Meetings

Cancer Research

Developmental and metabolic control mechanisms and neoplasia was the subject of the 19th annual symposium on fundamental cancer research, held at Houston, Texas, 4–6 March 1965.

R. M. S. Smellie (University of Glasgow) opened the meeting with a review of the enzymology of nucleic acid synthesis. DNA nucleotidyltransferases were discussed for similarities and differences with respect to the source of the enzyme. It was concluded that one of the most pressing questions concerns how the enzyme copies native DNA biosynthetically in the presence of histone. Even for simplified in vitro systems the nature of the products of these enzymes requires much more characterization for an understanding of the details of DNA synthesis. The most challenging problem cited with respect to RNA synthesis was the mechanism whereby the RNA nucleotide sequence is determined by the DNA (or RNA) sequence of the informational nucleic acid.

Richard Schweet (University of Kentucky) reported on the mechanism of peptide bond synthesis. (The paper was coauthored by R. Arlinghaus and J. Shaeffer.) Earlier studies furnished a dynamic model for protein biosynthesis which involves the addition of a ribosome to a polysome-mRNA unit. The newly added ribosome would be at the N-terminal position with respect to the protein to be synthesized. Degradation-inhibition studies with exonucleases indicated that this point of attachment is to the 3′-hydroxyl end of the mRNA. With each addition to the polypeptide chain the ribosome proceeds along the mRNA. Ultimately, the 5′-hydroxyl end of the mRNA is required for release of the ribosome associated with a particular polypeptide chain.

To study the steps in the synthesis of the peptide bond, Schweet and his co-workers used a synthetic mRNA (poly-U) and an in vitro system. Two steps are identified: (i) Binding of two phenylalanyl-sRNA's (in this case) to the ribosome-mRNA. This step requires GTP. (ii) A peptide synthetase step requiring only the enzyme. By fractionation of this enzyme from the system and the use of double-labeled tracers the stepwise incorporation of phenylalanine to form di- and tripeptides could be demonstrated.

Polysome organization as a control element was discussed by Hans Noll (Northwestern University). Polysome organization was shown to be the product of a complex dynamic state maintained by the interaction of several metabolic circuits. The size of the polyribosome is determined by the length of the mRNA involved and the spacing of monosomes along it. The spacing, in turn, is a function of readout rate and the ratio of monosomes to mRNA. The level of sRNA, nucleotide triphosphates, amino acids, charging enzymes, and transfer enzymes all affect the readout rate. It was concluded that attachment and readout are independently variable. The results also indicate that attachment of ribosomes to mRNA is not controlled simply by diffusion but very likely includes an enzymatic attachment factor.

Noboru Sueoka (Princeton University) spoke on heterogeneity of sRNA as a factor in biosynthetic control. The role of sRNA as a control element was considered from the point of view of alteration of acceptor activity, modification of codon recognition, or modification of the specificity of amino acid–acceptor activity. The involvement of sRNA in the *Escherichia coli* B-T2 phage system was studied. Following infection only the leucyl-sRNA showed appreciable difference in its behavior on a methylated albumin-kieselguhr column. Time studies indicate some of the host cell leucyl-sRNA is transformed to the new form. Much remains to be done to correlate these observations with the biosynthetic control mechanisms of the cell and relation to phage infection.

In a paper on mutation and protein structure in tobacco mosaic virus, H. G. Wittmann (Max-Planck Institut, Tübingen) discussed the known amino acid replacements in tobacco mosaic virus coat protein that have been produced by mutagens acting on tobacco mosaic virus. About 170 mutants produced by treatment with nitrous acid, fluoroacil, or hydroxylamine have been isolated and studied. In addition to amino acid replacements, the alterations in serologic and electrophoretic properties of the proteins with localized amino acid replacements were studied. In certain cases the ability of the viral subunit to aggregate was investigated. It was shown that replacement of only one amino acid was sufficient to prevent aggregation (under certain conditions), thus leading to a conditional lethality. The changes in protein in about one-third of the mutants studied were sufficiently extensive so that the serology was appreciably different.

The nature and action of repressors in the biosynthesis of proteins have been studied by John R. Sadler (University of Colorado Medical School, Denver) and Aaron Novick (University of Oregon). Using the Jacob and Monod model of repressor function as a starting point, Sadler and Novick showed that many aspects of the model fit very well the experimental observations for a lac operon and lac repressor in *E. coli*. To gain insight into the nature of these elements, mutants sensitive to temperature were studied. In one instance the lac repressor was heat labile, while in two other mutants the synthesis of the lac repressor was heat sensitive. By a combination of kinetic and genetic studies with these mutants it was shown that the product of the *i*-gene that regulates lactose metabolism either is the repressor or contains the repressor. The repressor is "growth unstable" with a mean life of one-fifth to one-tenth of a bacterial generation time. Inducers cause structural changes in the repressor in that the heat stability is altered.

Robert B. Hurlbert (University of Texas M. D. Anderson Hospital and Tumor Institute) spoke on the role of subcellular organization in the control of RNA synthesis. His work particularly concerned the action of the RNA synthesizing system in isolated nuclei

and nucleoli. Functional nuclei isolated from the Novikoff ascites rat tumor and rat liver incorporated P^{32}-labeled nucleotides into nuclear RNA. The composition of this newly formed, labeled RNA resembled that produced in living cells and had a moderately high content of guanine and cytosine. Inhibition by actinomycin D indicated that the labeled RNA was formed on a DNA template, which in these nuclei has a high content of adenine and thymine.

Autoradiographic localization of tritium-labeled RNA precursors showed about two-thirds of the newly synthesized RNA to be associated with the nucleoli. Further proof that the RNA polymerase functioning in this system is associated with nucleoli was provided by the preparation of isolated nucleoli capable of the synthesis of RNA under conditions comparable to those for nuclei. The results indicate that the isolated nucleoli are involved in the synthesis of ribosomal RNA and that histones may form part of the structural organization of the nucleoli necessary for the selective control of the "readout" of DNA by means of RNA polymerase.

Cellular organization and poliovirus infection was the subject of a paper by Sheldon Penman (Albert Einstein College of Medicine). Previous work with HeLa cells infected with poliovirus had shown formation of polyribosomes specific to poliovirus. Such polyribosomes are much larger than the polyribosomes of the host cell soon after infection (3½ hours). Another early effect after infection is the formation of virus synthesizing bodies. On the basis of pulse-labeling studies correlated with density gradient isolation, apparently all the functions leading to the production of progeny virus are associated with the virus-synthesizing bodies. These bodies contain the elements engaged in viral RNA and protein synthesis and virus particles in various states of assembly.

It was shown that the synthesis of new viral RNA was necessary to obtain a stimulation of choline incorporation. Thus, this latter phase correlates with the production of the virus-synthesizing bodies that contain lipoprotein.

Enzymes as molecular markers of cellular differentiation were discussed by William J. Rutter (University of Illinois). Two fructose diphosphate aldolases (A and B) were discussed. Each has a similar amino acid composition, the same number of active sites, and the same number of peptide chains; however, they are immunologically distinct and have different peptide maps. Thus they are concluded to be homologous, but coded by different genome regions. Catalytically, aldolase A functions best in glycolysis and aldolase B favors gluconeogenesis. In early embryos the A form predominates. During differentiation there is a changeover to a predominance of B (for liver and kidney). Rate of labeling in the two forms was studied in embryonic liver by using an immunochemical isolation system. The rate of synthesis of the A form drops following onset of the synthesis of the B form. The findings indicate the fundamental change in this differentiation is "locked" into the cells and not mediated by external inducers.

A quite different example was cited for differentiating pancreas which requires an "external" mesenchyme for normal development, including transition to cells producing the normal pancreatic enzymes.

D. D. Brown (Carnegie Institution of Washington) reported experiments showing that newly synthesized RNA is first detectable (P^{32} incorporation) at the late cleavage stage of the developing embryo of *Xenopus laevis*. This "dRNA" is similar to DNA in its base composition and is associated with the ribosomes. New 4*S* RNA is not detected until a slightly later stage just prior to gastrulation. At the dorsal lip stage of gastrulation, synthesis of 28*S* RNA was detected.

The foregoing observations are consistent with a pattern of synthesis of sRNA and mRNA (which are probably equated with the operationally defined 4*S* RNA and dRNA) to be used in protein synthesis supported by ribosomes present at fertilization. No new ribosomes are required until hatching because there is a very large content of maternal ribosomes in the original egg. A mutant strain of *X. laevis* whose cells contain no nucleoli cannot synthesize rRNA but will develop to the early swimming stage, consistent with this pattern of RNA synthesis during early development. Also correlated with this pattern of RNA synthesis is the absence of nucleoli during early development.

Hans Laufer (Johns Hopkins University) discussed relationships between chromosomal puffing and cellular function during insect development. The insect *Chironomus thummi* was studied during the larval-pupal transformation. Several enzymes were studied in the differentiating salivary glands. Although some of the enzyme changes correlated with puffing in the Balbiani rings of the chromosomes in the salivary gland, tracer studies and immunochemical isolations led Laufer to conclude that tissue specificity of the enzymes was not great enough to account for the specificity of the puffing. To circumvent this dilemma, Laufer proposes that the activity of the Balbiani ring correlates with the production of a transport system in the salivary gland, which in turn moves enzymes from other parts of the insect circulation and into the secreted product of the gland.

The detection of chemical factors in morphogenic induction was the subject of Stanley Cohen (Vanderbilt University). Methods that have been utilized were illustrated by specific examples—cartilage induction from explanted somites (Holtzer and co-workers), inducing action of nerve growth factor (Levi-Montalcini and Cohen), and the epidermal growth stimulating factor.

Lubomir S. Hnilica (Univeristy of Texas M. D. Anderson Hospital and Tumor Institute) reported on the role of nuclear proteins in hereditary mechanisms of higher organisms. The possibility that histones may act as gene suppressors or regulators induced many researchers to investigate such mechanisms. From several possibilities the most preferred is the assumption that the regulatory function of histones resides in the tissue and species specificity of their primary structure. However, in Hnilica's studies, analyses of histones rich in arginine and lysine from different tissues failed to reveal any specificity (differences) in their chemical composition.

It was speculated that the genetic expression and the differentiation may be controlled by variations in the stability of different histone fractions rather than by the genetically vulnerable mechanism exerted by changes in the primary structure of histones in different tissues. To investigate this possibility, the incorporation of lysine C^{14} into the four main histone fractions was studied in specimens of normal and regenerating rat liver and in Novikoff hepatoma. Different histone fractions were found to incorporate the lysine C^{14} to a different extent. These results led Hnilica to propose that histones play a passive role in genetic regulation, with anticipated

differences in turnover rates rather than differences in primary structure of histone fractions from various tissues or species.

Barbara E. Wright (Huntington Memorial Hospital of Harvard University) reported on recent studies on control of carbohydrate synthesis in the slime mold (*Dictyostelium discoideum*). In this system differentiation is induced by a change in environment, the removal of exogenous nutrients. In the terminal stage of the differentiation, preformed cellular materials, such as protein, are converted to two complex carbohydrates in the cell wall. It is possible to synthesize labeled carbohydrates with uridine diphosphoglucose-C^{14} in an in vitro system. The enzymes required for this synthesis are very labile in early stages of the differentiation but as differentiation proceeds the enzymes are more stable (or conversely less degradative conditions are encountered). For this reason, changes in enzyme levels cannot yet be measured. It has been established, however, that adequate enzyme levels are present long before cell wall polysaccharide accumulates. More closely correlated with the onset of cell wall polysaccharide synthesis is the accumulation of the precursor compounds, glucose, glucose-6-phosphate, and uridine diphosphoglucose. The level of the latter compound and the kinetics of the cell wall synthesis indicate it is one limiting factor in the cell wall biosynthesis. The work of Wright thus focuses attention on intermediary metabolites as significant factors in differentiation quite separate from any role in a feedback system. In order to prevent regarding this as an oversimplification, she called attention to the fact that differentiation was ultimately a composite of a multiplicity of limiting factors.

James B. Walker (Rice University) discussed metabolite-repressor: receptor interaction during embryonic development. Of the several programmed metabolic events in this system (the chick embryo), few have proven to be subject to external perturbation with physiological compounds. One system is remarkably susceptible, however, and this is the enzyme system for the synthesis of creatine. Walker has shown that the metabolite repressor : receptor interaction of creatine introduced into the developing chick embryo follows saturation kinetics consistent with a reversible interaction with a macromolecule.

J. R. Tata (National Institute for

Medical Research, London) spoke on growth and developmental hormones as tools for the study of biosynthetic control mechanisms. He reviewed the effects of hormones on nucleic acid synthesis. It is now apparent that several hormones promote RNA synthesis in target organs as one of the very early observable effects. Tata presented data for the thyroid hormone induction of metamorphosis in the American bullfrog. The lag period for the precocious production of proteins which appear following thyroid hormone was shown to be one of active RNA synthesis. Density gradient studies during this period (40 to 60 hours after administering) showed newly formed polysomes, presumably bearing mRNA for the new protein synthesis that was to follow. It was stressed that an increase in mRNA is not the only means for increasing new protein synthesis. Stimulation of other RNA synthesis (ribosomal and sRNA) would effectively increase the synthetic machinery for the synthesis and, in fact, may be as important to hormone action as mRNA synthesis. Labeling patterns in the sequence of RNA polymerase increase during the combined action of growth and thyroid hormones on liver in hypophysectomized rats indicate that the two hormones act at different initial sites. In conclusion, Tata stressed that the many levels at which hormones act make the hormones versatile tools in exploring regulatory mechanisms in higher organisms during development.

Ulrich Clever (Purdue University) reported on the control of gene activity as a factor of cell differentiation in insect development. Certain insects are particularly suited to the study of gene activity because the "puffing" phenomena may be correlated with an active gene. The use of the hormone ecdysone to induce differentiation provides still another dimension to such studies. Clever described changes induced in chromosome activity in *Chironemus tentrans* and those genes which were directly affected by ecdysone. The primary target of the hormone appeared to be the same in different stages of development and in different tissues even though the final cell reactions were not the same. Also, certain genes must be active in order for other genes to respond to ecdysone (temporal and sequential action). These effects may be shown in two ways. First, the age of the insect (larva-pupa) is important to the action of the

hormone. Second, the application of actinomycin D may alter the course of the response to ecdysone. Some "puffs" may be activated in the absence of protein synthesis (puromycin resistant), while others require protein synthesis for activation.

Jean D. Wilson (University of Texas Southwestern Medical School, Dallas, Texas) and Peter M. Loeb reported on their studies on control by estrogen and androgen of cell biosynthesis in target organs, delineating still further the site of action of the sex hormones in target organs. Wilson and Loeb have studied labeled-testosterone localization in the preen gland of the duck. They concluded that testosterone label localized in an area of the cell actively synthesizing RNA in this tissue.

Using the crested newt as test material, they showed by autoradiography that tritiated estradiol localizes in the lampbrush chromosomes in ovary nuclei, presumably the site of active gene transcription. These studies suggest the mechanism of action of estrogen and androgen involves the regulation of specific gene activity. In the case of testosterone, this regulation appears to involve some types of reaction with the histone or protein associated with the DNA.

Regulation of enzyme action by metabolites was discussed by Carl Frieden (Washington University, St. Louis, Missouri). The enzymes considered were those whose activity is influenced by metabolites or end products which are not substrates for the enzyme. Several such examples are now known. This form of enzyme control is involved in three types of regulation: (i) those enzymes whose control will influence the particular metabolic pathway relative to other possible metabolic pathways; (ii) those enzyme-metabolite interactions which will affect enzyme subunit interaction to affect enzyme activity and thus metabolic rate; and (iii) those enzyme-metabolite interactions which affect primarily enzyme kinetics. All three types of regulation may be explained as allosteric effects.

Some forms of human disease can be considered in terms of regulatory mechanisms that involve control genes and structural genes. Genetic regulatory mechanisms as exemplified by human disease was discussed by Alexander G. Bearn (Rockefeller Institute). Bearn cited genetic studies on the transferrins, gamma globulins, bisalbuminemia, and others as specific ex-

amples of structural gene mutations that have been studied for human plasma proteins. In the erythrocyte proteins a very impressive series has been accumulated, including the several known hemoglobin variants, Thalassemia (several suspected structural mutations, at least), and blood group substances. Several examples were also furnished for human disease that have been analyzed as control gene mutations (for example, high fetal hemoglobin, hemophilia A and B, and an α_1 antitrypsin disease which produces an unusual emphysema).

Altered template stability in rat hepatomas has been shown to be an important aspect of tumors by Henry C. Pitot and co-workers (McArdle Laboratory, University of Wisconsin, Madison). Studies reported by Pitot have shown enzyme template stability in several minimal deviation hepatomas to vary considerably. In some of these highly differentiated tumors half-life of the template, as studied by the duration of the actinomycin D resitant period, may be very near or even greater than the normal liver stability, but in others the corresponding value is much decreased.

Since it has been known for some time that hepatomas fail to respond to normal enzyme inductions (for example, synthesis induced by substrates or hormones), it is possible this defective control of enzyme synthesis in hepatomas is related to the altered template stability.

Marvin D. Siperstein (University of Texas Southwestern Medical School, Dallas, Texas) has made a comparison of feed-back mechanisms of cholesterol metabolism in liver and hepatoma. The normal liver is subject to control of cholesterol biosynthesis at the point of conversion of β-hydroxy-β-methylglutarate to mevalomate by an end-product inhibition. The control appears to be related to a site in the membrane portion of the liver microsome fraction. This control is absent in hepatoma for which Siperstein postulates a steric alteration of the site.

In a paper on regulatory steps in the replication of mammalian cell nuclei Gerald C. Mueller (University of Wisconsin) presented evidence for sequential steps in replication. Cultures of HeLa or human lymphocytes, synchronized by temporary amethopterin block, were studied. Some distinction in the replication cycle could be shown by inhibition of replication if the analog 5-bromodeoxyuridine were incor-

porated into early replicated DNA but not in late replicated DNA. This late replicated DNA is essential for cell division, because blockage by phleomycin is selective for this portion of DNA replication. Inhibition by phleomycin, puromycin, or parafluorophenylalanine showed that both RNA synthesis and protein synthesis were required for triggering replication.

A. Clark Griffin (University of Texas M. D. Anderson Hospital) made a comparison of protein-synthesizing systems from normal and tumor tissues. Studies were carried out on in vitro amino acid incorporating systems isolated from Novikoff ascites tumor cells, rat liver, and *Escherichia coli*. The ascites tumor and liver components were completely interchangeable in terms of amino acid activation or incorporation. Synthetases from tumor or liver would form the aminoacyl-sRNA (for sixteen amino acids that were tested) in the presence of sRNA isolated from liver or tumor. The tumor synthetase fraction catalyzed the formation of arginyl-sRNA in the presence of yeast sRNA while liver synthetase fraction failed to catalyze this reaction.

Specificity of the transfer enzymes was also studied. Carbon-14–labeled aminoacyl sRNA's were added to the ribosomes along with energy components. In each system the corresponding transfer enzyme fraction was essential for amino acid incorporation, as measured by insolubility in hot trichloroacetic acid. Tumor and liver systems were interchangeable while *E. coli* ribosomes would not respond to the mammalian transfer enzymes.

It is important that a means of studying gene combinations in cells of higher organisms be available. Hybridization of cells presents the most direct approach currently available for such studies. In discussing hybridization of somatic cells and phenotypic expression, Boris Ephrussi (Western Reserve, Cleveland, Ohio) reported on his work in this field.

From studies of the phenotypic expression of hybridized cells, examples of production of two forms of β-glucuronidase were cited, each form deriving originally from the parent strain. In other cases enzyme forms have been suppressed in hybrids by a regulatory interaction, because under other conditions of culture the enzyme (esterase) could be made to reappear. These findings are interpreted as consistent with the modern form of the deletion theory

of carcinogenesis which states that carcinogenesis involves deletion or alteration of a regulatory mechanism, not a structural gene.

Since 1950 a traditional highlight in this symposium is the presentation of the Bertner Award for Outstanding Achievement in the Field of Cancer Research. This year's recipient was Erwin Chargaff (Columbia University College of Physicians and Surgeons, New York). In the award presentation by R. Lee Clark (University of Texas M. D. Anderson Hospital and Tumor Institute) Chargaff was cited for his numerous contributions to nucleic acid chemistry, and particularly for the careful analytical studies which established the adenine-thymine and guanine-cytosine regulatorities in the base composition of DNA. After accepting the award, Chargaff spoke on the biological consequences of base-pairing in nucleic acids. The lecture began with some thoughtful comments on contemporary science, then briefly reviewed some of the contributions of Chargaff and his co-workers concerning base-pairing.

A final subject of the lecture concerned recently gathered evidence for the symparallel or antiparallel polarity of the strands in native DNA. Josse, Kaiser, and Kornberg have presented nearest-neighbor frequency studies of in vitro, enzymatically synthesized, DNA which indicate an antiparallel alignment. Chargaff's studies approach the problem using native DNA as the material investigated and a detailed analysis of the resulting isostiches containing one or two bases. The isolated isostiches can be analyzed for their sequence (for example, pApGp versus pGpAp) and the resulting distributions of isostich-two content compared with that expected for a syn- or antimodel of DNA strands. The data are in accord with the antiparallel model, thus affording experimental support for assumptions that have long been held for native DNA.

The symposium was supported by grants from the National Cancer Institute, U.S. Public Health Service, and the American Cancer Society, Texas Division. The full text of the papers will be published as a monograph entitled "Developmental and Metabolic Control Mechanisms and Neoplasia."

DARRELL N. WARD
Department of Biochemistry, University of Texas M. D. Anderson Hospital and Tumor Institute, Houston