Protein Structure and Function during Differentiation

Problems of developmental biology are proving increasingly attractive to biochemists and geneticists. The central importance of these problems has long been recognized but the intermittent efforts in the past to apply the tools of biochemistry or genetics to the solution of problems of development have not been very rewarding. Now, a breakthrough, particularly in the area of cell differentiation, seems imminent. One of the most profitable avenues of attack lies through an examination of the synthesis, structure, and function of macromolecules and in particular of the proteins.

At the recent Federation meetings the American Society of Biological Chemists brought together representatives of embryology, biochemistry, and genetics in a symposium (15–17 April) devoted to protein structure and function during differentiation. Three well chosen proteins were discussed: hemoglobin, glutamate dehydrogenase, and lactate dehydrogenase. Each illustrates same fundamental aspect of the process of cell differentiation. Park I. Gerald reviewed the extensive work on human hemoglobin. From this work has emerged the most complete description of the time and cell specificity of gene function in bringing about the synthesis of a specific variety of protein. Hemoglobin is a tetramer composed of two kinds of monomers, each under separate genetic control. At least four distinct genes, and perhaps more, encode the structure of corresponding monomers. The relative rates of activity of these various genes at different stages in development determine the availability of the monomeric subunits out of which the hemoglobin molecule is constructed. The subunit composition in turn specifies the functional properties of the hemoglobin, and these are normally in accord with the requirements of the organism, whether fetal or adult. Many mutant genes have been discovered through the aberrant types of hemoglobin they form, and because of the complete analysis of the primary structure of hemoglobin it has been possible to relate these mutations to the substitution of one amino acid for another in the primary sequence. Presumably the several distinct genes for the synthesis of the different hemoglobin monomers arose during the long course of evolu-

tion by duplication and gradual divergence through accumulated mutations. On this hypothesis the various polypeptide chains of hemoglobin and myoglobin may be placed in a plausible evolutionary sequence based upon the degree of chemical homology among them. From the viewpoint of developmental biology it is not the structure of hemoglobin may be placed in a plausible evothe fact that one kind of hemoglobin replaces another during development. The mechanism by which genes for protein synthesis are turned on and off are presently unknown, but Gerald described an interesting mutation in a gene which may be responsible for switching on the synthesis of the adult chains of hemoglobin. The location of this gene adjacent to the genes for adult chains of hemoglobin suggests that the well-known bacterial models for regulating gene activity may also operate in man.

The enzyme lactate dehydrogenase (LDH) has a quaternary structure analogous to that of hemoglobin. In fact, much of the work on the structure of this enzyme was stimulated by the prior analysis of hemoglobin. Some years ago Clement L. Markert and his associates at Johns Hopkins discovered that lactic dehydrogenase is a tetramer which may be dissociated into two different varieties of monomers. They originated the hypothesis that the five different forms, or isozymes, of this enzyme found in mammalian tissues were due to the association of the two kinds of monomers in all possible combinations of four. Thus, the formulas for the five isozymes could be written LDH-5 = A^4B^0, LDH-4 = A^3B^1, LDH-3 = A^2B^2, LDH-2 = A^1B^3, LDH-1 = A^0B^4. This attractive hypothesis has received general support by investigators working with lactic dehydrogenase, but critical proof was lacking. In this symposium Markert presented for the first time what appears to be a decisive verification of his hypothesis. LDH-1 and LDH-5, each of which according to the hypothesis should contain only one kind of subunit, were isolated as pure preparations from the usual mixture of isozymes. These two isozymes were then mixed, dissociated without denaturation, and allowed to reassociate. Random recombination of the two subunits should generate a mixture of all five isozymes in the proportions of 1:4:6:4:1. This definitive result was obtained.

Amino acid analyses of the different isozymes as presented by Markert also supported the hypothesis since LDH-3 showed a composition that was the average of LDH-1 and LDH-5. These latter two isozymes have very different amino acid compositions and thus their subunits must be under the control of different genes. The characteristic pattern of LDH isozymes found in each tissue at each stage of development can then be explained by different relative activities of the two genes for the A and B subunits of the enzyme.

Having obtained undenatured subunits of lactic dehydrogenase, Markert combined these subunits from different species to produce a wide spectrum of hybrid isozymes. These hybrid molecules, between mouse and cow or horse and rabbit, are not likely to be found in nature, but may have interesting potentialities for examining the monomeric characteristics essential to the formation of functional tetramers.

Nathan O. Kaplan (Brandeis University) emphasized the functional significance of the different isozymes, particularly LDH-1 which is predominant in heart and certain other muscle tissues, and LDH-5 which is predominant in most skeletal muscles. Kaplan commonly refers to these isozymes as the H and M types, respectively. These two isozymes are known to be differentially inhibited by high substrate concentrations; LDH-5 functions better at the higher concentrations. Moreover, LDH-5 is prevalent in tissues that function under conditions of relative anaerobiosis and that accumulate lactic acid. Kaplan presented an extensive analysis of the LDH composition of many different muscles from birds and other animals and demonstrated excellent agreement between the metabolism of the tissue, whether oxidative or glycolytic, and the isozyme composition. These analyses have been greatly facilitated by Kaplan's ingenious use of analogs of nicotinamide adenine dinucleotide as cofactors in enzyme tests. These analogs discriminate among the isozymes and allow a calculation of isozyme composition in a mixture from the overall relative rates of reaction with the different analogs.

Significantly, the isozyme composition of several tissues changes during embryonic development and may also be shifted by disease or by various treatments such as with hormones. These induced changes in LDH composition are in accord with the apparent functional significance of the different isozymes and also demonstrate

that differential synthesis of related polypeptides can be induced in specific tissues by specific agents. Whether these agents act directly at the gene level or at some later point in the sequence from gene to protein is not yet known.

The coordinate control of groups of enzymes has been extensively studied in microorganisms but little work of this nature has been done with vertebrates. Kaplan presented a novel and intriguing analysis of the distribution of α-glycerophosphate dehydrogenase (GPDH) with reference to lactic dehydrogenase. A close correlation exists between the level of GPDH and the relative abundance of LDH-5, generally prevalent in most skeletal muscles. In exceptional conditions as in the breast muscle of birds accustomed to sustained flying, however, both the LDH-5 and GPDH decrease greatly and LDH-1 becomes predominant. Thus some sort of coordinate control is suggested.

Gordon Tomkins (National Institutes of Health) discussed a different type of biological regulation brought about by molecular conformational changes. He has analyzed the properties of the enzyme glutamate dehydrogenase (GDH) at various states of polymerization and has found, remarkably, that the higher polymers have GDH activity, but as the state of aggregation decreases the GDH activity declines and a corresponding increase in alanine dehydrogenase activity occurs. Moreover, the state of aggregation is subject to regulation by steroid hormones, and this fact opens the prospect that some hormones may act through controlling polymerization of polypeptides and by this means regulate metabolic activity. Such mechanisms permit a very rapid, reversible shift in function without involving de novo protein synthesis. Tomkins finds that in addition to steroids, guanosine triphosphate and the reduced form of nicotinamide adenine dinucleotide, promote disaggregation, whereas adenosine diphosphate promotes aggregation. The availability of these small molecules is closely tied to cell metabolism and their action on protein polymerization bridges the gap between the metabolism of small molecules and the regulation of the activity of specific macromolecules. The specificity of the different polymers was further emphasized by his report that each level of polymerization of glutamate dehydrogenase is characterized by different antigenic properties.

It is interesting to note that each of

the proteins discussed in this symposium is a polymer and that variation in its subunit structure leads to altered function. Mechanisms controlling the synthesis of specific polypeptides or governing their association seem immediately related to the functional differentiation of cells and in fact may be fundamentally responsible for differentiation. It is a moot question as to whether all the characteristics of any organism are ultimately attributable to intrinsic molecular structure or whether pre-existing organization (as in the egg or embryonic cell) confers specific order on molecular populations independently of the molecular properties themselves. Some support for both points of view could be found in this symposium.

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Resonant Particles in High-Energy Physics

Recently discovered resonant particles were the main theme of a high-energy physics meeting held at Ohio University, Athens, 26–28 April 1963. The objective of the meeting, attended by about 90 physicists from the United States and Europe, was to achieve a better understanding of the status of the many resonance states and new particles that have been discovered or have shown some evidence of existence in recent experiments. More than 30 elementary particles or resonance states are now known to exist. Until recently there had not been a coherent picture that could serve as a framework to tie all these particles and states together.

Among the papers of general interest was a comprehensive survey of the isobaric states of mesons and baryons presented by R. Dalitz (University of Chicago). He reviewed the classification of these states into multiplet schemes based on the unitary symmetry model originally suggested by S. Sakata (Osaka University, Japan). This is essentially a symmetry scheme which is a generalization of the isotopic spin. Practically all the existing states fit into this representation very well, and form families of resonances whose structure can be conveniently studied.

Evidence for the existence of a particle which decays into two charged pions (π^+ and π^-) was presented by D. K. Robinson and E. O. Salant (Brookhaven National Laboratory) and W. D. Walker (University of Wisconsin). The mass of the particle was determined to be 782 Mev, which coincides with that of the known ω particle. Since the ω particle usually decays into three pions ($\pi^+\pi^0\pi^-$), this particle is interpreted as the two-pion decay mode of the ω. The branching ratio of the two-pion decay mode versus the three-pion decay mode:

$$\frac{\omega \rightarrow \pi^+ + \pi^-}{\omega \rightarrow \pi^+ + \pi^- + \pi^0} \sim 5 \text{ percent.}$$

G. Goldhaber and S. Goldhaber (University of California) presented evidence for the production of double resonances of the following reaction:

$$K^+ + p \rightarrow K^* + N^*$$

where K* and N* are the K meson resonance state (895 Mev) and the nucleon resonance state (1238 Mev), respectively. This subsequently decays into a four-particle final state:

$$\begin{aligned} K^- + p &\rightarrow K^+ + \pi^- + p + \pi^+ \\ &\rightarrow K^0 + \pi^0 + p + \pi^+ \\ &\rightarrow K^0 + \pi^+ + n + \pi^+ \end{aligned}$$

A one-pion exchange model with a form factor gives a good fit in the experimental angular distribution.

A. Thorndike (Brookhaven) reported the observation, for the first time, of antihyperons in a 20-inch hydrogen bubble chamber in the 3.69-Bev/cm anti-proton beam of the 33-Bev Brookhaven alternating gradient synchrotron.

More detailed information on the properties of $\varphi(K_1K_2)$, the most recent member of the family of newly discovered resonant particles, was presented by J. Leitner (Syracuse University) and N. P. Samios (Brookhaven). They reported the following information: mass = 1019 ± 1 Mev; parity, $P = -1$ $(= C)$; spin $J = 1$; width $\Gamma_T > 0$; isospin $I = 0$ or $G = -1$.

A report on high-energy elastic scattering and Regge pole predictions was presented by L. C. L. Yuan (Brookhaven). Slightly over a year ago a theory based on Regge pole hypothesis predicted that the diffraction pattern in high-energy elastic scattering shrinks with increasing energy. This means that the radius of interaction of a nucleon becomes larger at higher energies. It has also been proposed that all the strong interacting particles are associated with Regge poles and that these poles control the asymptotic behavior of scattering amplitudes. These predictions have aroused great excitement