THE GENERAL PROBLEM OF REGULATION OF CELL DIVISION AND MALIGNANT TRANSFORMATION 2. AN APPROACH TOWARDS UNRAVELLING OF THE UNDERLYING MECHANISM

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INTRODUCTION

IN the first part of this two-article series[1], I have pointed out four basic questions that relate to the mechanism of regulation of cell division and malignant transformation. These questions are:

(1) What is the switch—chemically, biochemically, morphologically and functionally defined—that is put on when a resting cell is triggered by a mitogenic agent/event into the division cycle?
(2) What is the language of the programme of the division cycle?
(3) What is the nature of the link between the switch and the programme?
(4) How does the switch get jammed in malignant transformation?

I have also pointed out that the above questions comprise two non-overlapping subsets: the first consisting of questions 1, 3 and 4, and the other of question 2. In this concluding part of the discussion, I will confine my attention only to questions 1, 3 and 4. In regard to question 2, I would only state that virtually nothing is known today about the language of the programme of the division cycle—that is, the mechanism responsible for the occurrence, in a highly programmed manner, of the large number of events that occur during the cell cycle, which culminate in the formation of two cells starting from one.

The method that I wish to follow is construction of a model—essentially a hypothesis—which attempts to provide tenable answers to the three questions and which makes unique, testable predictions. As the first step in the construction of models designed to provide explanation of natural events, is to look for clues which would constitute the basic premises of the model, some 15 years ago we set out to look for such clues in the plethora of information available in the area. It seemed that the following six observations might provide us the necessary clues.

(*a*) All cell types which can turn malignant, that is, which can exist in the normal and the malignant state, are, without exception, capable of existing in the resting and the dividing states. Further—and this is even more important—all cell types, without a single exception, that can exist in the dividing state and the true resting state that would satisfy the criteria already mentioned, are auxotrophic for a certain number of carbon-containing nutrients. Thus, mammalian cells require, for maintenance and growth, a certain number of aminoacids, termed the essential aminoacids, vitamins and unsaturated fatty acids (called the essential fatty acids). It is a point to ponder that animal cells—including human cells—need to have these 'essential nutrients' given to them in a preformed state as the cells are either not capable of making these nutrients at all or make them at extremely slow rates that would not take care of the cells' requirements. On the other hand, a lowly creature such as *E. coli*, can make all its carbon-containing compounds from a single carbon source, that is, glucose. Why has this discrepancy come about during the course of evolution? It does not seem unreasonable to argue that auxotrophy (requirement for certain preformed, carbon-containing nutrients, besides a primary carbon source such as glucose), while being a distinct disadvantage, might have conferred upon the organism also an advantage that could have simply balanced off the disadvantage from the point of view of natural selection. Indeed, the course of human history might have been different if human beings possessed the ability to convert cellulose into

glucose and to use glucose as the source of all carbon-containing compounds that the human organism needs for growth and maintenance! It may, therefore, be worthwhile to consider the possibility that the evolution of auxotrophy in higher organisms may be causally related to the evolution of the ability of the cell to exist in the resting and the dividing state, that is, to the mechanism that regulates growth—such regulation being clearly an advantage, as without such a regulation it simply wouldn't have been possible for higher organisms such as a human-being, to perform many of their important functions. Similarly, it is possible that the ability to be transformed malignantly that is found in cells of higher organisms, may merely be an inevitable consequence of the ability of the cells to exist in the resting and the dividing state, and that the disadvantage that has accrued as a result of the evolution of the ability of a cell to be transformed malignantly, might have been more than amply compensated by the ability of the cell to exist in the resting and the dividing states, in the case of higher organisms.

(*b*) The transport of the essential nutrients (that is, those nutrients for which the cell is auxotrophic), is essential for the maintenance of both the resting and the dividing states.

(*c*) The rates of uptake or transport (the two terms being used here synonymously, although there is a subtle difference between them) of essential nutrients in cells that are dividing, that is, going from one cell cycle to another without going through an intermediate resting state, are about an order of magnitude greater than the rates of uptake of the same nutrients obtained in the resting or the G_o cells[2-6]

(*d*) The increase in the rate of uptake of essential nutrients is an *early* event when a resting cell is triggered by a mitogenic agent or event into the division cycle. (It should, perhaps, have been mentioned earlier that when we talk of mitogenic events such as partial hepatectomy, it is most likely that the event leads to the generation of a mitogenic *agent* in the system.) In fact, no matter how early one looks, within the limitation of the techniques available, one finds that when a resting cell enters the division cycle, there is an increase in the rates of uptake of essential nutrients. No exceptions are known to this 'rule'.

(*e*) When a resting cell is triggered into the division cycle, the primary event appears to occur on the cell surface. It is thus possible to make a resting cell enter the division cycle by mitogenic agents which are bound to solid substrates, under conditions that they cannot enter the cell (or the amount that enters the cell as a result of cleavage from the solid substrate, is so small that, all by itself, it is unlikely to be able to initiate cell division).

(*f*) When a normal cell is transformed into a malignant cell, changes in the properties of the cell surface precede and are obligatory to the expression of the malignant phenotype, that is, the general characteristics of malignant cells.

THE MODEL

Are the above observations sufficient to allow one to construct a viable model which can then be tested? The answer to this question appears to be, yes. A model that is based on the six premises mentioned in the preceding section has been constructed and worked upon in our laboratory during the last twelve years or so. It is described in detail elsewhere[7-11]. In principle, the model proposes that regulation of cell division involves a regulation of the transition from the state in which the uptake of essential nutrients is low, to the state in which the uptake of essential nutrients is high, it being argued that the higher rate of the uptake of essential nutrients is an absolute prerequisite for the cell to be able to take in these nutrients at a rate that would allow it to double all its cellular material in the time that is generally taken by cells of higher organisms to divide, starting from the beginning of the G_1 phase to the end of the M phase.

The essential features of this model are as follows. The model postulates four chemical (*I*, Anti-*I*, SF1 and SF2) and two structural (Sites A and Sites B) entities. Sites A and B are functionally different transport sites on the membrane for essential nutrients. Sites A are open in resting cells and need a serum factor, SF1, for operation

at V_{max}. Sites B are closed in resting cells but open in dividing cells· the "on"→"off" control of Sites B is achieved through *I*, a protein with a high rate of turnover and two binding sites. Sites B are closed when *I* is bound to them; the affinity of *I* for Sites B increases when one molecule of *I* links two Sites B. A second serum factor (SF2), Anti-*I* (a postulated antagonist of *I* that generally stays in the cell) when realeased from the cells (*e.g.* as a result of tissue damage), and other external triggers for cell division (such as mitogenic hormones), destroy or inactivate *I*, or prevent its binding to Sites B. The opening of Sites B results in an enhancement of the rate of uptake of nutrients; the resulting increase in the intracellular concentration of one or more of the nutrients starts the programmed operation of events that culminate in cell division; two possible mechanisms for the initiation of this programme are suggested[9]. Growth ceases as a consequence of re-establishment of *I* function on the membrane. Malignant transformation is defined as an inheritable intracellular event, spontaneous or induced, which interferes with the production or activity of *I* and leads to a loss of the capacity for transition from the dividing to the resting state. The model is illustrated in figure 1

THE TESTING OF A PREDICTION

An attempt has been made in our laboratory during the last ten years to isolate the transport-inhibitory protein, *I*, postulated in the model, which protein is predicted to be responsible for the on-off transition of the switch referred to in

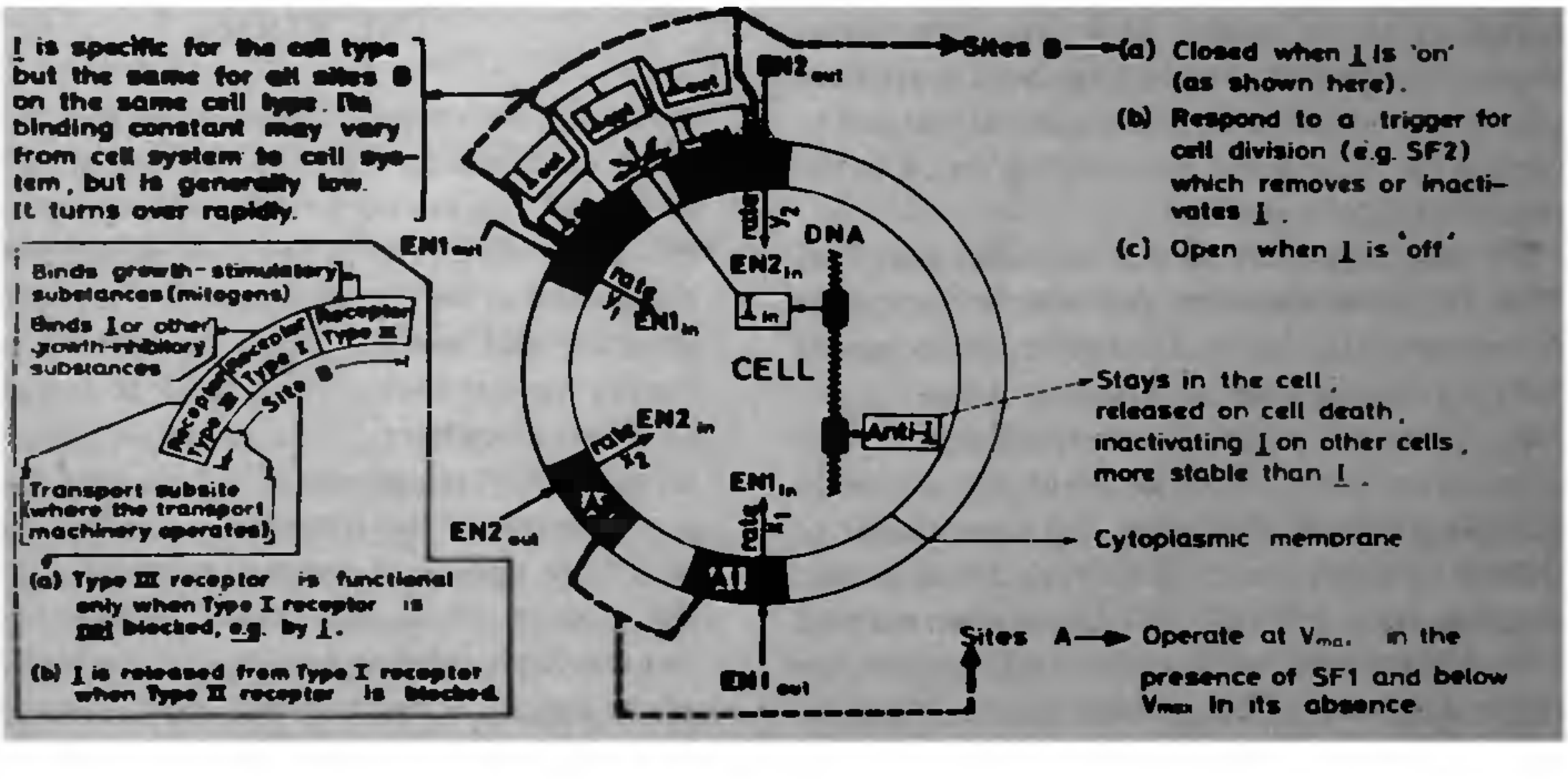

Figure 1. A model for the regulation of cell division through control of uptake of essential nutrients. EN1 and EN2, essential nutrients; Sites A1 and A2, membrane sites for the uptake of various ENs in resting cells (these sites may also be open in dividing cells); Sites B1 and B2, membrane sites for the uptake of ENs in dividing cells, *closed* in resting cells; rate *x*, the maximal rate (V_{max}) of uptake of an EN through a Site A; rate *y*, the maximal rate of uptake of an EN through a Site B; SF1, a serum factor necessary for transport of an EN through Site A at the maximal rate; *I*, an inhibitor of transport through Sites B, which comes out of the cell and acts from outside. Anti-*I*, an intracellular factor, functionally antagonistic to *I* and *normally* incapable of coming out of the cell; SF2, a serum factor functionally antagonistic to *I*. The main figure shows a resting cell. Sites B (inset left) are postulated to consist of three subsites, named Type I, Type II and Type III receptors; morphological overlap of these receptors is not ruled out (From ref. 9).

figure 1. The most important property predicted for this protein is that it will inhibit transport of essential nutrients in all types of dividing cells and bring it to the level obtained in resting cells, but it would not have any effect on transport of these nutrients in resting cells. The protein would, as already mentioned, be tissue-specific. Thus, the *I* factor for liver would not act on kidney cells.

During the current year, a protein that satisfies all the criteria for which it has so far been tested, out of those postulated for *I* in the above-mentioned model, has been purified to homogeneity in our laboratory, from rat liver[10,11]. Its properties are summarised below:

(*a*) It appears homogeneous on SDS and non-SDS polyacrylamide gel electrophoresis and on high performance liquid chromatography, as well as immunogenically.

(*b*) It appears to be a single polypeptide chain with a molecular weight of approximately 50000–55000.

(*c*) It appears to be a major liver protein, representing more than 0.1% of the total dry weight of rat liver.

(*d*) On dispersion of liver tissue to a suspension of liver parenchymal cells, as predicted by the model mentioned above, there is a many-fold increase in the net rate of uptake of essential aminoacids by the parenchymal cells when incubated in a dilute ($\sim 0.5 \times 10^6$ cells/ml) suspension in Krebs-Ringer phosphate buffer or in a tissue culture medium, using concentrations of aminoacids required for maintenance of growth. This protein brings this 'high' level of uptake to the level obtained in resting liver cells (*e.g.* in the tissue slices) or in high-concentration cells, as predicted by the model (see ref. 9 for the detailed rationale).

(*e*) It inhibits the ennanced uptake of aminoacids as well as DNA synthesis obtained in liver cells following partial hepatectomy, bringing the former to the level obtained in resting cells.

(*f*) It inhibits the 'high' uptake of certain aminoacids obtained in the Zajdela ascitic hepatoma, but in no case it brings down the uptake to a level *below* that obtained in resting liver cells.

(*g*) It inhibits the uptake of thymidine and its incorporation into DNA, in the Zajdela ascitic hepatoma.

(*h*) It has no effect on the uptake of amino acids in resting liver cells or kidney cells, or in Ehrlich ascitic carcinoma cells.

(*i*) Antisera raised in rabbits against this protein gives three precipitin lines with the Zajdela ascitic fluid/cells; the lines being generally more predominant in the fluid than in the cells. At least one of the proteins of the Zajdela ascitic fluid precipitated by the antisera, seems to be immunologically partially cross-reactive with the liver protein against which the antisera was raised. This ascitic fluid protein has been purified to near homogeneity following the same method as used for purifying the liver protein. The ascitic hepatoma protein comes out at the same place as the liver protein on the various columns used for the purification. The two proteins also show near identical behaviour on polyacrylamide gels. However, the ascitic fluid protein does not possess the transport-inhibitory properties mentioned above for the liver protein.

We are in the process of determining if the ascitic fluid protein that immunologically cross-reacts with the transport-inhibitory protein we have isolated from rat liver, is a mutated form of the normal protein—as would be predicted by the model. Such a finding would provide stronger support to the model mentioned here.

THE QUESTIONS REVISITED

In terms of the model, then, the switch is a permeability switch, its "off" position corresponding to a transport-inhibitory protein with a special set of properties, sitting on the cell surface, and the "on" position to the cell-surface receptors for this protein being unoccupied. That would be the answer to the first question. We believe we have a good candidate for this protein in the case of liver.

As regards the fourth question (how does the switch get jammed in the "on" postion in malignant transformation?), the model suggests that malignant transformation (chemical or viral, spontaneous or induced) leads to a condition

that makes the cell—at least phenotypically—I^-. Chemicals, for example, could lead to a mutation in the structural or a regulatory gene or nucleotide sequence for *I* or its receptor. Alternatively, the mutation could be in the regulatory part of a normal, host oncogene, such that it (the mutation) leads to increased production of the oncogene product, or in the structural part of a defective oncogene which can now (following the mutation) produce an active product; the product—before processing or after, alone or in conjunction with a host cell-coded product—may act on the cell membrane (or within the cell) in such a way that *I* can no longer be secreted or can no longer bind to its own receptor. The cells would then become incapable of reverting back to the resting state and continue to divide even in the absence of a trigger or mitogen. This view is supported by the fact that ultimate chemical carcinogens are known to be mutagens[9,12,13]. Oncogenic viruses could lead to the production, in the transformed cell, of a product that acts as mentioned above for the normal host oncogene product. We have, in our laboratory, through the isolation of what appears to be a mutated and inactive transport-inhibitory protein from a liver tumour, obtained evidence which supports the above view.

In regard to the third question (the link between the switch and the programme of the division cycle), the model is on more tenuous grounds. However, it is not difficult to envisage several possible mechanisms through which the influx of essential nutrients following the putting on of the permeability switch, could lead to the synthesis or the activation of a protein that is required to catalyse the first reaction of the programme of the division cycle[9].

Above all, the model provides a satisfactory basis for the evolution of auxotrophy—which would, *prima facie*, appear to be a disadvantage—in higher organisms such as man. It suggests that the ability of cells of higher organisms to exist in the resting state, even when all the required nutrients and auxiliary growth factors are available--an ability crucial to the existence and performance of the organism—would not have come about if the cells were not auxotrophic.

The model makes one important point: that both chemical carcinogens and oncogenic viruses converge at a definable point in regard to the mechanism which is responsible for a cancer cell behaving the way it does[15]. I must point out that tumours such as leukemias and teratomas which are due to a block in the normal pathway of differentiation, or plant tumours (the crown galls), fall outside of the scope of the model described here.

CONCLUSION

To conclude, what I have attempted to do in this and the earlier article of this series is to share with the readers how one of the most important problems in biology, that is central and crucial to many other interesting problems, can be looked at in a way that it relates to common experience, and how one can convert it into a set of well-defined and precise questions that allow one to construct viable models. Finally, I have presented here *one* model which attempts to provide tentative answers to three out of the four questions asked in these articles—the questions of which the total problem of regulation of cell division and malignant transformation from the mechanistic point of view is comprised.

So far, there does not appear to be any major finding which argues against the model briefly mentioned here. Further work on the transport-inhibitory protein that we have isolated from rat liver, and on what appears to be an inactive mutated form of this protein from a liver tumour, will, hopefully, allow one to determine whether or not the model that we had constructed sometime ago deserves further attention.

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(This references cited here are only representative and not exhaustive.)

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ANNOUNCEMENT

SEMINAR ON "AMPHIBOLITES: THEIR MINERALOGY, PETROLOGY, GENESIS AND GEODYNAMIC SIGNIFICANCE"

The Seminar is being organised at the Department of Geology, M.S. University of Baroda, during 28th November to 1st December 1983, with a view to bringing together all workers from various disciplines who are interested in the study of Amphibolites. The main objective of the Seminar is to provide a common platform of Mineralogists, Petrologists, Geochemists etc., to present their recent studies on the various aspects of the Amphibolites.

Geoscientists who are interested in participating in this Seminar are requested to contact Prof. S. S. Merh, (Director of the Seminar), Department of Geology, Faculty of Science, M.S. University of Baroda, Baroda 390 002.