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MECHANISMS IN CELLULAR METABOLIC REGULATION*

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Abstract—Studies on the regulation of genetic expression in mammalian systems have demonstrated that a significant amount of regulation occurs at the level of genetic translation. Studies in this report demonstrate that it is possible to measure the specific lifetime for a mRNA of a hepatic enzyme. Furthermore, the enzyme under study, serine dehydratase, was found to exist in two isozymic forms, the synthesis of each form apparently being regulated by different mechanisms. Such findings are in line with other studies in this area and demonstrate the complexity of the regulation of genetic expression in mammalian systems wherein one is dealing with multiple phenotypic populations of cells as opposed to prokaryotes which are self-sufficient.

IN A DISCUSSION of any topic the authors are logically forced into the position of beginning with a resume of known knowledge and progressing to its fringes as illustrated by work from their own laboratory. In using this approach to such a broad topic as "the mechanisms of cellular metabolic regulation", our most definitive and complete knowledge appears to reside in an understanding of these mechanisms in bacteria. Thus, one should logically consider the knowledge gained from experiments in microorganisms and then attempt applications of such facts to mammalian systems.

There appear to be several major differences between prokaryotic and eukaryotic regulatory mechanisms, one of the most striking being that of the levels of regulation utilized in the uni- versus the multicellular forms. Studies from several laboratories[1–3] have shown that mammalian systems, unlike many bacterial systems, have mechanisms for the regulation of genetic expression independent of RNA synthesis. Such systems are apparently involved in such diverse phenomena as differentiation, antibody response, carcinogenesis and hormonal response. Mammalian systems also exhibit the phenomenon of enzyme turnover which has been shown to be of considerable importance in the environmental determination of enzyme levels in multi-cellular organisms.[4] Although we do not know the entire mechanisms of enzyme turnover, evidence indicates that this may well be primarily a translational mechanism.

Another interesting difference between mammalian and bacterial systems is the extensive utilization of allosteric regulatory mechanisms wherein, especially in the case of multiple forms of an enzyme, each form may be regulated by different allosteric effectors.[5] This is not to say that there are no allosteric regulatory mechanisms in mammalian tissues, but rather that a number of enzymes having such regulation in microorganisms appear to have lost it during the evolutionary jump to the mammal.

If one grants the relative frequency of translational regulatory mechanisms in mammalian tissues, as well as the relative paucity of allosteric effects, (the latter statement being debatable in general but certainly true in specific cases) then the questions

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posed are (1) What is the mechanism of translational regulation? and (2) Does the mammal have mechanisms open to it other than allosteric effects to regulate enzyme activity and to answer the problem of multiple functions for one enzymatic reaction? In our laboratory we have been directing our efforts toward the answer of question 1 and only recently have been given some insight into the answer of question 2. As we have reported earlier,[6,7] several hepatic enzymes exhibit periods of enzyme synthesis

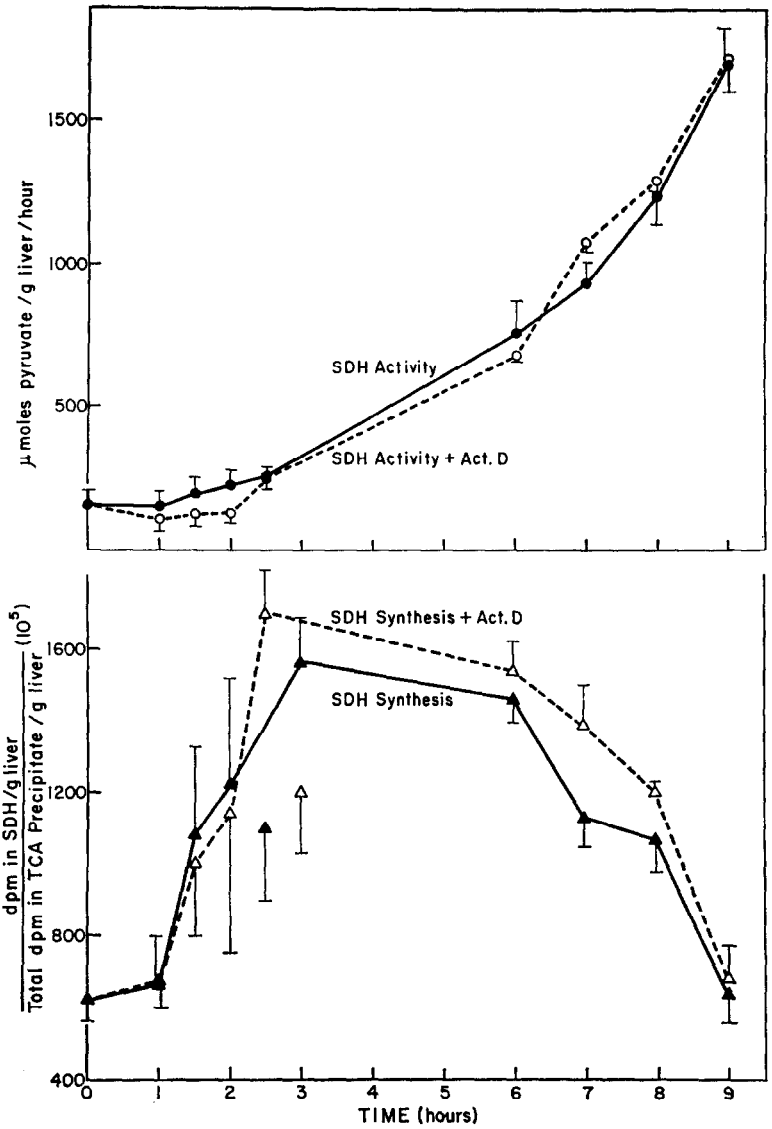

FIG. 1. Changes in serine dehydratase activity (upper) and rate of synthesis (lower) in the presence and absence of actinomycin-D after a single dose of casein hydrolysate. At each time point actinomycin-D (1 mg/kg) was given 45 min prior to sacrifice. [^{3}H]-leucine (50 μc) was administered intraperitoneally 30 min before sacrifice in order to obtain the data of the lower part of the graph. The immunochemical methods utilized, and the enzyme assays are described in refs. 3, 7, 8. The entire details of the experiment are described in ref. 8.

which occur in the absence of RNA synthesis. Furthermore, it has been possible to demonstrate that during these periods changes in the environment by hormones or substrates may actually alter the rate of synthesis of the enzyme demonstrating clearly a translational regulatory phenomenon.[7] Recent studies in our laboratory have shown that by utilizing such techniques it is possible to demonstrate the actual kinetics of translational control periods in liver. In Fig. 1 is demonstrated such an experiment wherein both the increase in enzyme activity and the actual rate of synthesis of serine dehydratase in the presence and absence of actinomycin-D is determined. The format of the experiment has been previously described.[8] In essence, the data indicate that the actual rate of synthesis of serine dehydratase increases rather dramatically at about the second hour after the initiation of induction reaching a maximal rate which then continues for approximately the next 6 hr rapidly returning to the initial rate at the end of this period. Actinomycin administration completely suppresses enzyme synthesis when given at zero time[6] or the first to second hour after induction is initiated. Also when given again at the ninth hour, further enzyme synthesis is completely curtailed. It should be noted that this period of actinomycin-D resistance is also indicative of the actual rate of synthesis of the enzyme. Thus, when no more stable template is available, enzyme synthesis drops to control levels. Other studies not presented here indicate that during the period of translational regulation, the rate of synthesis also may drop and then be regained by suitable administration of an inducing agent. Furthermore, the administration of glucose during this time period completely suppresses enzyme synthesis although other mechanisms have demonstrated that the template for the enzyme appears to remain in a stable condition despite the virtual absence of enzyme synthesis *per se*. Therefore, it would appear to be evident that translational regulatory phenomena are quite prominent in the synthesis of this enzyme, serine dehydratase. Other studies[3,9] have demonstrated the importance of this same phenomenon in the case of ornithine transaminase, tryptophan pyrrolase, aspartic transcarbamylase, and deoxythymidine kinase. A possible mechanism for such translational phenomena has been discussed previously.[11] In essence, the model presented argues that a membrane-bound messenger RNA-ribosome complex is in effect the functioning, regulatable translating unit, certainly in liver and possibly in many other tissues as well. This cytoplasmic translating unit has been termed the MEMBRON. The evidence for its structure as demonstrated in Fig. 2 has been previously considered[11] as well as the possibility that an alteration of the MEMBRON population is a major factor in the neoplastic transformation.

Although the MEMBRON concept suggests a mechanism for translational regulation, it does not *per se* suggest any answer to the second question posed above, that of the regulation of multiple functions for a single catalytic event. In bacteria this is carried out quite easily by synthesizing two or more enzymes having the same catalytic function but different allosteric effectors, e.g. threonine deaminase in *E. coli*.[12] Recent studies in our laboratory have suggested a possible alternate explanation for such phenomena in mammalian liver. In Fig. 3 is demonstrated a chromatographic eluent pattern of the enzyme, serine dehydratase, as seen in crude hepatic extracts. It will be noted that there are two peaks of enzymatic activity. One can demonstrate these two peaks of enzymatic activity and their separation on gel electrophoresis. The characteristics of the two forms of the enzyme, serine dehydratase, are quite similar. No immunological or kinetic differences can be found in the two proteins. Each has been

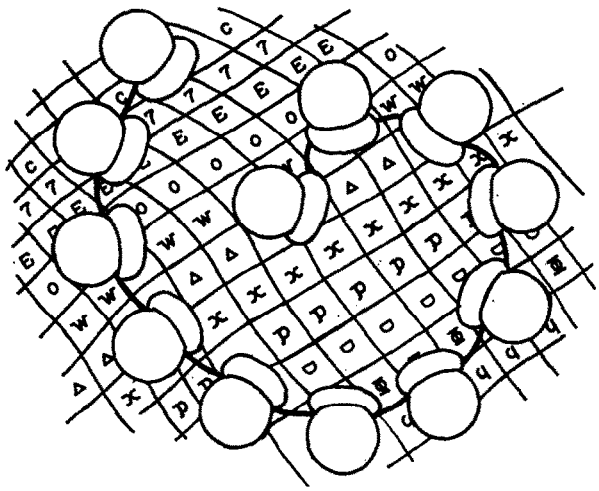

FIG. 2. Artist's conception of the MEMBRON. The mosaic pattern in the background denotes the surface of the endoplasmic reticulum while the 30 and 50 s ribosomal subunits and messenger RNA are seen from this picture. Transfer RNA and the growing polypeptide chains are not pictured for purposes of clarity. See ref. 11 for details. (Reproduced by permission of the AMA Archives of Pathology.)

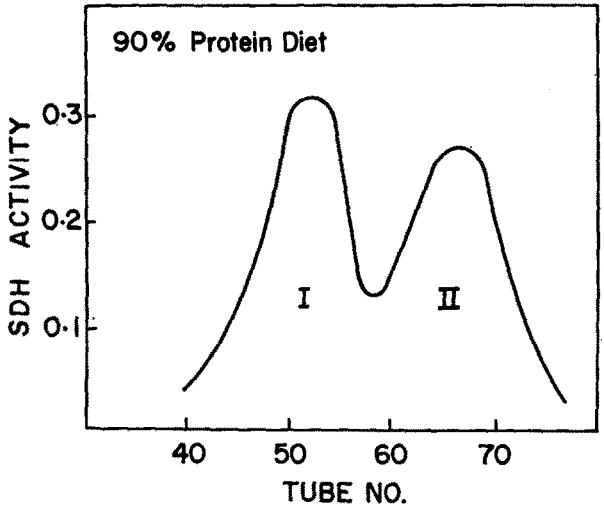

FIG. 3. Serine dehydratase isozymes in the liver of rats on a 90% protein diet. Chromatographic fractions are plotted on the X axis versus enzyme activity on the Y axis.

crystallized from rat liver. The most interesting facet of this phenomenon is seen in Table 1. Herein, it is demonstrated that the levels of the two forms of serine dehydratase vary under different environmental conditions. As can be seen from the table, the administration of glucagon increases only the second form of the enzyme whereas the administration of tryptophan increases both forms. In several hepatomas, enzyme 1 is the predominant form. Shortly after birth enzyme 2 appears with no simultaneous appearance of the other form.

These studies strongly indicate that the two forms of serine dehydratase, although being quite similar, are regulated by different environmental conditions. In a cell as

TABLE 1. PERCENTAGE DISTRIBUTION OF ISOZYMIC FORMS OF SERINE DEHYDRATASE IN LIVER UNDER SEVERAL HORMONAL AND DIETARY CONDITIONS AND IN THE MORRIS 7800 HEPATOMA

Treatment	Type of SDH	
	I	II
	%	
Normal (chow)	0	100
Hepatoma (Morris 7800)	80	20
90% Protein Diet (7 days)	55	45
Glucagon (0·5 μg/rat)	8	92
Diabetes (Alloxan-induced)	2	98
Cortisone (10 mg/rat)	5	95
Starvation (48 hr)	6	94
Newborn (1 week after birth)	0	100

complex as the mammalian cell it is entirely possible that there is a compartmentalization of cellular enzyme synthesis and that certain aspects of the environment may affect the synthesis of one form of an enzyme and others another form. This requires, in the MEMBRON concept, a certain spatial arrangement of these functioning units within the cell. In actual fact, this may be predicted from the concept. Thus, it would appear that the mammalian cell may have an even more sensitive mechanism for regulating multiple functions of a single enzymatic capability. Recent studies by Oliver and his associates[13] have demonstrated a similar phenomenon for the enzyme, tyrosine transaminase. Other studies on isozymes of hexokinase, lactate dehydrogenase, malic dehydrogenase, etc. are well known. The possibility that spatial arrangements of MEMBRONS in the cell may be a regulating factor in environmental control is relatively new, but certainly opens an interesting vista of research in biochemistry of the future.

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