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Genes and Nucleoproteins in the Synthesis of Enzymes[1]

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PREVIOUS PUBLICATIONS (*14, 16*) reported examinations of substrate-induced enzyme formation in genetically controlled yeast strains in an attempt to employ this phenomenon as a tool for the analysis of the gene-enzyme relationship problem. Some of the pertinent facts which emerged from these studies may be summarized briefly as follows: (1) Potentiality for enzyme formation can segregate in a Mendelian fashion; (2) substrates, rather than genes, critically control enzyme activity levels; and (3) an enzyme (melibiase) can be maintained in the presence of substrate for an indefinite number of cell generations in the absence of the gene necessary to the initiation of its synthesis.

These findings suggested the existence of a cytoplasmic self-duplicating entity as being involved in enzyme synthesis. Whether the entity was the enzyme itself or something mediating its formation could not be decided on the basis of the observations. However, because of its simplicity the former was adopted as a tentative working hypothesis. In any case, it was evident that, in so far as enzymatic constitution was concerned, the cytoplasm possessed a degree of autonomy from the genome not easily reconcilable with the more classical views of gene-enzyme relationships in which the gene is considered as the only self-duplicating unit in the cell.

Subsequent experiments were designed to obtain more information on the mechanism of enzyme formation with particular reference to energy requirements. It is proposed to report here certain of the results obtained as being particularly suggestive for the gene-enzyme problem and to discuss their implications.

It has been found (*15*) that enzyme synthesis under anaerobic conditions could be prevented either with NaN_3 or dinitrophenol without disturbing the rate of carbohydrate metabolism. The ability of these substances to prevent utilization of metabolic energy for synthetic activity is apparently quite general, having been demonstrated for such diverse processes as cell division (*8*), carbohydrate and ammonia assimilation (*18*), and cell regeneration (*9*).

On the assumption that phosphate-bond energy, as generated by the glycolytic system, forms the primary source of energy for cell function and growth under anaerobic conditions, experiments were undertaken to examine the effect of these agents on phosphorus metabolism, using radioactive phosphorus (P^{32}) as a tracer.

The most consistent correlation between phosphorus metabolism and protein or enzyme formation was found in the flow of phosphate *from* the nucleoprotein fraction (NP). This latter is the residue phosphate remaining after successive extractions with water, cold trichloroacetic acid, alcohol, and hot alcohol-ether (3:1).

The behavior of the phosphate in this fraction was followed under various conditions, employing P^{32} in the following manner: Cells were grown in the usual media at 30° C. in the presence of P^{32} (activity, 5×10^5 cts./min./mg. P). This resulted in complete equilibration of the labeled phosphorus in all fractions. After 48 hours these cells were harvested, washed three times in unlabeled M/15 KH_2PO_4, resuspended in unlabeled M/15 KH_2PO_4 with 4 per cent glucose, and allowed to ferment the carbohydrate under completely anaerobic conditions. No budding or increase in protein nitrogen is observed in such suspensions. Samples were withdrawn at intervals for activity measurements. It was found that within 4 hours about half of the total activity was lost. After fractionation it was found that (except for 1 or 2 per cent) this loss in activity could be completely accounted for in the acid-soluble fraction which forms about 50 per cent of the total P content of the cells. The total activity (P^{32} content) as well as total P^{31} of the nucleoprotein fraction had actually increased slightly (8 per cent) during this period, indicating flow of phosphate into this fraction. These data clearly showed that rapidly metabolizing but nondividing cells did not lose phosphate from the NP fraction even though the major portion of the remaining phosphate

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was being rapidly equilibrated. Since activity of the phosphate in the acid-soluble fraction of such cells was about one-fourth that of the NP phosphate, they were favorable material for the further study of exchanges between the two fractions. Allowing such cells to ferment carbohydrate for longer periods of time (up to 6 hours) again left the *total* activity of the NP fraction unchanged, although the specific activity was decreased due to dilution by the flow of low specific activity phosphate from the acid-soluble fraction.

The entire behavior of the NP fraction was changed, however, when such cells were induced to synthesize new protein either by adding ammonia or by forcing the synthesis of a new enzyme. The results obtained in a typical experiment are exemplified by the data

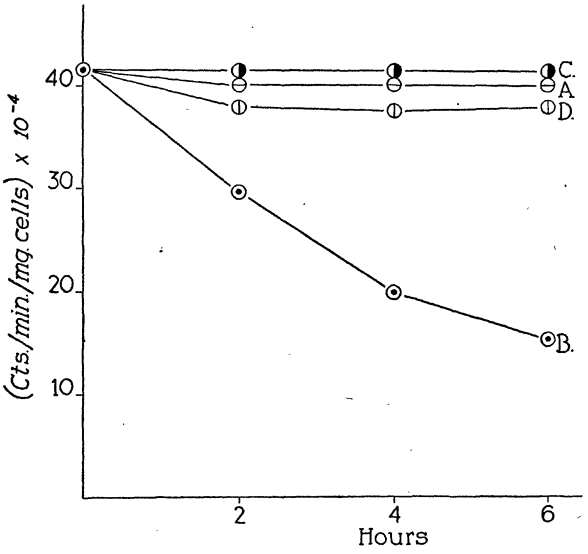

FIG. 1. Behavior of NP phosphate when new protein is being synthesized (B) and when protein formation is prevented by absence of nitrogen supply (A), presence of sodium azide (C), or dinitrophenol (D).

in Fig. 1. In this experiment cells were suspended in physiological saline[2] containing: (a) glucose, (b) glucose + $(NH_4)_2SO_4$, (c) glucose + $(NH_4)_2SO_4$ + NaN_3, (d) glucose + $(NH_4)_2SO_4$ + dinitrophenol. The amount of $(NH_4)_2SO_4$ was equivalent in nitrogen to 50 per cent of the nitrogen content of the yeast. The concentrations of NaN_3 and dinitrophenol were 5×10^{-3} and 5×10^{-4} M, respectively—sufficient to completely inhibit enzyme formation.

It will be noted that with glucose alone there was no change in activity, whereas when ammonia was present, with consequent budding, the nucleoprotein P dropped to 38 per cent of its original total activity, indicating a flow of phosphate from this fraction. It is evident that the azide and, to only a slightly lesser extent, the dinitrophenol were able to prevent this utilization. Except for the fact that the transfer of less phosphate was involved, the same phenomenon was observed when cells were induced to form a new enzyme. Thus, in an experiment in which cells were adapted to maltose, a 34-per cent drop in activity of the nucleoprotein phosphate was observed. Again azide and dinitrophenol in the above concentrations prevented both the formation of the enzyme and the transfer of phosphate from the NP fraction.

These findings provide us with the following correlations between protein or enzyme synthesis and the transfer of phosphate from the NP fraction: (1) Rapidly metabolizing cells which are not synthesizing new protein do not transfer phosphate from the NP fraction; (2) synthesis of new protein or enzyme is paralleled by a marked transfer of phosphate from the NP fraction; (3) agents which are effective in inhibiting enzyme formation and protein synthesis also prevent flow of P from the NP fraction. To these must be added the fundamental observations of Caspersson and his collaborators on yeast (*2*), as well as many other cells, which point to a rigid connection between nucleic acid metabolism and protein synthesis.

In the light of the data presented here, which introduce nucleoproteins as controlling elements in enzyme synthesis, it seems highly desirable to reexamine our previous experiments and interpretations. Several problems are raised, among them the function performed by the nucleoprotein and, closely related, the nature of the relation of the nucleoprotein to the gene, on the one hand, and to the enzyme whose formation it mediates, on the other. An obvious opportunity exists here for a rational linkage of these three fundamental units.

Modern biochemical research (*7*) which has emphasized the role of organic phosphate bonds as sources of energy for synthetic activities provides a foundation upon which may be based a reasonable hypothesis of nucleoprotein function in enzyme synthesis. Of particular value here is the mechanism of complex polysaccharide synthesis, the elucidation of which we owe to the work of the Coris (*3*) and their collaborators. It is found that in the synthesis of a glycosidic bond, glucose-l-phosphate rather than glucose is the reactant involved. The unique feature here is the conversion of an energy-requiring synthetic step into a spontaneous reaction by supplying the necessary energy *in the molecular structure of one of the reactants.*

From this it would appear that the quantitative energy requirement for a particular synthetic reaction is not the crucial issue. There are many phosphory-

[2] The use of exogenous inorganic phosphate decreases quantitatively the flow of phosphate from the NP fraction as observed by activity measurements, since there is a tendency for the cells to utilize the more recently esterified phosphate for synthetic purposes.

lated compounds (e.g. glucose-6-phosphate, adenosinetriphosphate) which have more than sufficient energy to form a glycosidic link if there actually existed some mechanism for 'feeding' it into the reaction. However, the energy content of these compounds cannot be used for this purpose. Clearly, a further requirement is what may be called "specificity" of bond energy. Thus, the actual nature of the bond, and the compound carrying the energy, will determine its suitability for driving a particular reaction. The energy generated by the 'catabolic wheel' and trapped in such energy accumulators as adenosinetriphosphate or creatine-phosphate is too 'generalized' to be used in driving the various synthetic mechanism of anabolism. The energy contained in such compounds must be transferred to others which can act as specific energy donators for particular synthetic reactions.

This concept unifies and simplifies the problem of biological synthesis, since it avoids separating the problem of synthesis into one involving the reactants and another concerned with the source of the "coupled" driving energy.

From this point of view it is not surprising to find that nucleoproteins are controlling elements in protein formation. We may further plausibly suggest that these phosphorus-containing proteins are the specific energy donators which make possible reactions leading to protein and enzyme synthesis.[3]

It would be hazardous at present to attempt to offer a definitive formulation of what we mean by a 'self-duplicating' unit. However, one attribute it is likely to possess is the ability to transform and accumulate energy within its own molecular structure which can be used for the synthesis of similar units. At any rate, it is relatively easy to show that the growth kinetics of such 'energy accumulators' is of the self-duplicating or autocatalytic type. Of the known proteins, those containing phosphorus would be the most likely to possess this property.

In the light of the data presented here and the above discussion it seems reasonable to adopt the tentative working hypothesis that the cytoplasmic self-duplicating entities previously found (*14, 16*) to be involved in enzyme formation are nucleoproteins rather than the enzyme itself. Such an hypothesis would be in harmony with the findings that all accepted self-duplicating units (with but one or two isolated exceptions) have been found to be inseparably linked with nucleic acid-containing compounds. Among such entities may be mentioned genes (*12*), plastogenes (*19*), viruses (*5*), and the pneumococcus 'transforming principle' (*1*).

[3] H. J. Muller (personal communication) independently suggested that nucleoproteins might provide the energy for protein synthesis in his Pilgrim Lecture in London, November 1945 (in press).

From the assumption that the nucleoprotein rather than the enzyme is the cytoplasmic self-duplicating unit several experimental consequences follow. It would be expected that cells not possessing the initiating gene for a particular enzyme could still retain capacity for synthesis of such an enzyme, even in the absence of this enzyme, providing an adequate number of nucleoprotein units were present. Experiments with melibiase (*16*) are consistent with this point of view. In some of the clones missing the mel + gene, irreversible loss of potentiality for melibiase synthesis was not obtained until about 20 hours subsequent to the disappearance of all measurable enzyme. Thus, for a considerable period of time these cells retained the capacity for the synthesis of this enzyme in the absence of any evidence for its presence in the cytoplasm. Recent experiments (*6*), suggesting that cytoplasmic transmission of the capacity to form enzyme can occur in the absence of any measurable enzyme activity, would also support this idea.

In view of the presumed chemical similarity between the two, it seems almost necessary to conclude that the self-duplicating nucleoprotein which mediates protein and enzyme formation is derived from the gene. The value of this conclusion resides in the fact that it provides us with an experimentally analyzable entity which can bridge the gap between the gene in the nucleus and the enzyme in the cytoplasm.

We are thus led to propose the following concept of gene action: Genes *continually* produce at different rates partial replicas of themselves which enter the cytoplasm. These replicas are nucleoprotein in nature and possess to varying degrees the capacity for self-duplication. Their presence in the cytoplasm controls the type and amounts of proteins and enzymes synthesized. These cytoplasmic self-duplicating units, like all such entities, would compete with each other, and the outcome of such competitive interaction would then determine the enzymatic make-up of the cytoplasm. Inherent in this concept is the possibility of changing the ultimate result of this competition by varying the conditions under which it takes place.

The unique feature of this kind of theory is that while supplying a link between gene and enzyme it at the same time predicts that cells with identical genomes need not possess identical enzymatic constitutions. Whether a particular character will be transmitted from one cell generation to the next in a Mendelian fashion will, thus, depend on the relative rates of duplication of the controlling cytoplasmic units as compared with their rate of production from the gene. If the latter is quantitatively determining, Mendelian inheritance will be observed; if the former is determining, the Mendelian picture will be obscured to varying degrees. As a tentative working hypothesis, it has

the advantage of providing a unified point of view from which such diverse and apparently contradictory phenomena as classical Mendelian genetics, cytoplasmic inheritance, cellular differentiation, and enzymatic adaptation may be analyzed.

The basic problem of cancer involves explaining the appearance of a sudden *heritable* change in somatic cells analogous in several ways to enzyme adaptation or cellular differentiation. It is, therefore, not surprising that cancer investigators (*11, 17, 20*) were one of the first groups of biological workers to strongly support the suggested existence of a cytoplasmic hereditary unit. An entity of this kind, by being self-duplicating, provides them with another level at which a mutation can take place and be subsequently transmitted via the cytoplasm from one cell generation to the next.

More or less similar views have been proposed by geneticists. Wright (*21*) in particular emphasized several difficulties in trying to explain either growth or differentiation in terms of the classical Mendelian concept of the gene. Thus, assumption that every time a new protein molecule is formed during growth the gene on the chromosome must intervene as a kind of model implies that growth would proceed linearly from a relatively minute portion of the cell. The kinetics of cell growth follow an autocatalytic law and so are not consistent with this thesis. He therefore suggested that perhaps "duplicates or partial duplicates of genes reach the cytoplasm when the nuclear membrane disappears in mitosis and that these can produce duplicates in turn, and so on, permitting exponential increase." To explain the fact that cytoplasmic inheritance is rarely observed he assumes that the self-duplicating capacity of these free genic replicas is subject to decay. Those that retain this capacity indefinitely he called "plasmagenes."

Again, in connection with cellular differentiation Wright (*22*) points out that the heritable stability of the differentiated state is more easily understood if we assume the existence of self-duplicating cytoplasmic components (plasmagenes) which can undergo controlled mutations. Stimulated by the fundamental observations of Sonneborn (*13*), Darlington (*4*) also postulated the existence of a cytoplasmic self-duplicating unit which he called the "plasmagene" and which he assumed controls heredity at the "molecular level."

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Obituary

Enoch Karrer

1887-1946

The field of textile research lost a valuable physicist in the death of Enoch Karrer on 27 March 1946 at Marine Hospital, New Orleans, Louisiana, following a short illness. Dr. Karrer was in charge of the Physics Section, Cotton Fiber Research Division, Southern Regional Research Laboratory.

Dr. Karrer came in 1936 to the Cotton Division, Bureau of Agricultural Economics, U. S. Department of Agriculture, in which the cotton investigations were then being conducted. The investigation of the physical properties of cotton fibers was a relatively new field to Dr. Karrer, who had given many of his earlier years to problems connected with light, optics, physiological stimulus of animals, rheology of rubber, and the like. This work was subsequently moved to the New Orleans laboratory.

Enoch Karrer was born at Rich Hill, Missouri, on 23 May 1887. After completion of grade and high school at Ellensburg, Washington, he attended the state university at Seattle, where he received the A.B. and M.A. degrees in 1911 and 1912, respectively. During 1913 and 1914 he was a fellow at Johns Hopkins University and was awarded the Ph.D. degree at the end of the latter year.

For several years following the completion of his formal education Dr. Karrer was associated with the United Gas Improvement Company, Philadelphia,