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RESPONSE OF CELLS TO INHIBITION OF SYNTHESIS OF DNA, RNA AND PROTEIN*

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Abstract—Fundamental to our eventual understanding of the cellular biochemistry of disease is a knowledge of how different cells react to discrete and selected biochemical lesions. This presentation is restricted to an analysis of the response patterns of some cells in the intact animal to the inhibition of the synthesis of three major macromolecules—protein, RNA and DNA.

Proliferating cells respond to inhibition of protein synthesis by interruption of the cell cycle early in the G-2 phase, resulting in complete disappearance of all mitotic figures without cell death. In the liver, the major superficial response is accumulation of triglyceride (fatty liver) due to the interference, with the triglyceride-secretory function of the liver.

Two patterns of response to inhibition of RNA synthesis have been observed—a rapid dissociation of nucleolar components, with a slower disaggregation of free polysomes (liver) due to inhibitors that interact primarily with DNA and a progressive fragmentation of the nucleolus with wide scattering of fragments in the nucleus, seen with inhibitors that do not act via DNA. It is considered that the first pattern may be the result of a triggering of RNA breakdown.

It is generally considered that interference with DNA synthesis leads to cell death. However, recent evidence indicates that protein synthesis is essential for cell damage induced by DNA inhibitors, thus suggesting that the relation between DNA and cell death is an indirect one.

IT IS eminently clear that virtually every pathologic process and disease must have a clearly defined biochemical base before we can admit to any significant understanding of that entity. Yet, it is also clear that most naturally-occurring disease, be it induced by chemicals, viruses, bacteria, dietary deficiencies or other environmental alterations, is extremely complex at the level of the chemistry of the cell and may often be impossible to unravel with current knowledge. This is in part due to the existence of many interlocking regulatory control mechanisms which allow for integration and modulation of the metabolic activity of the cell.

These considerations require a body of fundamental knowledge about how cells react to a change, be it increase or deficiency, in the level of a single step in important metabolic pathways before the more complex picture in naturally-occurring disease can be fully understood.

It was with this in mind that we have concentrated many of our research efforts over the past several years upon the response patterns of selected cells, such as liver and intestine, to inhibition of synthesis of major types of macromolecules—protein, RNA and DNA. Although we realize that the full clarity of a response pattern may be obscured by the simultaneous interference with large numbers of macromolecules of

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one type, such as proteins, we nevertheless feel that this kind of information is basic and necessary.

Inhibition of RNA synthesis

There are two known general response patterns of cells to inhibition of RNA synthesis—(1) a rapid dissociation of the nucleolar components accompanied by a much slower disaggregation of free polyribosomes (at least in the liver) and (2) progressive fragmentation of the nucleolus with scattering of pieces of the nucleolus widely in the nucleus. The best example of the former is actinomycin (cf. 1), although other compounds that combine with DNA such as aflatoxin,[1] 4-quinoline-*N*-oxide,[2] proflavin[3, 4] daunomycin[1] and ethidium bromide[1] also induce the same changes. The available evidence suggests that it is not the inhibition of RNA synthesis *per se*[1, 5] but some other property such as interaction with DNA that may be responsible for the nucleolar change. The polyribosome change is almost certainly due to the relatively slow loss of messenger RNA from the cytoplasm.

Ethionine[6–8] α-amanatin[9] and perhaps azaserine in part[1] produce the second change, the fragmentation of the nucleolus. These appear to act not by combining with DNA but rather by an effect on nuclear ATP concentration[7, 8, 10] or by direct inhibition of RNA polymerase.[11] The striking feature of this type of response is the formation of precursor structures during reformation.[7–9] This offers an interesting model to get at some aspects of the important problem of the organization of a nuclear organelle, the nucleolus, so important in ribosome synthesis and perhaps also in m-RNA metabolism.

These reaction patterns to RNA synthesis inhibition raise many interesting questions concerning the role of DNA in controlling the metabolism of RNA in addition to its template function for transcription. The possibilities have been suggested that DNA may control the activity of nuclear ribonucleases or may protect newly synthesized RNA molecules from degradation by nucleases until they are appropriately coated or used.[10]

Inhibition of protein synthesis

In this phase of macromolecular synthesis, our knowledge is less, mainly because it appears that the response patterns may vary from cell to cell. However, there is one response pattern that appears to be general, at least in somatic cells in the intact rodent—interruption of the cell cycle with at least one block early in G-2.[12, 13] In the intestine and liver and probably other organs also, the inhibited cells continue their progression uninterruptedly through G-2 and M and into G-1. This results in a virtual disappearance of mitotic figures and in a partial synchronization of the cells without any loss of cells through cell death.[12, 13] It appears that a high degree of cell synchronization *in vivo* may become possible through the use of inhibitors of protein synthesis.

In the liver, inhibition of protein synthesis appears to be a major biochemical lesion for several types of fatty liver—CCl_4, ethionine, puromycin, etc. This story is now quite well known and requires only a brief reference. The liver plays a major role in the conversion of free fatty acids from the blood to triglyceride (TG) which is then put back into the blood as plasma lipoproteins. The real biological significance of this physiologic function is not yet completely clear. However, the role of the liver in the trans-

formation is a major one. For this is required rapidly turning over protein, phospholipid, cholesterol and perhaps some polysaccharide as well as TG. The inhibition of protein synthesis cuts off the supply of the essential protein with the consequent increase in liver TG, since there is no apparent feedback inhibition of TG synthesis under such conditions. Large amounts of TG can thus accumulate. There are many other features which can be found in fairly recent reviews.[14–16]

However, recent work with cycloheximide[17] and other inhibitors of protein synthesis suggest that the story may be more complex than outlined above. Despite a high degree of inhibition of protein synthesis (over 95 per cent), only slight increases of liver TG are seen with cycloheximide. Also, the male rat given ethionine shows considerable inhibition of protein synthesis *in vivo* without a large increase in TG such as seen in the female with ethionine. These puzzling phenomena remain to be explained. One feature which appears to correlate so far is the state of aggregation of the ribosomes. With ethionine, puromycin and CCl_4, inhibition of protein synthesis is accompanied by a disaggregation of polysomes, probably free and bound, with the concomitant appearance of free monosomes and ribosome subunits. In contrast, with cycloheximide and the male rat treated with ethionine, the inhibition of protein synthesis is not associated with any significant degree of disaggregation of polysomes.[18, 19] Is it possible that the maintenance of the intact polysome in close association with the organelle synthesizing the TG, the endoplasmic reticulum, may allow a regulatory control of TG synthesis, such as a negative feedback, which is lost when the polysome is no longer *in situ* on the membrane? The absence of a significant TG accumulation with Actinomycin D could conceivably fall into the same class, since recent work has shown that this antibiotic affects only free and not bound polysomes.[20]

Thus, the results so far obtained with the analysis of the response of cells in the intact animal to inhibitors of protein synthesis are sufficiently interesting and intriguing to suggest that their further study in depth may give new insight into several important aspects of cell metabolism and structure, especially from the point of view of integration of metabolic pathways in the intact cell.

Inhibition of DNA synthesis

It is generally considered that interference with DNA synthesis or metabolism leads to death of the affected cell. This was first clearly indicated by the work of Cohen and Barner who described unbalanced growth or thymineless death in bacteria.[21, 22] This was subsequently extended to many other cells including eukaryotes (see ref. 23). This tentative generalization has been one of the guiding principles in the design of chemical agents for cancer chemotherapy.

However, certain results have recently been described which raise serious doubts concerning the validity of this hypothesis.[23]

Inhibition of protein synthesis is consistently accompanied by inhibition of DNA synthesis in proliferating cells. Yet, inhibitors of protein synthesis, such as cycloheximide and tenuazonic acid do not lead to cell death in cells such as crypt cells of the intestine which are sensitive to inhibitors of DNA synthesis such as arabinosyl cytosine (ara-C, "cytosine arabinoside"). This suggested that inhibition of DNA synthesis *per se* may not be sufficient to induce cell death and that perhaps protein synthesis may be required to kill cells by inhibition of DNA synthesis.[23] This reasoning led to studies on the effects of inhibitors of protein synthesis, such as cycloheximide

and tenuazonic acid on cell damage induced by ara-C, nitrogen mustard (HN2) or X-irradiation. This study has clearly shown that inhibition of protein synthesis of about 75 per cent or more will selectively prevent cell damage of intestinal crypt cells induced by the three methods just mentioned and that the inhibitor can be given even *after* the maximum initial biochemical lesion has occurred and still be effective. There is a high degree of selectivity in all of these phenomena since lymphoid cells are killed by inhibition of RNA, DNA or protein synthesis and no protective effect has yet been evident in these types of cells. This differential response pattern may be responsible for the high susceptibility of some lymphoid neoplasms to therapy in the face of the resistance of so many other neoplasms.

It thus appears that protein synthesis, perhaps as enzyme induction, may be necessary for cell damage by interference with DNA synthesis or metabolism. The nature of the protein(s) required and of the coupling between interference with DNA and protein synthesis seem to be potentially important areas for further exploration of this phenomenon.

COMMENTS

It is evident from this abbreviated review of the response of different cells to selective interference with the synthesis of groups of macromolecules that further study of this field may well uncover many interesting new facets concerning cell organization and behavior. Some of these could remain hidden in the complexity of the interlocking nature of the metabolic pathways of the cell and only be observed and studied by focussing our attention on how cells react to selective interference with the synthesis of macromolecules.

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