Progress in Experimental Tumor Research

Series Editor: F. Homburger

Vol. 19

Immunology of Cancer

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Volume Editor: V. RICHARDS, San Francisco, Calif.

50 figures, 62 tables, 1974

S. Karger · Basel · München · Paris · London · New York · Sydney

Series Editor's Foreword

The field of immunology of neoplasia moves so rapidly that another volume on this subject is timely only four years after Robert S. SCHWART, as Guest Editor, compiled Volume 13 of this series (Immunological Aspects of Neoplasia). Whereas the earlier volume concerned itself with broad, general comments, the present collection of papers arranged by Victor Richards addresses itself in more detail to specific instances of immunological considerations which modify certain forms of neoplasia.

This succession of volumes on one aspect of tumors, as seen from widely different points of view, illustrates the value of the guest editor system now employed in compiling this series. The faithful reader of *Progress in Experimetal Tumor Research* will acquire through the years an overview of the field as it appears to a large number of qualified reporters. We hope that, ultimately, the ingredients of true progress will become apparent through retrospection.

Again, the series editor expresses his gratitude to the guest editor, the contributors, and his faithful editorial assistant Mrs. MARY MILLER.

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Immunology of Cancer. Progr. exp. Tumor Res., vol. 19, pp. 1–22 (Karger, Basel 1974)

Oncoduction. A Unifying Hypothesis of Viral Carcinogenesis

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Any hypothesis concerned with normal or altered information transfer must take cognizance of the well understood and ordered concepts of the bacterial operon system [37]. Assuming a basic conservation of this model in eukaryotic cells as a starting point, several 'super-operon' theories of structure and regulation were developed to explain coordinated and concerted action of many operons [15, 19, 26]. One of these also postulated that some DNA viruses may transform cells because they integrate at a locus which deals with control rather than structural elements [26]. A number of parallel aspects of cellular control was found to exist in normal differentiating cells and tumor cells which led to the interpretation that neoplasia is a disease of cellular differentiation [44]. The present hypothesis attempts to explain both cellular differentiation and virus mediated carcinogenesis within the framework of a more complex super-operon model which has additional levels of control. This view holds that some DNA or RNA viruses can be regarded as tumor viruses because they contain aberrant control information within themselves and transduce it to the cell, thus rendering the cell neoplastic.

Role of Viruses in Neoplasia

The plethora of viruses, their dissimilarities, and the multiple host system involved in virus-induced carcinogenesis make for a complex scenario. Many seem to be absolutely inconsequential as tumor-inducing agents in the natural host, and the spectre of merely a casual association haunts the causal relationship. There does not seem to be a specific type of virus, virus antigen, or virus nucleic acid that is mandatory for provoking neoplasia.

The DNA tumor virus group itself is composed of quite different agents ranging from the very large poxvirus, intermediate adeno- and herpes viruses, and down to the small papova virus group. Some very closely related members may or may not be suspect, e.g. herpes type 1 and 2 [23, 39]. Some herpes-like viruses, such as Marek's disease agent or the *Herpesvirus saimiri*, are extremely good tumor inducers in avian and primate systems, respectively, and seem to fulfill Koch's postulates [36, 39]. The ultimate pragmatic criterion – the absolute prevention of disease – has been realized recently by immunization of chickens with attenuated Marek's disease virus or with a related nononcogenic virus from turkeys [17, 49]. Papova virus DNA is particularly amenable to investigation because of its physically small genome size, and studies of temperature sensitive mutants have clearly determined that a viral function must continue to be expressed if cell transformation is to continue in culture [24]. These salient features – namely, that viral integration does occur, that a direct expression of viral information is mandatory, and the prevention of tumor induction after immunization with the virus – militate strongly for a direct role rather than merely a co-carcinogenic event [5, 17, 49, 51]. The specific DNA viruses in tumorigenesis and the especially fascinating relationship of EB virus and its DNA to Burkitt's lymphoma of man have been reviewed [39].

RNA tumor viruses have a similarly impressive pedigree of direct causal relationship to tumor induction. The life cycle of these viruses is still unknown, but the role of DNA synthesis and a transcription of viral RNA into DNA by a DNA polymerase dependent on viral RNA are apparently central to their neoplastic properties [59, 61]. Oncorna viruses which can induce many kinds of neoplasms in their natural host are widespread in vertebrate systems, and apparently all normal cells of a species contain all or a part of a viral genome in their DNA [9]. Cells transformed by temperature-sensitive mutants of RSV are also clearly dependent on a specific viral function for maintenance of the transformed state [65].

The apparently natural and widely prevalent oncorna virus presence engendered two basic hypotheses of viral carcinogenesis which in their basic forms seem to be testable and mutally exclusive. Each of these hypotheses relegates a primal role for these viruses in the general phenomenon of oncogenesis. The dominant concepts of each theory have been reviewed [60, 64]. The 'oncogene' theory states that each cell has within its own DNA the information for a complete oncogenic RNA virus. This viral copy has evolved evolutionarily as an intrinsic part of the cellular DNA. The critical part of the virus is the gene responsible for cell transformation (oncogene),

and associated with it but not necessarily linked to it are viral genes [64]. The strongest feature of the theory which has been demonstrated is that such oncorna viruses behave like temperate phages and can be 'turned on' by various derepressive stimuli – such as halogenated pyrimidines – from cells of many species. Cellular represssive mechanisms keep this viral 'oncogene' in check.

The provirus theory states that (1) there is a net gain of exogenous viral DNA by the cell infected with RNA virus, (2) this gain is recent, and (3) all viral genes are linked. The protovirus modification states that reverse information transfer is part of normal development and that a protovirus evolves as new DNA sequences are formed [61].

Recent experiments by HILL and HILLOVA [34] have clearly shown that mammalian cells which have no avian oncorna virus DNA information can gain such information after virus infection. They also show that this DNA from transformed mammalian cells can be infectious for susceptible avian cells and can transmute itself again into the original oncorna virus in the natural host [69]. Another finding is the BAXT and SPIEGELMAN [10] experiment which shows that human leukemic cells but not their normal counterparts possess some new DNA sequences. Thus, some experiments support the protovirus theory, although the model for protovirus evolution to the natural virus state seems to be very weak. Neither theory is complete; both theories ignore DNA tumor viruses, and both hypotheses state that a positive viral genetic function, when expressed, results in the transformation of susceptible cells.

The hypothesis presented below attempts to coordinate these disparate observations and to link them to a specific derangement of normal cellular control mechanisms and differentiation processes.

The Oncoduction Model

The term 'oncoduction' is loosely coined in the wake of the 'oncogene' and is, furthermore, used loosely hereafter in a dual sense. Firstly, it is used to describe mechanisms by which viruses can induce heritable, neoplastic changes in a host cell; secondly, it is also used to describe the transfer by virus of information responsible for the tumor state, regardless of the origin of this information. The word transduction is normally qualified by the terms 'specific' and 'nonspecific' which designate two quite different mechanisms of information transfer by bacteriophage. The words transduction and transduce are used herein as a general term, without further qualification as to

specific mechanisms involved to describe the transfer of 'nonviral information' by animal tumor viruses.

An integral aspect of the hypothesis is that many groups of animal viruses can pick up aberrant cellular control information (discussed below) and transduce it to normal cells in the course of infection.

Relevant to the mechanistic aspects of the model is the behavoir of bacterial episomes which display a variety of effects on their host cells, many of which results from replication errors. The behavior of tumor viruses loosely parallels that of an episome or a lysogenic phage; that is, it can infect a cell and subsequently become integrated into the host's genome. There is no reason to doubt that the interactions of such 'episome-like' elements with mammalian cells should be less complex than microbial systems. Specific types of episome-host interaction relative to the 'oncoduction model' include the following:

(1) Insertion of episomes into the host chromosome produces a mutation at or near the point of insertion. Striking examples of this type are the phage μ [16] and the control elements in maize described by MCCLINTOCK [46].

(2) Excision of an episome is often imperfect, resulting in acquisition by the episome of certain host determinants and also a residuum of the episome remaining in the host chromosome. Well-studied examples include F′ factors and λdg. These modified elements then transfer their newly acquired properties to subsequent host cells with a resulting phenotypic change in the host. One interesting corollary of this is that certain cells retain a 'memory' of previous F factor insertions and that this 'memory' results in the subsequent preferential insertion of F at the previous site [4, 52].

(3) Recombination subsequent to the integration of an F-genote in the region of genetic duplication in *E. coli* can give rise to an alteration between the attached and detached states of the episome, but inversion of the duplicated region prevents episome detachment [3].

We propose that temperate viruses of vertebrate species can also interact with their host's genome in an abnormal or disruptive fashion and are therefore potentially oncogenic. Based on the above mentioned behavior of episomes, the following types of virus cell interactions might be expected.

Nondisruptive Integration

Virus integration occurs in such a way that cellular information is not disrupted. The virus is normally repressed by its control genes or cellular mechanisms and remains silent until actuated by some specific mechanism.

Disruptive Integration

Regions of eukaryotic genomes are believed to function as control regions responsible for the specific activation of large numbers of structural genes (see below). Integration of episomes into these control regions, therefore, could potentially result in pleiotropic effects if the integration event is disruptive. Two discrete types of disruptive integration effects are given as extremes in a spectrum of interactions.

Negative disruption. In this case, a cellular control gene is disrupted by virus insertion. This results in failure of the control region to respond to its appropriate command. Thus the structural genes normally activated by this control region do not function.

Positive disruption. The control gene is altered in such a way that it is always activated. This results in expression of structural genes normally inactive in a particular cell type.

Either of these disruptive integration events could result in what is generally defined as cell transformation. This type of virus-cell interaction could be a very low probability event. However, once a cell experienced this type of integrative event, virus induction could result in a particle containing deranging control sequences because of errors in excision discussed above in relation to λ and F–. The virus could then carry this disruptive information as part of its genetic material. This virus could now be called a transforming virus and transfer altered control information to new host cells with a higher order of probability because of a greater area of homology with host DNA.

It is seen that this model not only describes one mechanism for cell transformation but also provides a mechanism for evolution of tumor viruses. The model is purposely broad in concept, and we would expect that different viruses interact with many different control regions of cells giving rise to widely variable alterations in these cells. However, one specific prediction of the model is that all tumor viruses will have a direct effect on cellular genetic expression at the transcriptional level.

Also implicit in the model is a nonspecific 'dilution effect'. It seems reasonable to assume that any given cell type requires a certain continuing expression of a finite number of structural genes to maintain that differentiated state. It is also reasonable to assume that the cell has a finite capacity to anabolize with a comfortable margin over that required for its maintenance. If such a cell is faced with any situation in which more regions of its genome are actively transcribing, or where a single region (either

viral or cellular) is transcribing greater quantities of RNA, the required RNA species will be diluted by competition and the cell may undergo a phenotypic change. Such cells might continue to produce their characteristic products but at a reduced rate (e.g. the reduced production of myoglobin by rhabdomysarcoma cells). In addition, other types of transformed cells might either produce a wide variety of products characteristic of other cell types (e.g. structures in teratomas) or great quantities of one or a few gene products (e.g. myeloma proteins).

It is very tempting at this point to broaden the model by including translational control mechanisms such as altered transfer RNAs, initiation factors, etc. However, this broadening detracts from the more directly testable production of altered transcription within tumor cells.

The Episomal Nature of Tumor Viruses

Normal cellular or viral mechanisms accomplish an integration of the DNA virus and its putative transduced control information into cellular DNA, and specialized reverse transcriptase mechanisms in some RNA viruses make a DNA copy which is then inserted into cellular DNA. An example of such an event has clearly been documented for the papova DNA virus group where covalent linkage of viral and cellular DNA is demonstrable [5, 53]. Transfers of infectious DNA from mammalian tumor cells made neoplastic originally by RSV have shown that a complete copy of nonendogenous viral RNA must have been inserted into cellular DNA [34, 69]. Recently, at least part of a suspected human oncogenic virus, Herpes type 2, was found to be associated with cervical tumor cell DNA [23]. The *de novo* presence of EBV genetic information as part of cellular DNA seems to be a prerequisite for cellular growth and maintenance [39].

That at least some tumor viruses pick up cellular information in a manner analogous to transducing bacteriophage has been established [5, 66]. If such a DNA or RNA tumor virus were to pick up disruptive cellular control information or if, by nature of homologous complementary sequences, it would merely place itself at a critical cellular control region, gene disruption of normal cellular controls could theoretically occur. Such a virus would gain selective advantage at several levels. If the transduced element, for example, displaced a viral replicative late function, the virus need not be lytic for the cell but its genome could multiply with each cellular division. In time such viruses would become detectable and of interest to

biologists who would select such a specialized virus variant as representative of the phenomenon at large. A transducing virus variant with a complete set of replicative functions would be most easy to detect, whereas various defective variants would need correspondingly more techniques (e. g. use of helper virus) to propagate the transducing variant. Historically, transforming viruses were first detected as complete, competent forms of RSV; but as tumor virologists become less defective in their armamentarium, nonreplicating forms of transforming viruses were detected [32]. In this sense, certain laboratory strains of tumor viruses can be considered as artifactual forms derived after multiple generations of selected evolutionary pressure. These viruses, however, now represent genuine tumor viruses and can insert deranging control information into a new host's DNA. In time this type of a 'tumor' virus can become established with cellular replicative mechanisms. The integration event could have transpired many generations previously and have been well controlled so that the virus becomes evident only after rigorous steps are taken to elicit it from the cellular DNA.

Some of the strongest evidence apart from the bacteriophage transduction is the apparent promiscuity of transforming information found in murine sarcoma virus (MSV). The MSV genome can transform and cause sarcomas in several mammalian species. The virus can become firmly established within the host cell [32, 35]. The normal host range of MSV can be modified by acquiring viral envelopes from different species of helper viruses [21]. On a molecular level, MSV can associate with helper type viruses such as the RD-114 and feline leukemia virus (FeLV) which are molecularly dissimilar from each other, from MSV, and from MuLV as determined by DNA·RNA hybridization experiments [31]. The MSV-specific information, however, becomes faithfully associated with its host cell and upon rescue from different mammalian cells with molecularly dissimilar helper viruses nonetheless maintains its MSV-specific sequences [HAAPALA, in preparation]. Thus the transforming information of MSV may be transduced by these very dissimilar helper viruses which presumably supply replication and/or integration functions mandatory for survival of the MSV genes. When this transforming RNA virus becomes established in the cell, virus specific information such as group specific antigens can, but need not, be coordinately expressed with the transforming moiety [35, 50, 58].

The genetic interactions of SV40 and adenoviruses have been extensively investigated, and it is clear that adenovirus can transduce part of all of the SV40-specific DNA, which can then exert its oncogenic potential in susceptible host cells [13].

Relationship of Gene Regulation, Differentiation, and Oncoduction

The progressive sequential, unidirectional, and patterned series of biochemical events during development which transmute a pluripotential cell to a highly specialized differentiated state are indubitably of great complexity. Differentiation in well-known systems such as hemoglobin synthesis is generally manifest by concerted gene action, but the evidence indicates that, in contrast to bacterial structural genes which are often expressed in sequence, the expression of contiguous mammalian genes might be mutually exclusive [38]. Mammalian gene regulation is a concerted phenomenon involving selective activation of noncontiguous genes. Often manifest is a pleiotropic response to small control molecules as steroids and polypeptide hormones which induce changes in many enzyme systems within a cell in a series of well-defined patterns of expression [15, 38]. A widely held basic assumption is that the differentiated cellular genome is repressed in many of its potential functions by histone-like proteins and that pleiotropic responses to a hormone are mediated by a *de novo* activation of repressed sequences [40]. A control model based on requirements of regulatory phenomena – i.e. integration of physically unlinked genes – and pleiotropic responses has been proposed by BRITTEN and DAVIDSON [15]. The model also attempts to correlate biological data with the molecular nature of eukaryotic DNA and the complex controls assumed to exist. Simply stated, the model proposes: (1) a sensor gene which acts as a target for a stimulus (e. g. a hormone) in the cell and which in turn activates (2) an integrator gene. The integrator gene then produces a series of (3) specific activator molecules (perhaps RNA or proteins) which diffuse through the nucleoplasm to interact sterically with (4) regulatory or promotor sites. The regulatory site then activates (5) the structural or producer genes. It may be seen that parts 4 and 5 of this model are essentially descriptive of the o, p, and the x, y, and z genes of the lac operon [71]; the integrator gene may be thought of as an intermediary coordinator of several operons in response to a stimulus from a single receptor.

This model seeks to assign a function for the families of similar nucleotide sequences which are interspersed throughout the genomes of all eukaryotes [14]. These reiterated sequences function as the effector and receptor control regions implicit in the model. GEORGIEV [26] has presented evidence that rapidly metabolized and highly repetitive sequences form the 5′ end of newly transcribed giant nuclear RNA. However, when this giant nuclear RNA is processed into messenger RNA, only the 3′ end of the molecule remains [68].

Thus the highly repetitive information at the 5′ end seems to function during the transcriptional process but probably does not contain structural information. This type of RNA fits the description of the theoretical integrator gene products which are required to activate various groups of structural genes distributed throughout the eukaryotic genome [15].

To concatenate differentiation with gene regulation of higher organisms led us to postulate another superior order of control mechanisms which must determine a concerted action of many integrator sets (fig. 1). The initial dividing cell is pluripotential, but the progression of internal events coupled to external and largely unknown (organizer?) stimuli rapidly results in a repression of manifold potential directions of differentiation and a canalization of the cell into a particular irreversible direction. The intital stimuli can be as simple as the pin prick of the unfertilized ovum which results in a haploid frog. Subsequent cells must develop an insensitivity to such nonspecific stimuli and acquire a positive recognition for complex chemicals or structures. Differentiating cells of one type (e.g. neuron precursor), which result in highly specialized organs, must choose to call up series of coordinated integrator sets which will be different from those sets found in organs with totally different functions (e.g. muscle). A conservative view would assume the presence of genes whose products would control the *choice* of further integrator gene activity. Evidence for such supraorganizational control genes is inherent in the process of transdetermining insect imaginal disks, whose primordial cell rests, which would normally give rise to a given structure, result in totally different organ sets. A *single* gene mutation can activate all other genes needed to differentiate a leg disk in a blastema normally supposed to form head structures [25]. Accordingly, a single gene must control the function of multiple integrator sets.

We tentatively term these stimulus-responsive primal control genes as 'trigger sets' and visualize a tree diagram of such trigger sets interacting with each other. During development, trigger set products activate a series of sensors for integrative genes in a sequence programmed by evolution. The nature of the trigger product may be RNA; certainly much intrachromosomal RNA does not become dissociated from DNA as in chromomeres in giant chromosome puffs, and control functions have been proposed for this RNA [12, 26]. Figure 1 depicts a series of repressed, closed trigger sets whose products at one time acted on integrator genes responsible for activating gene batteries producing embryonal proteins. The same closed (repressed) trigger sets could have had a stimulating effect on certain integrator genes which are still active. Active nonrepressed 'trigger set' products

influence the sensor-integrator complexes in a manner compatible with multiple concerted responses previously detailed [15]. In the simplest sense the 'trigger sets' and their sensors represent a series of critical and primal

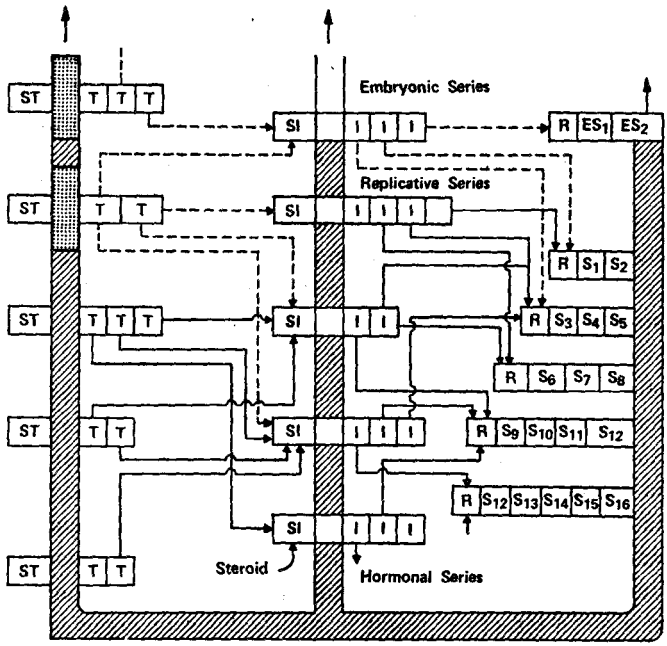

Fig. 1 A 'super operon' model of eukaryotic genetic control. The basic block is an operon-like unit which consists of a regulator (operator-promoter region) (R) and a contiguous structural gene battery (Sn), all of which are transcribed as one piece. A single product of an integrator gene (I) can cause initiation of multiple regulators. The product of several integrator genes can effect a given regulator allowing for fine quantitative control. Feedback loops of gene products, which can promote or repress, are possible at each level. Integrator genes have sensor gene moieties (SI) which receive the recognition molecules responsible for commencement of integrator gene transcription. To determine which integrator-structural gene profile is to be active at given times in the developmental program is the responsibility of trigger genes (T), most of which are turned off in a differentiated cell, as indicated by darker ST-T blocks and hatched lines. In a developing embryonic cell, these trigger genes, which ultimately call for embryonal proteins (ESn), become progressively closed and unavailable for further transcription. Trigger genes also have a sensor part (ST) which is responsive to some product(s) of the intracellular milieu by initiating a round of transcription. The nature of the integrator gene or trigger gene product for their specific sites is unknown, but the large amounts of apparently untranslated chromosomal RNA in several eukaryotic cell systems may have such a function (cf. text).

control junction points which a normal cell traverses in its path to a differentiated state. The ordered progress from the pluri- to the unipotential state may results from a vertical series of primal trigger sets progressively eliminating other secondary trigger sets. Therefore, the disruption of a very primal trigger set may result in a pluripotential disorder. Ideally the pleiotropic and aberrant responses manifest in neoplastic behavior could be explained by disruption of a 'trigger site' superposed on and controlling integrator genes involved in cellular differentiation. In this sense, neoplasia could be viewed as a trigger set error, and the derangement of primal trigger sets could result in tumors such as teratomas, which in miniature represent a grouped parody of differentiated tissues. A disruption of secondary trigger sets can be visualized as a neoplastic cell arising from more specialized tissues and retaining at least a part of its specialized structure and function.

Mechanisms of Control Site Disruption

The trigger control site DNA could be disturbed in any number of ways each of which would result in an altered information sequence leading to an imperfect product interacting aberrantly with subsequent control sensor sets. The trigger gene product may interact with or bind to secondary trigger or integrator sensor sites and the resulting response may be a permuted expression of unexpected differentiated or embryonal gene batteries. This event could also conceivably result in abnormal structures and functions if products of different genes must quantitatively interact to form multimeric enzymes, control or structural proteins [71]. The simplest event of this type is a point mutation within a trigger gene. Physical or chemical agents causing frameshift mutations in bacterial systems which become firmly fixed are good candidates because carcinogens typically mutagenize bacteria in this manner (fig. 2A) [6]. If the control site is general enough, embryonal proteins may be expressed [2, 8, 30, 57, 62]. Figure 2B further considers that associated with a trigger site may be a DNA version of an endogenous virus (see below) which may share homology at, or in close proximity with the trigger. If the site is normal and if the endogenous virus is induced by halogenated pyrimidines, the virus is derepressed and may continue to replicate, depending on the function of other controlling genes [50, 58]. It is fascinating that several lines of evidence now link halogenated pyrimidines not only to oncorna or herpes-like virus induction but also as to a controlling role in differentiation

of normal embryonic and malignant cells [18, 54]. Herpes virus type 2 specific RNA sequences seem to reside in cervical carcinoma cells in direct association with the repetitive DNA sequences which have been postulated to be the control series [15, 23]. Such an endogenous virus transducing part

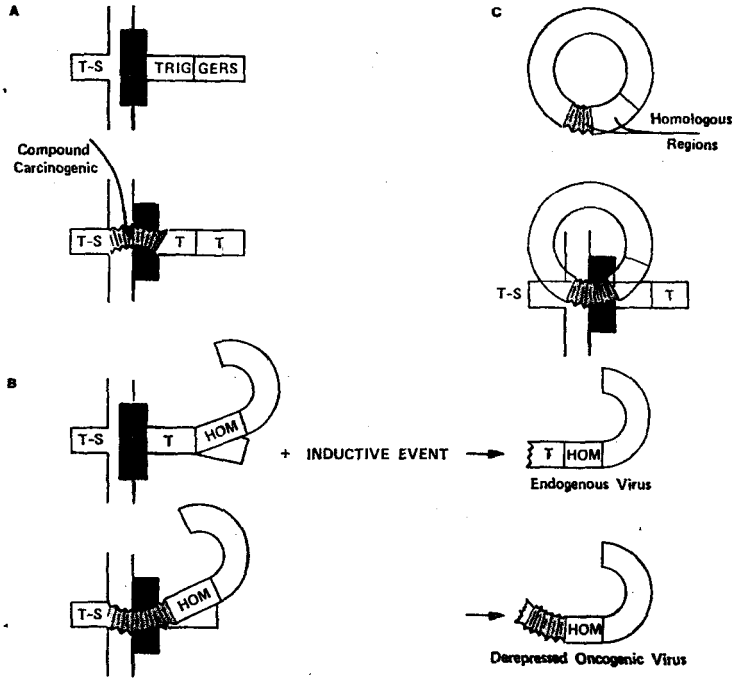

Fig. 2. Potential disruption at the closed trigger gene complex. The sensor element of the trigger gene has been blocked by a feedback product which functions as a repressor. *A* The simplest derangement may be visualized as a frameshift mutation (e.g. by a chemical carcinogen) which renders the repressor ineffectual, thereby causing trigger gene transcription and activation of previously repressed integrator-structural gene function. *B* The locus in a normal cell where an integrated 'endogenous' virus resides. Incorporation of halogenated pyrimidines may cause a release of the virus with part of the cellular region attached. If the cell is a tumor cell whose particular trigger gene function is abnormal, then a contiguous natural virus may pick up the deranged region and become a free virus on induction. Thus the tumor virus will have the disrupted trigger site as a major new area of homology with the cell DNA analogous to transducing bacteriophage λdg. Such a virus can now transduce these disruptive trigger gene sequences to a normal cell and render it neoplastic (C). The sequence from B to C can recapitulate itself. Eventually a 'tumor' virus can either integrate nonproductively into the homologous trigger gene or concurrently continue to be replicated on a DNA or RNA level.