Meetings

Macromolecules

While the method of manufacture of the protein and nucleic acid components of cells was the general subject of discussion at the 28th Cold Spring Harbor Symposium on Quantitative Biology 7–13 June, other topics included: the structure and synthesis of DNA, RNA, and proteins; the role of the enzymes, ribosomes, and transfer RNA in the syntheses; regulatory mechanisms within the cell; relation of tertiary protein structure to function; and the coding relations between RNA and protein.

Evidence continues to grow that the central doctrine of macromolecular biologists is essentially correct: that in the living cell the sequence of nucleotides in the relatively stable DNA is transcribed into a corresponding sequence in the transient messenger RNA and this in turn is translated into the sequence of amino acid residues in proteins. The transcription is under genetic control, as are certain aspects of the translation. The able servants of the process are, at least at the macromolecular level, the enzymes, the ribosomes, and the transfer RNA. Although most of the details of the mechanisms have come from studies on microorganisms there are indications that the process may be universal.

After some stimulating remarks by F. Crick on the role of theory and models in molecular biology, the first consideration was given to DNA synthesis. C. Richardson presented increased evidence that in the in vitro replication of DNA (with the aid of DNA polymerases) the sequential arrangement of the nucleotides of the DNA primer determines the sequence of the replicated polymer; the enzymes have no specificity for sequence determination. The newly synthesized polymers are readily separated from the primer; they are better "annealed" (after heating and fast cooling) than primer DNA. Richardson also showed that branching may occur during replication; this indicates that it may be initiated on both strands of the primer which in turn goes on to unwind as the process continues.

Sequential replication of DNA in vivo was demonstrated by the radioautographic results of J. Cairns. The chromosome of *E. coli* is unequivocally in one piece and is most likely a single loop. Replication appears to start at one point, goes continuously around the loop, and ends with the formation of two joined loops. Genetic evidence of sequential replication of DNA came from N. Sueoka who compared the frequency of genetic markers in rapidly growing cells of *B. subtilis* with the frequency in stationary cells. The ratio of the frequency of markers that replicate first to those that replicate last is greater in the rapidly growing cells than in the stationary ones; this would be expected for sequential replication around much of the chromosome. J. Hurwitz discussed the copying in the DNA-dependent reaction of RNA polymerase, and indicated that, at least in local regions, the transcription may be quite precise. The primer was DNA of *D. pneumoniae* and carried the genetic marker of sulfonamide resistance. The replicated RNA temporarily transformed sulfonamide-sensitive cells to resistant ones, an indication of faithful copying. M. Chamberlin, in summarizing extensive work on the action of RNA polymerase in a system with single-stranded ϕX174 DNA as primer, noted that hybrids of DNA and RNA form first and then later free RNA accumulates. The hybrids are irreversibly separated upon heating; they can serve as primer and the newly formed RNA displaces most of the RNA strand of the hybrid, as if a "semiconservative" replication mechanism were important.

Everyone still wants to know the nucleotide sequences of transfer RNA's. Primitive steps in analysis indicate that there are differing nucleotide sequences in different amino-acid-specific transfer RNA's. Methylated and other "odd" bases are quite common; methylation is performed by species-specific and base-specific methylating enzymes after the transfer RNA is polymerized. The natural role of the methylating enzymes is still unknown, although E. Borek speculated upon their potential oncogenicity.

Messenger RNA was considered in reference to the manner of its transcription from DNA, its role as a primer in the RNA-polymerase reaction, and its instability in the living cell. As a result of hybridization experiments in which messenger RNA formed a complex with only one strand of heat-denatured DNA, it was concluded that, in vivo, only one strand of the DNA is apparently copied in forming messenger RNA. (It is assumed that hybridization is evidence of faithful copying and vice versa.) Identification and partial purification of an RNA-primed RNA polymerase from cells infected with RNA viruses have been achieved. Thus, as with DNA, RNA can serve as its own primer. There is no evidence that this type of copying exists within the uninfected cell. Messenger RNA is far from immortal; its half-life has been assessed at 1 to 3 minutes, a time sufficient to read off about 15 polypeptide chains.

In connection with protein synthesis and with amino acid incorporation, messenger-RNA synthesized in the DNA-dependent, RNA-polymerase reaction was found (W. Wood) to vary in its incorporative activity depending upon what sort of DNA it had been copied from. If copied from single-stranded DNA, either natural or host-denatured, it was inactive; if copied from double-stranded DNA that had been obtained naturally or formed by replication or renaturation, it was active. The physical properties of the active and inactive RNA differed; in fact, the inactive material is distinctly shorter than the active one and is less readily bound to ribosomes. Polyribosomes stood out as the most active form in which ribosomes act as the banker of protein synthesis (J. Darnell, W. Gilbert, R. Schweet, A. Rich). It is increasingly apparent that they are connected in vivo by messenger RNA strands, and that during in vitro amino acid incorporation the strands break, thus allowing the "normal," individual ribosomes to complete the job as best they can.

A model of biosynthetic regulation was presented by F. Gros in which uncharged RNA serves as a "physiological switch" to regulate the transcription of

DNA to bulk cellular RNA through inhibition of the RNA polymerase. This model accounts for the observation that a cell with a scant supply of amino acid makes fewer ribosomes and less protein. There is regulation of the translation of DNA to specific messenger RNA's (G. Attardi) because in the induction of both prophage and β-galactosidase the amount of specific messenger RNA was notably increased.

Based on experiments with bacterial mating and with prophage, F. Jacob proposed a model to account for the fact that each daughter cell gets only its fair share of DNA upon division. His model assumes coordination between the replication of DNA and the synthesis of cell wall, perhaps through an attachment of the two at one phase. When the DNA is fully replicated (in the form of two joined loops) the intruding cell-wall septum would destroy the joining point and thus deliver one complete loop to each daughter. Another aspect of regulation, given by B. Ames, refers to the ten-enzyme system of the histidine pathway. The ten enzymes are in different molar amounts in the cell, and the genetic evidence is that they are transcribed from one long messenger RNA. The suggestion is that the messenger RNA is read from one end to the other and that it contains occasional triplets for scarce transfer RNA's so that synthesis of enzymes from the far end of the messenger RNA occurs less frequently than at the starting end.

Allosteric interactions occur when a small molecule affects the catalytic activity of an enzyme by binding at a site other than its active one and causing a conformational change. Inhibition by end-products, and protection from this inhibition, can be achieved by allosteric effects. The conformational changes may extend even to varying degrees of aggregation and disaggregation of the enzyme molecule. Along similar lines is D. Koshland's model in which the substrate itself can induce conformational changes at the active site of the enzyme to increase its activity.

Complementation, as observed in enzyme systems, was shown by J. Fincham to exist in GDH formed by mutants of *Neurospora*. Incompetent GDH from mutants can be aggregated in mixtures to produce active enzyme, a result made plausible by the observation that wild-type GDH is an aggregate of several sub-units. D. Perrin and D. Zipser reported similar aggregation and activation phenomena with β-galactosidase; Zipser indicated possible in vivo complementation taking place on ribosomes. Complementation of alkaline phosphatase was shown by A. Garen, while M. Schlesinger found that complementation could be accomplished even when the defective enzymes came from different bacterial species. Evidently complementation of enzymes as observed in genetic crosses can be accounted for by a simple aggregation of two or more sub-units that are defective in different respects.

Two review papers on the RNA-protein coding problem were given by M. Nirenberg and J. Speyer. About 50 of the possible 64 RNA triplets are now assigned, a situation that clearly indicates degeneracy in a triplet code. Whether or not the code is ambiguous (one RNA triplet coding for more than one amino acid) was discussed, and some tentative evidence implied that an unambiguous code is inadequate. G. von Ehrenstein found that three separable leucine-accepting transfer RNA's (probably different in their codon triplets because of differing amino-acid incorporating activity), when tested with synthetic polyribonucleotides, incorporated leucine in the same position in synthesized hemoglobin and also in the coat protein of MS-2 phage. Such results indicate that a single triplet in the messenger RNA may be recognized by several transfer RNA's with differing codons. Difficulty has been encountered in fitting results on the analysis of the A protein of tryptophan synthetase of *E. coli* with the messenger RNA code letters derived from in vitro work (C. Yanofsky). In the A protein from mutants and revertants, seven different amino acids are substituted at the same site in the protein. No scheme of permuted, or changed, nucleotide composition in a messenger RNA triplet coding for this site, based upon the current coding scheme, accounts for the amino acid substitutions. An analogous situation exists in the analysis of the protein formed by nitrous-acid mutants of TMV-RNA, where, although there are no genetic data, H. Wittmann has not found it possible to fit a consistent coding scheme to all the 150 mutants whose proteins he has analyzed.

The field represented by the symposium subject is in feverish activity and it is both stimulating and not surprising that moderately conflicting results were reported.

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Hurricanes and Tropical Meteorology

The impact of the meteorological satellite, of high-speed digital computers, and of new and more sophisticated facilities for experimentation and analysis was evident in the recent technical conference on hurricanes and tropical meteorology held at Mexico City from 6 to 12 June. Attended by 125 scientists from the United States and from Central and South America, this was the third conference of this type to review research progress since the establishment of the National Hurricane Research Project by the United States in 1956. The meeting was sponsored jointly by the American Meteorological Society, the American Geophysical Union, and the Mexican Geophysical Union.

The 11 scientific sessions were devoted to discussions of large-scale flow patterns in the tropics, convection and air-sea-earth exchanges, precipitation and weather modification, and the development, structure, and prediction of hurricanes.

In reviewing the role of the tropics in the general circulation, Herbert Riehl presented the results of recent computations which indicate that, in the tropics, the meridional circulation is self-supporting and does not depend upon the energy of perturbations for its maintenance.

The development of more powerful computers has led to a more intense search for an effective means of describing numerically the circulations of the tropics. Roy Endlich described a dynamical method for deriving stream functions from layer mean winds by using contour-height data for first approximations in areas where wind data are sparse. Joe Vederman described statistical procedures now being used to augment wind observations in objective multilevel machine analyses of the Pacific area. A means of extending