Cell Transformation by Viruses and the Role of Viruses in Cancer

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The frantic race to discover human cancer viruses that is now going on is justified by the importance of the practical consequences that such discoveries might have for cancer prevention. The state of the results, however, is confusing. Even that apparent stalwart of human viral cancer, the Burkitt lymphoma, has recently lapsed into uncertainty since it has been found to harbour not one but two different viruses: the Epstein-Barr herpesvirus and a leukovirus (Kufe, Magrath, Ziegler & Spiegelman, 1973). I think that much of this confusion comes from disregarding what is known in tumour virology and from the weakness of the theoretical bases which have motivated some of the experiments and justified some of the conclusions.

I would like to set the stage for considering the problem of viral oncogenesis in man by reviewing the available information concerning the biology of oncogenic viruses, using results obtained with two small viruses containing DNA—polyoma virus and SV40. These two small viruses will be referred to collectively as polyomaviruses, a family name. The studies carried out for some years in our own and other laboratories have yielded a considerable amount of information at the molecular level, which allows a number of conclusions to be drawn. The conclusions are applicable to other oncogenic viruses including those containing RNA, because in the cells these RNA viruses build a DNA replica of their genome, and their biological properties probably derive from the characteristics of that replica. The replica is built by an RNA-dependent DNA polymerase, or reverse transcriptase (Baltimore, 1970; Temin & Mizutani, 1970), which is present in the virions and in several steps synthesizes a double-stranded DNA, using as template the viral single-stranded RNA. Since no other RNA viruses are oncogenic it appears that oncogenesis is an attribute of DNA genomes within the cells. There may be several reasons for this correlation but, as we shall see, the most important may be to allow recombination with the cellular DNA.

Polyomaviruses are highly suitable as experimental tools because they have a very small genome, probably containing five or six genes. Furthermore, their DNA is circular and this fact, besides being useful in the experiments, confers on the viruses important properties which I will discuss.

In tissue cultures polyomaviruses multiply in permissive cells, which are killed. The oncogenic activity is especially evident in non-permissive cells which undergo a non-productive infection and are not killed. Some, usually a minority of the non-permissive cells, undergo a stable alteration (called transformation) and generate clones of cells that are similar to cancer cells in many properties. Transformation, however, is not an exclusive prerogative of non-permissive cells since identical changes occur in the lytic infection of permissive cells before they are killed (Dulbecco, 1970). This transient transformation affects all infected cells and therefore is very useful for biochemical and molecular investigations. A transient transformation also occurs in non-permissive cells after infection, and

is known as abortive transformation (Stoker, 1968). After a few divisions these cells again become normal.

The main event of transformation is a change of the regulation of cell growth. The fibroblastic cells that are widely used in transformation work require high concentrations of serum for multiplication and when this is exhausted they enter a resting stage. This event is especially evident in crowded cultures. The resting cells are usually in a postmitotic holding phase, sometimes called *G₀*; but I shall call this phase H-1 (from holding). From H-1 the cells re-enter the cycle upon serum addition or after wounding a crowded cell layer. It is likely that there is a regulation switch between H-1 and G-1 (the H-1 switch), which is activated by the serum.

The transformed cells have a much lower and different serum requirement (Holley & Kiernan, 1971) and they are unaffected by crowding (Dulbecco, 1970a). When the serum becomes exhausted they go more slowly through the cycle but do not enter the holding phase. In these cells the H-1 switch seems stuck in the direction of the cycle; hence they do not require the serum factor that turns the switch toward the cycle in the untransformed cells. The numerous differences between transformed and normal cells in growth properties, cell and culture morphology, surface glycosylation and agglutination by lectins probably all derive from this single difference. In fact, the differences tend to vanish when cultures of transformed cells are compared with uncrowded cultures of normal cells. The concomitant variation of all these cellular features suggests that they are produced by a common mechanism. Cyclic AMP seems to be intimately involved in their control, since its concentration is elevated in dense cultures of normal cells, but is low in actively growing cells as well as in transformed cells (Johnson & Pastan, 1972). The roles of cyclic AMP and serum on the growth of normal cells suggest that cell growth regulation is initiated at the cell surface membrane. In fact, the serum factors, being large proteins, are unlikely to penetrate through the surface, and cyclic AMP is generated and destroyed by membrane enzymes. Hence in normal regulation the serum may interact with specific receptors at the cell surface which are frozen in the active state in transformed cells. However, these receptors have not been identified. They can be postulated to be identical to the H-1 switch, which also appears to be the target of transformation.

Another important event in stable transformation is the integration of the viral DNA in the cellular DNA. The viral DNA present in the cells is recognized by hybridization with radioactive viral DNA or RNA (Westphal & Dulbecco, 1965). Most transformed cells contain between one and six to eight copies of viral DNA; but in some transformed cells only a fraction of the viral genome is present. In several lines of transformed cells the viral DNA has been shown to be integrated, i.e. covalently bound to the cellular DNA, and this result probably applies to all or most cell lines transformed by polyomaviruses. Integration has been demonstrated by fractionating the DNA extracted from transformed cells, for instance by sedimentation in an alkali sucrose gradient, which separates the large cellular DNA from the smaller free viral DNA, if present (Sambrook, Westphal, Srinivasan & Dulbecco, 1968). After fractionation the hybridizable viral DNA is found in the long cellular DNA molecules to which it must be bound covalently. An important feature of integration is that in cells containing more than one copy of viral DNA, each copy is integrated individually. This was shown by experiments in which hybrids formed by the extracted cellular DNA with synthetic radioactive viral RNA were tested for their ability to stick to nitrocellulose filters (Haas, Vogt & Dulbecco, 1972). In order to stick, the hybrids must be bound to unhybridized single-stranded DNA. In fact, hybrids formed by pure viral DNA saturated with viral RNA do not stick, since they are entirely double-stranded. The hybrids formed by

the DNA of the transformed cells were found to stick to the filters even at saturating amounts of viral RNA, owing to their attachment to cellular DNA which remained unhybridized. If before hybridization the extracted DNA was broken to fragments of decreasing size, at a certain size the hybrids began to be lost. Evidently this can happen when the fragments are equal in length to the integrated DNA. This critical length was found to correspond to the length of a viral DNA molecule, showing that the molecules are individually integrated.

In many non-permissive transformed cells the integrated DNA is unable to replicate independently of the cellular chromosome and to generate infectious virus; therefore the infection is latent. Virus production can however be induced by chemical agents such as mitomycin, or by fusing the transformed cell with a permissive cell (Koprowski, Jensen & Steplewski, 1967; Watkins & Dulbecco, 1967).

The latency of the viral genome in transformed cells of the non-permissive type is generally due to incomplete transcription (Oda & Dulbecco, 1968; Sambrook, Sharp & Keller, 1972); its activation after cell fusion probably occurs because the permissive cell supplies suitable transcription factors lacking in the non-permissive cells. This observation brings forth another important feature of the transformed cells, i.e. that the expression of the transforming genome is controlled by cellular genes. This control may be very extensive since in addition to factors the cell may supply the promoter where transcription of the viral DNA begins. This possibility is suggested by the observation that in SV40-transformed 3T3 cells the viral messenger RNA is in long molecules which are partly cellular (Lindberg & Darnell, 1970; Tonegawa, Walter, Bernardini & Dulbecco, 1970). If transcription of these hybrid messengers begins on the cellular DNA, a viral genome can only be transcribed if it is included in a transcribed segment of cellular DNA. A viral genome integrated in an untranscribed cellular DNA segment, such as heterochromatin, would remain unexpressed and would not cause transformation.

Not all integrated genomes are complete: defective genomes can also be integrated and may cause transformation, as, for instance, in cells in which hybridization detects less than one polyoma genome (Berg & Stoker, personal communication). This and other results show that the activity of only a fraction of the viral genome is required for transformation; this fraction contains genes for viral DNA replication and for altering the regulation of cell growth but not for capsid proteins.

The consequences of integration are most evident in non-permissive cells where integration allows the formation of stably transformed clones; however, it occurs also in lytic infection of permissive cells, especially if carried out at high multiplicity (Lavi & Winocour, 1972). In these cells integration causes the emergence of circular DNA molecules containing covalently linked cellular and viral sequences, which can be encapsulated to form viral particles. The two types of sequences can be recognized in electron micrographs of heteroduplex DNA resulting from the annealing of defective and standard molecules. This technique has revealed that the viral component sometimes is a small fraction of a viral genome (Robberson & Fried, personal communication). An intriguing possibility emerging from the study of heteroduplexes is that the viral DNA may combine with the cellular DNA preferentially at certain points. A similar regularity is suggested by adenovirus-SV40 hybrids which contain fragments of the SV40 genome of various lengths, all beginning at the same point. Whether this is due to preferential breaking points on the viral DNA or to functional requirements is not known.

The results of the integration studies have important implications. They show that the viral DNA is able to integrate reversibly as in bacterial lysogeny. It is therefore a 'provirus'. As in lysogeny, the detachment of the provirus from the host chromosome can be precise,

yielding an infectious molecule, but often is not, producing a defective molecule, in which a segment of viral DNA has been replaced by cellular DNA. Such defective polyomavirus molecules replicate in cells co-infected by infectious virus which acts as helper, and after serial passages at high multiplicity they may constitute most of the yield. Defective molecules containing a little viral DNA, corresponding to perhaps one or two genes, are probably generated by a sequel of integrations and detachments during serial passage.

Defective molecules that multiply (in the presence of helper) must evidently contain the origin of autonomous DNA replication; however, proviruses without replication origin may also be formed. Such highly defective viral genomes would not be recognized in lysates where they would be present in very small proportions since if excised from the host chromosome they cannot multiply. They could only be perpetuated as proviruses; in genetic tests they would appear as true cellular genes. Their viral origin may still be demonstrable by hybridization with a viral nucleic acid; but if they were produced by an unknown virus, such a test would be impossible and the genes would be considered cellular. It is clear, therefore, that the exchanges between viral and cellular DNA represent a powerful evolutionary mechanism for both the virus and the cells. The viral genome can incorporate cellular genes which can then evolve to fulfil viral functions, and conversely the cellular genome can incorporate viral genes as bona fide cellular genes.

Viral functions in transformation. Two polyoma genes control transformation: one affects the growth regulation of the cells, the other probably affects integration. These results were obtained by studying temperature-sensitive mutants of polyoma virus, which multiply at 32 °C but not at 39 °C. Similar results are being obtained with SV40.

The cellular growth control is affected by the *ts3* mutation of polyoma virus (Dulbecco & Eckhart, 1970). A characteristic of this mutant is to be temperature-dependent for viral multiplication only in some cells, such as those of the BALB/c-3T3 line in confluent layers (W. Eckhart & R. Dulbecco, unpublished data). In contrast, in secondary mouse embryo cells or in transformed cells the mutation is almost completely temperature-independent. The degree to which viral multiplication depends on the function of this gene in different cell types seems correlated with the sensitivity of the cells to growth regulation. This correlation can be explained by the simultaneous replication of viral and cellular DNA: the viral DNA may be unable to replicate unless the cellular DNA is also replicating. In confluent cultures strongly regulated cells (such as BALB/c-3T3) are in the H-1 phase and need the transient transformation induced by the virus in order to restart their own DNA synthesis; hence they may require the function of the viral gene affected by the *ts3* mutation. In contrast, cells less sensitive to regulation (such as mouse embryo cells or transformed cells) still maintain a substantial DNA synthesis without viral stimulation.

With non-permissive BHK cells, the *ts3* mutant causes regular transformation at low temperature, when the mutated gene is active; but these cells lose most of their transformed characters when the temperature is raised and the gene becomes inactive. If the temperature is later lowered again, full transformation is again restored. Changing the temperature also changes the concentration of cAMP in the cells (W. Seifert & L. Dulbecco, unpublished data), which is higher at high temperature, when the cells are similar to untransformed cells. These results suggest that the *ts3* mutation affects the viral gene that controls the growth regulatory mechanism of the cells. I shall call this gene, of crucial significance in transformation, the 'transforming gene'. The cyclic AMP result further suggests that the product of the transforming gene interacts with the postulated receptors for growth-controlling substances on the cell surface, altering their function. However, more precise understanding of the mode of action of the transforming gene must wait for a more complete molecular

analysis of the consequences of the mutation and possibly the isolation of the product of the gene.

Mutations in a second viral gene affect transformation, probably by altering the integration or the detachment of the provirus. Such mutants, of which *tsa* is the prototype (Fried, 1965) are in DNA replication genes since they are unable to replicate their DNA at high temperature. They do not cause stable transformation of non-permissive cells at high temperature; but the cells they transform at low temperature remain fully transformed when shifted to high temperature. Hence the function of this gene is required only transiently for stable transformation, suggesting that it controls integration. The transforming gene is certainly normal in these mutants because at high temperature they cause transient transformation of permissive cells (Fried, 1970) and abortive transformation of non-permissive cells in a regular way (Stoker & Dulbecco, 1969). That mutations in the DNA replication gene also affect the detachment of the provirus is suggested by the behaviour of a line of permissive 3T3 cells transformed by the *tsa* mutant (Vogt, 1970). At high temperature the cells of this line are stable. They contain several proviruses but most of the cells do not release virus. However, when the temperature is lowered, reactivating the gene function, most of the cells enter a lytic phase culminating in virus release and cell death. Clearly, lowering the temperature must allow provirus detachment from the cellular chromosome, implying a requirement for the function of the DNA replication gene.

As to the product of this gene, there are two suggestions. One is that it is a highly specific endonuclease which nicks the viral DNA at a single site (Cuzin, Blangy & Rouget, 1971), thereby initiating the recombination leading to integration or excision; the nick might be also required at some stage of viral DNA replication. The other possibility is that the gene product is a DNA-denaturing protein, similar to the product of T4 gene 32, which might be required both in DNA replication and recombination.

Other oncogenic viruses

The conclusions reached with polyomaviruses form the basis for understanding the biology of other oncogenic viruses; however, some differences are introduced by properties of these viruses that are not found in polyomaviruses. For instance, DNA-containing viruses of larger size, such as herpesviruses, contain genes for blocking host macromolecular syntheses; and these genes can mask the action of the transforming gene. In fact, transformation by herpesvirus has been obtained only by using u.v.-irradiated virus, in which presumably genes blocking host syntheses were inactivated (Duff & Rapp, 1971).

Concerning the RNA-containing leukoviruses, it is important to realize that they are a heterogeneous group, including viruses with different properties. Most pathogenic are the sarcoma viruses which, like polyomaviruses, transform every cell they infect, and have a transforming gene defined by temperature-sensitive mutations (Martin, 1970). A second class of leukoviruses includes viruses able to induce various forms of leukaemia or mammary cancer. In contrast to the sarcoma viruses, they have low pathogenicity since only a very small proportion of the cells they infect become transformed. Thus leukaemia viruses can grow in many cells in an organism without appreciably affecting their functions or developmental capability; and leukaemia may initiate a long time after most cells in an organism have become infected. Why a cell may be transformed while a vast number of others remain unaffected is not known. A possible explanation is that these viruses can only transform cells in which the cellular gene balance has been altered in a certain way by rare virus-independent genetic events. This suggestion is based on the recognition that the balance of cellular genes affects transformation by polyomaviruses, sometimes bringing about a reversion of the

transformed state. It seems likely that leukoviruses of low pathogenicity do not possess a transforming gene; they are intrinsically non-transforming and are merely co-oncogenic. A third class of leukoviruses contains non-pathogenic viruses.

All leukoviruses can integrate, forming proviruses; and the expression of the provirus is under the control of cellular genes (Payne & Chubb, 1968; Taylor, Meier & Meyers, 1971; Taylor, Meier & Huebner, 1973).

Biologically, leukoviruses differ from polyomaviruses because they do not need the function of the transforming gene for their replication. In fact, even in sarcoma viruses, which possess a transforming gene, transformation and replication are independent functions and are affected by different mutations. The rationale for this difference is that the DNA synthesis requirement for polyomavirus replication is high, but is very low for the leukoviruses, whose progeny is RNA. This would make the transformation-dependent activation of DNA synthesis irrelevant for the reproduction of sarcoma viruses.

According to some theoretical views, leukoviruses or their proviruses are the agents of spontaneous cancers. In order to discuss this possibility it will first be useful to examine certain properties of leukoviruses, which have been extensively investigated in the last decade. They concern the relation of leukoviruses to their hosts. The most striking finding is the widespread presence of proviruses of the non-transforming leukoviruses in many animal species, in the absence of any symptom of infection (Baluda, 1972). Some of these proviruses are transmitted hereditarily from an animal to its progeny just like regular cellular genes (Bentvelzen, Daams, Hageman & Calafat, 1970; Weiss & Payne, 1971). Others are transmitted congenitally, i.e. through the embryo (Burmester, Gentry & Waters, 1955) or shortly after birth through the milk (Bittner, 1940). The proviruses are often silent and can be activated to express their functions and to yield infectious virus by BuDR or by some carcinogens (Lowy, Rowe, Teich & Hartley, 1971; Aaronson, Todaro & Scolnick, 1971; Weiss, Friis, Katz & Vogt, 1971). In contrast, sarcoma viruses, like polyomaviruses, exist as provirus only in tumours or in transformed cells and not in the genome of normal animals. Presumably they cannot exist permanently in the genome because their accidental activation, by initiating a cancer, would be lethal.

Another important finding is that the ability of leukoviruses to infect new cells is limited by strict host specificities determined by cellular genes (Pincus, Hartley & Rowe, 1971a; Pincus, Rowe & Lilly, 1971b; Payne, Pani & Weiss, 1971). In some cases the virus is unable to infect the cells of the same species or even the same animal strain by which it is produced under the influence of BuDR, but can infect cells of different hosts (Aaronson *et al.* 1971; Livingston & Todaro, 1973).

These two special host-virus relations probably have been selected in evolution to minimize the danger of widespread infection by leukoviruses, even those of low pathogenicity. Thus their proviruses might be silent because they have been confined to untranscribed parts of the DNA; and the limitations on spreading of the virus by infection are a second line of defence, in the event the provirus breaks through. The second defence may be the more important one because if the virus does not multiply after activation in some cells, it is unlikely that it will cause any transformation, since the probability of the event is so low.

The familiar inbred mouse strains such as AKR in which the virus can exist both as provirus and as infectious virus (Rowe *et al.* 1971) are probably unusual and result from artificial selection in the laboratory.

Sarcoma viruses. We have seen that these viruses have a transforming gene, which is however inessential and probably not an intrinsic part of the genome. We should now inquire into the origin of the transforming gene. It is a rather striking observation that many sarcoma

viruses arose in the laboratory. Thus the murine strains were isolated either from cells of transplanted tumours, which tend to collect viruses in their serial passages in different hosts, or from mice exposed to very high doses of a leukaemia virus. Genetically, sarcoma viruses differ profoundly from other seemingly related leukoviruses, such as leukaemia viruses. The sarcoma virus RNA is longer, and contains an extra piece probably not homologous to the leukovirus genome in hybridization experiments. What is the extra genome piece present in a sarcoma virus, and how has it been collected? Since many sarcoma viruses are defective (requiring other leukoviruses as helper) the extra genome piece has probably been incorporated by recombination, in exchange for a segment of the normal viral genome. In nature this event would be futile for the virus, giving it a transforming gene which it does not need and in exchange making it defective. However, the defect is not too damaging since it can be compensated for by co-infecting widespread leukoviruses. Nevertheless, the unnatural virus is unfit and is basically perpetuated by the experimenter who supplies the selective advantage denied by nature.

The acquired transforming gene must have pre-existed in the cell in which it was picked up, either in the cellular genome, as a cellular gene for malignancy, or oncogene, or in the genome of another virus. The question of the origin of the gene can be examined using as background the results obtained with polyomaviruses, which suggests several points. A first point is that a transforming gene is probably related to a cellular growth regulatory gene. Since it blocks a cellular regulatory function, its product probably interacts with a cellular constituent (such as the postulated receptors for growth-regulatory substances). Hence the transforming gene may derive from a cellular gene, just like genes specifying enzymes similar to cellular enzymes which are recognized both in animal and bacterial viruses. The viral gene would differ from the corresponding cellular gene in details that improve its usefulness for viral reproduction. If this assumption is correct, how has the transforming gene evolved? It is unlikely that it has evolved as part of a cellular chromosome, because the evolution would not confer any advantage on the organism of which it was a part; if anything, a disadvantage. And it cannot be assumed that the gene evolved while it remained unexpressed, since then there would be no driving force for meaningful evolution. In order to assign a goal to the evolution of the transforming gene, I suggest that it took place in a DNA virus. For instance, a cellular regulator gene first became incorporated in the circular DNA of a precursor of the present-day polyomaviruses. There, its evolution was driven by the advantage conferred on viral replication. After evolution, the transforming gene was available for transfer to the cell's DNA or to other viral DNAs.

It is doubtful in my mind whether the transforming gene was transferred to cellular DNA, since animal cells do not contain sarcoma proviruses as permanent constituents of their genomes. However, in the cells the transforming gene may be available in the DNA of exogenous viruses or plasmids from which it may be picked up when a sarcoma genome is formed.

These considerations affect the evaluation of the oncogene theory of Todaro and Huebner (Huebner & Todaro, 1969; Todaro & Huebner, 1972) which would account for spontaneous cancers through the activation of leukoproviruses, i.e. proviruses of leukoviruses. This theory arose when a leukoprovirus and a transforming gene could not be well differentiated. The centre of that theory is that the activation of a leukoprovirus will transform the cell in which it occurs, irrespective of infection of other cells. But presently this idea is untenable. Let me restate the reasons. Although leukoproviruses are extremely common and perhaps universal in animals, they do not contain transforming genes, and generate viruses of low or no pathogenicity. These viruses can transform with an appreciable frequency only if they

spread by infection to a very large number of cells. The viral aetiology of the neoplasias that are induced by such spreading viruses is not in doubt. On the other hand, transforming genomes which contain the transforming gene are never hereditarily transmitted, and the transforming gene itself may be a regular constituent only of viral DNAs. The previous considerations about the transforming gene suggest that spontaneous cancers may also be generated by mutations in cellular regulatory genes without viral intervention. The transforming gene thus produced would then be lost with the death of the cancer or of the organism.

Viruses in human cancer

I believe it is useful to consider what impact the results I have reviewed may have on the search for viral agents of human cancer. This is a field in which theoretical and even political considerations are crucial in determining the direction of research. I will bring forth several points deriving from the experimental work, in the hope that they may help in bringing things into the right perspective.

The first point is that in animals, naturally occurring cancers can be induced both by exogenous viruses propagated from one animal to another by infection and by endogenous proviruses after they are activated and spread by infection through the organism. Experimentally oncogenic viruses are always exogenous to the cell they transform. It is therefore unwise to neglect exogenous viruses as possible human cancer agents, as some politicians of science would have us do. In man the Epstein-Barr herpesvirus (Epstein & Barr, 1964) is the exogenous virus most strongly implicated so far as oncogenic, as the possible agent of Burkitt lymphomas. Its role is supported by the ability of similar viruses experimentally to induce a similar disease in monkeys (Melendez *et al.* 1969; Ablashi *et al.* 1971) and chickens (Biggs & Payne, 1963), and by the beneficial effect of viral vaccination in chicken (Purchase, Okazaki & Burmester, 1971). Some of the results implicating herpesvirus in cervical cancer (Nahmias, Josey, Naib, Luce & Guest, 1970) must also be taken very seriously. However, in neither case can we be sure of the aetiology. In fact, the aetiology of the Burkitt lymphoma has recently become questionable since it has been reported that the tumour also contains some leukovirus (Kufe *et al.* 1973). How serious a blow is this to the idea of a herpesvirus aetiology? This question, which cannot be answered, leads me to the second point I wish to make: finding a virus or a viral genome in a cancer has no aetiological connotation. Viruses can multiply better, and proviruses can become preferentially active in cancer cells (Todaro, 1972). Therefore, the slogan: 'Virus induces cancer' must be paired to the counterslogan: 'Cancer induces virus' (which was invented by R. A. Weiss). If the virus is exogenous its role may be established by vaccination used as an experimental tool. This is another good reason for concentrating much effort on exogenous viruses.

The third point I wish to make can also be illustrated using the Burkitt lymphoma. In this cancer we find that the putative agent is very widespread, so that there must be important concurring factors when it rarely causes cell transformation. The same is true for leukoviruses in the induction of natural tumours in animals. These two examples are probably representative of a general situation; therefore it is likely that human cancers will not be found to be induced by highly oncogenic viruses present only in the cancer cells. Rather, we will have to cope with a widespread virus causing rare cancers. This will make aetiological assignments very difficult. On the other hand, the conditions allowing the occurrence of the rare transformations will become highly interesting, perhaps even more than the virus itself, and will deserve a considerable research effort.

The fourth point is that the animal models may not closely apply to man. Leukoviruses especially may not have the same role as in mice or chickens. One reason could be human

longevity. Longevity is perhaps only possible because in evolution man has developed to a higher degree those genetic mechanisms that already limit the oncogenic activity of leuko-viruses in mice and chicken, both in terms of provirus activation and infectious spreading.

The final point is that at this time the impetus to discover a human cancer agent makes people forget what microbiologists have learned over many years of experience, namely that microbes are powerful model systems for getting at the roots of a great many baffling problems of life. Cancer is such a baffling problem, whose roots are still well hidden. Using suitable viruses we should be able to go very far in uncovering these roots. Thus further studies of the transforming gene, the product it specifies, the cellular constituents it interacts with, their mode of action and their role in cell regulation are all workable problems whose answers should come within a reasonable time, if effort is continued. I suspect that learning these answers would take us very far in understanding viral carcinogenesis and probably even beyond the viruses, to the heart of the problem of cancer itself.

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