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Virus-Cell Interactions and Viral Antimetabolites

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VIRUS-CELL INTERACTIONS AND VIRAL ANTIMETABOLITES

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Edited by

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Preface

The development of appropriate vaccines has resulted, in many countries, in the virtual elimination of such viral diseases as polio and smallpox. But the vital problem of combatting viral infections already established in the organism is still very much with us. At the moment we can only take consolation in the fact that current research efforts appear to hold out the promise of at least some success in this direction in the near future. This was one of the reasons which dictated the selection of the subject of this symposium at the 6th FEBS Meeting in Madrid, two years previously, i.e. even prior to the exciting new advances in the field of the RNA oncogenic viruses, with their striking confirmation of the earlier proposals of Temin regarding the existence and role of reverse transcriptase in the mode of action of oncornaviruses. These developments, apart from the new perspectives they have opened up in the search for effective agents against tumour and other viruses, have in turn had a profound impact on the whole of molecular biology, the full implications of which cannot as yet be assessed.

It is obviously not feasible in a Symposium such as this to cover more than several facets of the multitude of specialized methods of approach to the study of virus-cell interactions and the problem of viral diseases. But, as will be seen from the contents of this volume, an attempt has been made to gather a sufficiently varied number of participants involved in different disciplines to provide a reasonably broad outline of progress both in basic research and in the development and clinical evaluation of the newer anti-viral agents. It is our hope that, as in the past, the presentations will also prove reasonably informative to the non-specialist.

The division of this Symposium into two distinct sections, one on Virus-Cell Interactions, the other on Viral Antimetabolites, was clearly dictated by reasons of convenience. It is unrealistic to consider a viral antimetabolite as an isolated entity and, in fact, only an adequate understanding of the various phases of virus-cell interactions, and the accompanying biochemical processes, can be expected to render possible a more rational approach to the design of therapeutically effective antiviral agents, as compared to the laborious screening techniques still widely employed, but which nonetheless have provided us with such promising drugs as 5-iododeoxyuridine, the rifamycins, the arabinosyl nucleosides, amantadine, etc.

These introductory remarks would be incomplete without an expression of appreciation to the Bulgarian Biochemical Society for the hospitality extended to the Symposium participants, and to the co-organizers, Dr. D. Naskkov and Dr. E. Golovinsky, for their untiring efforts prior to, and during the course of, the meetings.

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Interactions Between Host Genome and Oncornaviruses in Oncogenesis

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Tumours can be induced in animals by various factors such as ionizing radiation, a great variety of chemical compounds, viruses and so on. But the most interesting are those tumours which arise spontaneously. Many inbred strains of laboratory animals have been developed which display great differences in incidences of spontaneous tumours in the various organs. This initially led to the concept that such tumours are hereditary. Indeed there are a few cases of clear-cut inheritance of a neoplastic disease such as pulmonary tumours [12], ovarian tumours [56] and mammary carcinomas in mice [4, 47] and renal adenomas in rats [21], but in most cases the genetic situation proves to be far more complex [29]. In view of the inducing factors mentioned above, the idea of cancer as a genetic disease has been given up in general, although it is recognized that host genetic factors will play some role in either spontaneous or induced development of a tumour.

From some spontaneous tumours oncogenic viruses have been retrieved in which RNA-containing viruses are prevalent. It must be emphasized that the list of "natural" tumour viruses is small. Well established oncogenic RNA viruses (oncornaviruses) are the agents of leukaemia in chickens, mice, cavias and cats, of sarcoma in chickens, mice and cats, and of mammary carcinoma in mice. In contrast to the oncogenic DNA viruses, these agents constitute a homogeneous group of viruses with regard to structural, biochemical and biological characteristics [49]. The virions are spheres about 100 nm in diameter, having a spherical internal electron-dense structure located at a restricted site, and lipid-rich outer membranes, which are formed at the cell membrane from which the virions are liberated by a budding process. Their genome consists mainly of a large single-stranded RNA molecule (10^7 daltons), which presumably consists of four or five subunits held together by hydrogen bonds. They contain a specific RNA-dependent DNA polymerase and other enzymes associated with the production of a double-stranded DNA using viral RNA as initial template. The

oncornaviruses are oncogenic although not necessarily exclusive to their natural hosts, in contrast to several oncogenic DNA viruses. They are in general not cytopathic, insofar as neoplastic conversion is not regarded as pathologic to the cell itself. The transmission of these viruses is mainly vertical, often prenatally and then in close association with the host genome. For a review of this group of viruses see not only Nowinski *et al.* [49], but also Vigier [65] and Montagnier [45].

Virus particles which have an appearance and biochemical properties similar to these definite cancer viruses have been found in several other vertebrate species including man, but proof is lacking for oncogenic activity in their hosts. Oncornavirus particles have also been observed in pulmonary adenomas by Rabotti [55], in hepatomas by Maca *et al.* [42] and in tumours of pituitary and adrenal glands in mice by Mitchell *et al.* [44]. It is very premature to assume that these particles would be aetiologically involved in the tumours where they have been seen. Murine leukaemia viruses, which have the same appearance, replicate in many different tissues such as the mammary gland, but we have never found any evidence for the induction of a mammary tumour by a leukaemia virus.

Oncornaviruses have been retrieved from some murine lymphomas and mammary tumours induced by radiation or carcinogenic chemicals by Kaplan [32] and Timmermans *et al.* [64]. However, the negative results in the search for tumour viruses in chemically induced neoplasms, including lymphomas and mammary tumours (also in my own laboratory), are too numerous to accept an all-viral theory of the origin of cancer as such. Nevertheless we are of the opinion that studies on interactions between host genome and oncornaviruses are highly relevant to the problem of spontaneous development of tumours.

Genetics of neoplasia has to be considered at two separate levels: (1) genetic susceptibility of the host to develop tumours of a certain type, and (2) the cellular genetic changes underlying neoplastic transformation. On this basis we try to review the following relationships between host genome and oncornaviruses: (a) genetic susceptibility to an oncornavirus; (b) genetic transmission of an oncornavirus; (c) integration of an oncornavirus into the host genome; (d) cellular genetic changes under influence of an oncornavirus with regard to neoplastic transformation; (e) influence of epigenetic state of the host cell on oncornavirus functions including neoplastic transformation.

The first two relationships concern mainly host genetic factors, whereas the three others are at the cellular genetic level.

GENETIC SUSCEPTIBILITY TO AN ONCORNAVIRUS

The first studies in this field, as far as we know, were made by Korteweg [33, 34] with the mouse mammary tumour virus. His results indicate that only a few genes control the difference between highly susceptible and highly resistant.

More detailed studies by Heston *et al.* [30] and Dux [18] are in accordance with this view. Susceptibility to murine leukaemia viruses often seems to be governed by a single gene [2, 37, 51], although more complex relationships also have been observed by Lilly [39]. The observation by Lilly [39] in the murine leukaemia system that genes can control susceptibility to only one strain of virus has also been made with the avian tumour viruses [17, 52, 66].

By means of mammary gland transplantation, Dux and Mühlbock [19,20] established that major susceptibility to the mouse mammary tumour virus is localized in the gland itself. In a few resistant strains humoral factors seem to play a role [48] and they are probably of an immunological nature [5]. An interesting observation is that genetic resistance to the virus is associated with a poor replication of the agent [5]; the rate of virion production corresponds well with susceptibility [9, 28].

Since, in the avian tumour virus system, genes control susceptibility to viruses with the same coat antigens [66], genetic resistance seems to be a block in an early phase of the infection. There is no difference in the rate of virus absorption between different genotypes [16, 54] suggesting that the block will be at the level of penetration or uncoating of the virus. Mutations, which affect haemopoiesis, also influence the response to leukaemia viruses. In addition Odaka and Matsukura [50] proved, by means of bone marrow transplantation, that susceptibility is expressed at the level of the haemopoietic cells. Genes, which control membrane components in mice (the so-called H-2 antigens), strongly influence susceptibility to leukaemia viruses [38]. These genes do not seem to facilitate penetration of the virus but influence later events related to the disease. An attractive hypothesis is that these membrane components have an influence on crucial cell surface alterations associated with neoplastic behaviour.

It seems unlikely that different gene-physiological systems would control susceptibility to the three oncornavirus groups. For instance, a more extensive search may demonstrate the existence of genes in birds which affect replication of a leukosis virus, and so on. As a tentative hypothesis we assume that in all three systems susceptibility to the oncogenic effect of an oncornavirus is achieved by separate genes for virus penetration, replication and noninterference with alterations of the cell membrane.

GENETIC TRANSMISSION OF AN ONCORNAVIRUS

The possibility that tumour viruses can be part of the genetic make-up of a vertebrate organism was first suggested by Lwoff [40] in his classical review on lysogeny in bacteria. There is as yet no evidence for naturally occurring vertical transmission of oncogenic DNA viruses as in the case of several oncornaviruses [26, 31, 35, 46, 47].

Leukemia of the AKR mouse strain behaves as a hereditary trait in crosses with low-leukaemia mouse strains [15]. The hereditary nature of this disease was also obvious from the finding of Fekete and Otis [23] that AKR ova, transferred to low-leukaemic mouse strains, produce mice which subsequently become leukaemic. The discovery of a leukaemia virus in this mouse strain by Gross [24] was in apparent conflict with this postulate. Because introduction of this virus into other mouse strains leads only to milkborne transmission instead of transmission by the gametes, it was concluded that this leukaemia virus is transmitted as a genetic factor in its natural host, the AKR strain [26, 35].

The spontaneous release of virus is a feature which does not fit well into the accepted scheme if the presumed genetic transmission of the virus is compared with lysogeny. The recovery of leukaemia viruses from lymphomas induced by irradiation in otherwise low-leukaemic mouse strains [25, 36] better supports the resemblance between both situations as Lwoff [40, 41] had in mind. In this respect the retrieval of leukaemia viruses from chemically induced neoplasms [for review see Kaplan (32)] is important, since several carcinogenic drugs are also inducing agents in lysogenic bacteria [22,41].

We have extensively studied the possibility of genetic transmission of mouse mammary tumour viruses (MTV) for several virus variants and mouse strains [4, 6, 9, 11, 47]. The results can be summarized as follows: In five genetically very different mouse strains MTV-variants were observed which are vertically transmitted by the gametes. Introduction of an MTV-strain into a mouse strain, to which it is not indigenous, does not lead to gamete-borne transmission but only to transfer by the milk. There seems to be a very close relationship between the host genome and MTV-variants with regard to transmission by the germ cells. We may exclude the possibility that susceptibility genes could be involved in this phenomenon. The BALB/c mouse strain is more susceptible to MTV-L than the C3Hf strain, which is the natural host of this virus. Nevertheless only in the C3Hf strain is MTV-L transmitted by the sex cells.

This exclusive relationship between host genome and virus strain with regard to transmission by the gametes is easily explained by the transmission of these viruses as genetic factors of their natural host. We assumed that in one of the mouse chromosomes a DNA copy of the viral RNA is present, which under certain circumstances can be transcribed, giving rise to viral RNA and eventually complete virus particles (Fig. 1). Usually spontaneous virus release proves to be a recessive trait except in one case (the GR strain), where it is dominant. Genetic analysis proves this property to be controlled by a single gene. Every cell of the GR strain seems to contain viral activity, which indicates that information for the virus is part of the hereditary material of this mouse strain.

In those mouse strains which do not show spontaneous release of virus, the appearance of MTV virions or antigens can be induced by X-rays or urethan. An interesting phenomenon is that these antigens also appear in aged animals. The

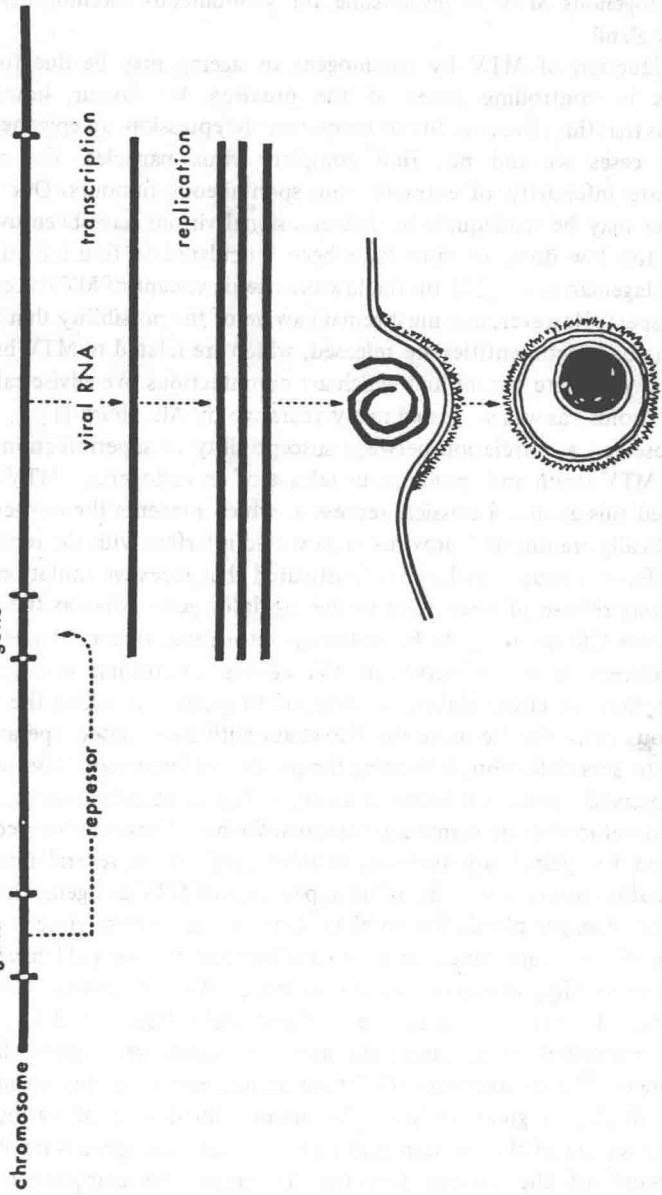

Figure 1. Scheme of genetic transmission of an oncornavirus.

time of appearance corresponds rather well with the susceptibility to spontaneous development of mammary tumours, suggesting that age-dependent switching on of an endogenous MTV is responsible for spontaneous carcinogenesis in the mammary gland.

The induction of MTV by carcinogens or ageing may be due to somatic mutations in controlling genes of the provirus. We favour, however, the hypothesis that this release is due to temporary derepression, an epigenetic event. In many cases we did not find complete virus particles, nor could we demonstrate infectivity of extracts from spontaneous tumours. Our technical procedures may be inadequate in that occasional virions have been overlooked and that too low doses of virus have been inoculated to find infectivity. The work of Hageman *et al.* [27] on the low-oncogenic variant of MTV is exemplary in this respect. However, one must remain aware of the possibility that in several mouse strains genetic entities are released, which are related to MTV but unable to produce complete virions and which are noninfectious. We advise calling such entities "viroids" as was suggested many years ago by Altenburg [1].

We observed a correlation between susceptibility to superinfection with the standard MTV strain and spontaneous release of an endogenous MTV. We have interpreted this as that a classical repressor, which prevents the transcription of the genetically transmitted provirus, also would interfere with the replication of a superinfecting virus. Further, we postulated that recessive mutations causing spontaneous release of virus were in the regulator gene, whereas the dominant lesion in the GR strain would be in the operator gene. In accordance with this whole concept is our observation that genes controlling susceptibility to superinfection are either linked or identical to genes controlling the release of endogenous virus. Furthermore the GR strain with its mutated operator gene is resistant to superinfection, indicating the presence of repressor. Also remarkable is the observed correlation between susceptibility to spontaneous and urethan-induced development of mammary tumours. We have likewise observed a similar correlation for pulmonary tumours in mice [10]. As in several instances the administration of urethan leads to the appearance of MTV-antigens, the observed correlation strongly pleads for an all-viral or, perhaps better stated, all-viroidal aetiology of mammary cancer in mice. Huebner and Todaro [31] have launched a similar general hypothesis concerning all modes of carcinogenesis. They suggest that certain determinants (oncogenes) of genetically transmitted C-type viruses (usually associated with leukaemia and sarcomas) are responsible for the development of most tumours. The many inbred mouse strains we have at our disposal display a great variation in tumour incidences of various organs. Therefore we are of the opinion that not one single oncogene is involved in the development of the various tumours. It cannot be completely excluded, however, that organ-specific expression genes control the switching on of one oncogene.

Huebner and Todaro [31] emphasized the frequent partial expression of the oncornaviruses. This may be reflected by independent appearance of internal or coat antigens of the virus or virus-coded cell-surface antigens and by the production of noninfectious entities, capable of transforming their host cell. In my laboratory we have failed to isolate a sarcoma virus from chemically transformed cells. However, my collaborator Sylvia Offers succeeded in retrieving such a virus when the sarcoma cells were infected with a leukaemia virus. Most likely the sarcoma virus resulted from the mixing (either phenotypically or by genetic recombination) of the sarcoma viroid with the infectious leukaemia virus.

An interesting example of partial expression of a provirus is the age-dependent release of MTV-O virions in BALB/c mice [27]. This mouse strain does not have a repressor causing immunity to superinfection with MTV. Nevertheless virions of the endogenous virus are not released in young adults but only in aged animals. Some virus-specific antigens are released throughout the life span. Obviously other control mechanisms can regulate the expression of some virus traits. It is remarkable that, upon passage of MTV-O in BALB/c mice, there is no interference with the production of complete virions.

On the basis of the repeatedly observed partial expression of oncornaviruses, Temin [61] developed the protovirus theory, which suggests that information for a whole virus is not necessarily present in the host genome. Furthermore he believes that virus release is a mutational event, whereas Huebner and Todaro [31] believe it to be epigenetic in nature. Techniques are as yet inadequate to discriminate between these postulates.

INTEGRATION OF AN ONCORNAVIRUS INTO THE HOST GENOME

The finding of an RNA-dependent DNA polymerase in the virions of oncornaviruses by Baltimore [3], Temin and Mizutani [63], Spiegelman *et al.* [57] strongly substantiate the initially rather unorthodox theory of Temin [59] that a DNA copy is made from viral RNA. Since cellular DNA synthesis is needed before the virus can replicate [60], it seems logical to assume that this DNA copy is integrated into the host genome and will then serve as a template for synthesis of new viral RNA.

In the opinion of several authors the discovery of the reverse transcriptase warrants also the hypothesis of transmission of oncornaviruses as genetic factors of the host [49, 52, 57, 58]. In our opinion the provirus for vertical transmission (germinal provirus) does not need this enzyme for its continuity. Otherwise replacement of one germinal provirus by another following superinfection must be possible. We have never observed this in the MTV or murine leukaemia virus systems. If the RNA-dependent DNA polymerase were involved

in vertical transmission, simple mendelian ratios, as found by Payne and Chubb [53], Bentvelzen [4], Bentvelzen and Daams [7] and Stockert *et al.* [58] would be impossible.

At first glance the hypothesis of the germinal provirus seems to be completely incompatible with the Temin postulate of the somatic provirus being produced after infection. In Fig. 2 a new hypothesis is presented, which reconciles both concepts. Ordinarily transcription of the germinal provirus is repressed. After temporary derepression induced by, for instance, radiation, viral RNA is released. Thereafter a new provirus is made and is inserted at a site less accessible to repressor molecules. This process accomplishes continuous production of viral RNA.

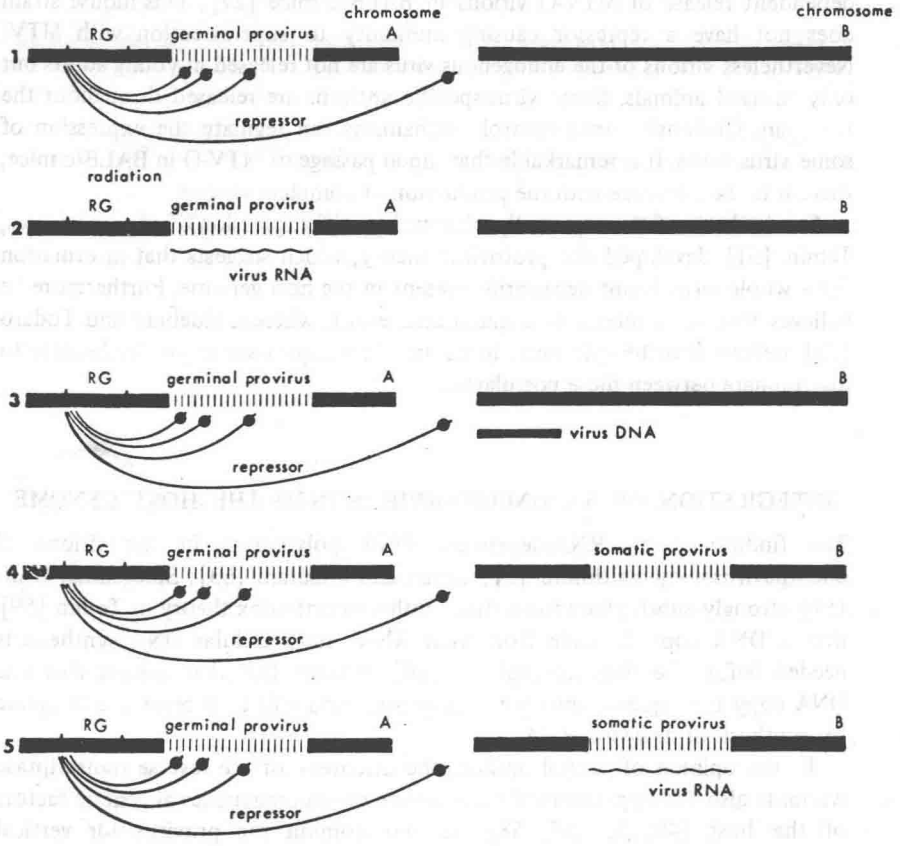

Figure 2. Combination of theories on germinal and somatic provirus: (1) repressed germinal provirus; (2) momentary derepression following radiation giving rise to transcription of germinal provirus; (3) restoration of repression; production of DNA copy of virus RNA; (4) insertion of DNA copy (somatic provirus) into another chromosome; (5) continuous transcription of somatic provirus.

CELLULAR GENETIC CHANGES UNDER INFLUENCE OF AN ONCORNAVIRUS WITH REGARD TO NEOPLASTIC TRANSFORMATION

A source of heated discussions in cancer research is whether cancer is due to somatic mutations or epigenetic changes. One might argue that the establishment of a somatic provirus is a special form of somatic mutation, but in the case of endogenous oncornaviruses this would have been preceded by an epigenetic change.

Burdette and Yoon [14] found Rous sarcoma virus (RSV) to be mutagenic to *Drosophila*. It is very unlikely, however, that RSV would be oncogenic by the induction of point mutations. Macpherson [43] observed that the reversion of RSV-transformed hamster cells to normalcy is accompanied by the loss of the viral genome. The persistence of the viral genome seems to be necessary for maintenance of the neoplastic condition, which would not be the case with virus-induced mutations.

The correlation observed between virion production and susceptibility to carcinogenesis by MTV suggests that production of much viral RNA is necessary before neoplastic conversion can take place. An as yet unresolved problem is whether this conversion is the result of direct action of viral genes or the consequence of changes in expression of the host genome induced by viral products.

INFLUENCE OF EPIGENETIC STATE OF THE HOST CELL ON ONCORNAVIRUS FUNCTIONS INCLUDING NEOPLASTIC TRANSFORMATION

Upon infection with the mammary tumour virus, infectivity can be retrieved from various tissues but only in secondary sex organs of the male and mammary glands of the female are complete virions produced. The virus has an oncogenic effect only in these female organs. In the lymphoid tissues some virus-specific antigens can be found which are not observed in the erythrocytes [9]. This demonstrates the considerable influence of the epigenetic state of the cell on various oncornavirus functions.

Murine leukaemia viruses can replicate in many different tissues but will transform only the haemopoietic ones. Our work with Rauscher leukaemia virus suggests a very subtle interplay between epigenetic events and the virus genome in the induction of erythroblastosis [13]. With antisera to the virus we could demonstrate the presence of the virus in haemopoietic stem cells. Various stimuli such as antigens, anti-platelet serum, which promote the proliferation of this stem cell, also enhance the leukaemic response. The kinetics of both processes closely resemble one other. The radiosensitivity of the stem cell parallels the

effect of pre-irradiation on the leukaemic response to the virus. All these data suggest that the leukaemic process finds its origin in the stem cell. However, virus-infected stem cells are capable of normal functions: they can differentiate into thrombocytes, granulocytes or lymphocytes. It seems that the differentiation stimulus into the erythroid direction leads to tumorous derailment.

CONCLUDING REMARKS

The following interactions between host genome and oncornaviruses have been discussed:

Genetic susceptibility to oncornaviruses is achieved by a low production of antibodies to the virus, a good penetration of the virus into the cell and subsequent uncoating, a good replication of the viral genome and tolerance to cell membrane alterations leading to neoplastic conversion.

Genetic vertical transmission of oncornaviruses is explained by the continuous presence of a DNA copy of the viral genome in one of the host chromosomes. Transcription of this germinal provirus takes place under the influence of germinal mutations in the controlling genes, after irradiation or treatment with carcinogenic drugs or ageing. Often only partial expression of the provirus is found.

After infection, oncornaviruses seem to make a DNA copy of their RNA, which becomes integrated into the cellular DNA. In the case of endogenous oncornaviruses the establishment of such a somatic provirus might be the escape from the repressor associated with the germinal provirus.

The persistence of the viral genome is needed for the maintenance of the neoplastic condition. The cell has to be in a certain epigenetic state before the virus can exert its oncogenic action.

It is not yet known whether neoplastic transformation is the consequence of direct viral action or of changes in the expression of the cellular genome induced by some viral products.

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