cancer by looking for its DNA in the cancer cells. I think that much more extensive surveys than those carried out so far are warranted, but they should have a sensitivity sufficient to detect fragments of viral DNA of about 1 million daltons, which is within the reach of modern technology, even with the most difficult viruses. A positive finding would be significant because DNA viruses do not appear to exist in widespread endogenous forms.

Another deduction is that somatic mutations are one of the fundamental ingredients of cancer, although they appear to require the occurrence of several other events not yet understood. The role of mutations in turn suggests that the incidence of cancer in man could be reduced by identifying as many promutagens as possible, and by eliminating them from the environment. One important feature of this approach to cancer prevention is that it can be started now, since these substances can be identified with simple bacterial tests suitable for mass screening (*30*). The feasibility of prevention is shown by the fact that the promutagens already identified in a preliminary screening, such as tobacco or some hair dyes, are inessential for human life (*31*).

However, it is practically difficult to achieve a substantial reduction of the use of these substances, as shown by the example of tobacco. According to epidemiological evidence, tobacco smoke is the agent of human lung cancer, which in Britain is responsible for one in eight of all male deaths (*32*). Yet only mild sanctions have been imposed on tobacco products, such as a vague health warning on cigarette packets which sounds rather like an official endorsement. Any limitation on the use of tobacco is left to the individual, although it is clear that the individual cannot easily exercise voluntary restraint in the face of very effective advertisements, especially as one does not usually appreciate the danger of a cumulative action over a long period of time.

The lax attitude of governments toward tobacco probably also derives from the difficulty of appreciating epidemiological evidence, especially since this evidence is contradicted from time to time by single-minded individuals who use incomplete or even erroneous analyses of the data and whose views are

magnified out of all proportion by the media. However, the recent recognition that tobacco smoke contains promutagens contributes direct experimental evidence on the dangers of tobacco smoke, evidence on which there cannot be any equivocation. I, therefore, call on governments to act toward severely discouraging tobacco consumption, and to act now because it will be at least 30 years before their action has its full effect.

Although tobacco smoke is a striking example of an environmental carcinogen, many others are known and probably many more remain to be identified. Identification by conventional tests is difficult because they are costly and laborious, but they can now be replaced by the bacterial tests for promutagens. Since the tests are easy and inexpensive it should be possible to investigate many normal constituents of the environment, and every new compound before it is offered to the public. The feasibility of such a program is borne out by the finding that most of the commonly available substances are not promutagens (*31*). Given the strong correlation between mutagenicity and carcinogenicity (*29*), any promutagen is suspect and, if at all possible, should be withdrawn.

In fact, this is precisely the attitude that scientists have taken for themselves concerning the experiments in genetic engineering, which carry the theoretical possibility of creating new viruslike molecules endowed with carcinogenic activity. Although the danger is only hypothetical, experiments that might be very useful for science and society have been postponed until they can be carried out under the strictest safeguards (*33*). Governments have accepted this position and are eager to impose severe restrictions on the performance of these experiments. While I fully approve of their concern, I cannot help noticing that they follow a double standard: if there is any doubt you must discourage experiments, but if there is any doubt you cannot discourage cigarettes.

Biologists and Society

This discussion about cancer prevention is a development of the experimental results obtained in the field of oncogenic viruses, but it is also strongly influenced by the new social conscience of many scientists. Historically, science and society have gone separate ways, although society has provided the funds for science to grow and in return science has given society all the material things it enjoys. In recent years, however, the separation between science and society has become excessive, and the consequences are felt especially by biologists. Thus, while we spend our life asking questions about the nature of cancer and ways to prevent or cure it, society merrily produces oncogenic substances and permeates the environment with them. Society does not seem prepared to accept the sacrifices required for effective prevention of cancer. The situation is clearly unacceptable, and we biologists would like to see it corrected. We have ourselves begun to put our house in order, by banning some experiments that may contain a risk for mankind. We would like to see society take a similar attitude, abandoning selfish practices that are dangerous for society itself. We would also like to see a new cooperation of science and society for the benefit of all mankind and hope that the dominant forces in society will recognize that this is a necessity.

References and Notes

1. R. Dulbecco and G. Freeman, *Virology* **8**, 396 (1959).
2. M. Vogt and R. Dulbecco, *Proc. Natl. Acad. Sci. U.S.A.* **46**, 365 (19→ R. Dulbecco and M. Vogt, *ibid.*, p. 1617.
3. J. D. Smith, G. Freeman, M. Vogt, R. Dulbecco, *Virology* **12**, 185 (1960).
4. R. Dulbecco and M. Vogt, *Proc. Natl. Acad. Sci. U.S.A.* **50**, 236 (1963).
→ J. Sambrook, H. Westphal, P. R. Srinivasan, R. Dulbecco, *ibid.* **60**, 1288 (1968).
→ K. Oda and R. Dulbecco, *ibid.*, p. 525.
7. M. Stoker, *Nature (London)* **218**, 234 (1968).
8. P. Berg and M. Stoker, personal communication.
9. H. Koprowski, F. C. Jensen, Z. Steplewski, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 127 (1967); J. F. Wakins and R. Dulbecco, *ibid.*, p. 1396.
10. S. Tonegawa, G. Walter, A. Bernardini, R. Dulbecco, *Cold Spring Harbor Symp. Quant. Biol.* **35**, 823 (1970).
11. D. M. Lindstrom and R. Dulbecco, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1517 (1972).
12. P. A. Sharp, U. Petterson, J. Sambrook, *J. Mol. Biol.* **86**, 709 (1974); P. A. Sharp, P. H. Gallimore, S. J. Flint, *Cold Spring Harbor Symp. Quant. Biol.* **39**, 457 (1974).
13. M. Fried, *Proc. Natl. Acad. Sci. U.S.A.* **53**, 486 (1965); W. Eckhart, *Virology* **38**, 120 (1969); P. Tegtmeyer and H. L. Ozer, *J. Virol.* **8**, 516 (1971).
14. R. G. Martin, J. Y. Chou, J. Avila, R. Saral, *Cold Spring Harbor Symp. Quant. Biol.* **39**, 17 (1974); J. S. Butel, J. S. Brugge, C. A. Noonan, *ibid.*, p. 25; G. Kimura and A. Itagaki, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 673 (1975).
15. P. H. Black, P. W. Rowe, H. C. Turner, R. J. Hubner, *Proc. Natl. Acad. Sci. U.S.A.* **50**, 1148 (1963).
16. P. Tegtmeyer, *Cold Spring Harbor Symp. Quant. Biol.* **39**, 9 (1974); M. Oxman, K. K. Takemoto, W. Eckhart, *Virology* **49**, 675 (1972); D. Paulin and F. Cuzin, *J. Virol.* **15**, 393 (1975).
17. R. B. Carroll, personal communication.
1→ ——, L. Hager, R. Dulbecco, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3754 (19→ D. Jessel, J. Hudson, T. Landau. D. Tenen, D. M. Livingston, *ibid.* **72**, 1960 (19→ S. I. Reed, J. Ferguson, R. W. Davis, G. R. Stark, *ibid.*, p. 1605.
19. A. E. Smith, S. T. Bayley, T. Wheeler, W. F. Mangel, in *In vivo Transcription and Translation of Viral Genomes*, A. Haenni and J. Beaud, Eds. (Institut National de la Santé et de la Recherche Médicale, Paris, 1975).
→ R. Dulbecco, *Proc. R. Soc. London Ser. B.* **189**, 1 (1975).
21. A. Novick and M. Weiner, *Proc. Natl. Acad. Sci. U.S.A.* **43**, 553 (1957).
→ R. Dulbecco, L. H. Hartwell, M. Vogt, *ibid.* **53**, 403 (1965); L. H. Hartwell, M. Vogt, R. Dulbecco, *Virology* **27**, 262 (1965).
23. J. H. Coggin, *J. Immunol.* **105**, 524 (1970).
24. F. Wiener, G. Klein, H. Harris, *J. Cell Sci.* **8**, 681 (1971).
25. B. Mintz and K. Illmensee, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 3585 (1975); V. E. Papaionnou, M. W. McBurney, R. L. Gardner, M. J. Evans, *Nature (London)* **258**, 70 (1975).
26. M. Vogt and R. Dulbecco, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 367 (1962).
27. R. E. Pollack, H. Green, G. J. Todaro. *Proc. Natl. Acad. Sci. U.S.A.* **60**, 126 (19→ H. C. Renger and C. Basilico, *ibid.* **69**, 109 (1972).
28. H. F. Stich, R. H. C. San, Y. Kawazoe, *Nature (London)* **229**, 416 (1971).
29. J. McCann, E. Choi, E. Yamasaki, B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
→ B. N. Ames, F. D. Lee, W. E. Durston, *ibid.* **70**, 782 (1973).
→ L. D. Kier. E. Yamasaki, B. N. Ames, *ibid.* **71**, 4159 (19→ B. N. Ames, H. O. Kammen, E. Yamasaki, *ibid.* **72**, 2423 (1975).
→ R. Doll, *J. Roy. Stat. Soc. Ser. A* **134**, 133 (1971).
33. P. Berg, D. Baltimore, S. Brenner, R. O. Roblin, M. F. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1981 (1975).