Meetings

Evolving Genes and Proteins

A symposium on evolving genes and proteins was held 17 and 18 September 1964 at the Institute of Microbiology of Rutgers University. Sessions on the evolution of pathways, of enzymes and other proteins, and of nucleic acids were chaired by P. H. Abelson, C. B. Anfinsen, W. Braun, P. Doty, R. D. Hotchkiss, E. L. Smith, K. V. Thimann, and C. B. van Niel. T. M. Sonneborn presented an evening lecture on the genetic code in relation to evolution, with D. Stetten presiding.

In the opening address, E. L. Tatum gave a thought-provoking analysis of molecular biology in the context of evolution. As a contrapuntal theme, the evolution of molecular genetics was characterized. Tatum distinguished four periods: primitive (pre-Mendel), classic (Morgan), renaissance (one gene-one enzyme), and modern (age of DNA). Going beyond the current era, he envisaged possibilities of controlled evolution and insight into the evolution of mind.

Anfinsen indicated that, to the biological evolutionist, evolution is organismal. Indeed, there was some hesitation in applying the term evolution to biochemical constructs (pathways) or to macromolecules (genes and proteins). However, pre-Darwinian uses (for example, by Hale in 1677) of the word "evolution" in a biological sense, in reference to traits rather than to organisms as such, are recorded. Much of the discussion during the meeting revolved around views of the relation between macromolecular and organismal evolution.

The atmosphere of the meeting was lively because of an unusually fruitful exchange among biochemists, molecular biologists, evolutionists, geneticists, taxonomists, exobiologists, and microbiologists. By way of a significant clarification, Hotchkiss pointed out that taxonomy is a manner of thinking—a convenience—and not subject to right or wrong. Phylogeny, however, refers to relation through genetic descent (C. A. Williams) and can have right interpretations (Hotchkiss). Nevertheless, there clearly is a strong affinity between phylogeny and taxonomy. In regard to classification, E. Mayr called the gene level an objective level, and J. Buettner-Janusch felt that the best taxonomy for primates is one that is phylogenetic in all possible aspects; both of these participants, though, cautioned against reliance on single traits in classification. The taxonomic utility of a character—chemical or other—cannot be assessed a priori (A. Cronquist).

Evolutionary Variability of DNA

The evolutionary variability of DNA was considered from many points of view. The idea of gene duplication as a process for increasing the amount of genetic material available for evolutionary change was favorably discussed. For instance, E. Zuckerkandl and L. Pauling illustrated the inferred separate evolution of duplicated genes in proposed lines of descent of hemoglobin α and non-α chains of various mammals, including cattle.

Gene duplication was held by N. H. Horowitz to account for the clustering, discovered by M. Demerec and coworkers, of functionally related genes in enterobacteria. Horowitz considered a series of localized gene duplications more likely than scattered duplications followed by juxtaposing rearrangements. Demerec stressed that, no matter what the origin of such clusters, they must offer selective advantages to the organism (for example, *Salmonella typhimurium, Escherichia coli*) that contains them; indications are, however, that higher organisms or even some other bacteria do not use this kind of gene cluster to nearly the same extent as do the enterobacteria studied.

In a paper from the laboratory of the late D. M. Bonner, J. A. DeMoss and S. E. Mills presented a detailed comparison of tryptophan synthetases from various microorganisms with the corresponding *E. coli* system, which had been found by C. Yanofsky and his colleagues to consist of two protein components coded by independent but clustered cistrons. *Neurospora* tryptophan synthetase, on the other hand, has only a single protein component, although a variety of biochemical, genetic, and immunochemical studies show that the *Neurospora* and *E. coli* enzymes are very similar. It was suggested that a change from a system consisting of two interacting polypeptides to a single-polypeptide system, which would correspond to a change from clustered cistrons to a single genetic locus, has played an important role in the evolution of enzymes. A coalescence of clustered genes, which may have arisen by some type of duplication, is thus contemplated.

A mechanism that can be regarded as a model for augmenting cellular DNA supply was announced by A. Kornberg: in the presence of DNA polymerase and certain deoxynucleoside triphosphates, but in the absence of template, polymers are produced after a lag; this probably reflects *de novo* synthesis of template, followed by autocatalytic replication. Certain AT (adenine-thymine) oligomers prime the synthesis of large AT polymers. The replication of the oligomer template was thought to be followed by slippage of the resulting helix by an AT dinucleotide pair; many successive steps of replication and slippage would result in a reiterative polymer synthesis. The formation of such polymers is also promoted by AT-rich DNA's. Occurrences in vivo of reiterative events (which may be associated with distinctive mutations in protein amino acid sequences) are reflected in AT-like components of crab testis DNA.

Duplicated genes which have evolved separately are homologous by virtue of common origin. Such genes are likely to show structural similarities, that is, molecular homology. In fact, at the meeting, the word homology was used both in an evolutionary and in a molecular sense. Demerec studied the fine structure of the *S. typhimurium* and *E. coli* chromosomes. The genetic maps of these two bacteria are very much alike, and there is good reason to conclude that corresponding genes of these organisms are homologous in the evolutionary sense and functionally similar. Yet, in investigations on the incorporation of pieces of *E. coli* chromosome into the *S. typhimurium* chromosome, Demerec found a relatively low degree of homology in the fine structure of these chromosomes; the degree of fine-structure homology apparently is not uniform throughout the genome, and there may be fairly abrupt changes from one region to another. These results are understandable in terms of the degeneracy of the genetic code, which allows a considerable variation in the base composition of nucleic acid without a corresponding variation in the amino acid composition of protein. The methods used by Demerec were essentially genetic.

Another method for exploring the structural homology of nucleic acids is the hybridization technique. "From DNA molecules to human society, much can be learned from studying the conditions under which complementary pairs separate and reform," said Doty. Hybridizations of DNA and RNA in vitro were considered by S. Spiegelman. Ribosomal RNA was shown to hybridize with DNA sequences other than those that correspond to messenger RNA. True hybrids are characterized by resistance to ribonuclease. It is noteworthy that there is a relatively high degree of homology between the ribosomal RNA of *S. typhimurium* and that of *E. coli*. For some bacteria more distantly related than these enterobacteria, no ribonuclease-resistant hybrids were detected, although there was evidence of some interaction.

J. Marmur described his work on the homology between the DNA of three phages and that of their host, *Bacillus subtilis*. The phages were of an induced, a transducing, and a virulent type. The homology between the DNA of the host and that of the induced phage was high; it was less for the transducing phage, and least for the virulent one.

The degree of homology among DNA species from various organisms was examined by E. T. Bolton and B. H. Hoyer, who used single-stranded DNA immobilized in agar to trap labeled DNA fragments by hybridization. All mammalian DNA species tested exhibited some homology; primate DNA's showed a comparatively high degree of homology, which is in general agreement with taxonomy. DNA's from different tissues of the same animal showed no significant variation in these experiments.

E. Freese and A. Yoshida pointed out that the variation in the base composition of DNA is much less pronounced in higher organisms than it is, for example, in bacteria. That even a sizable variation in base composition does not necessarily argue against the universality of the genetic code was stressed by N. Sueoka; such variation is attributed to frequency differences among codewords for each amino acid, in the light of universality and degeneracy, taken as empirical features.

Reflecting on degeneracy, Sonneborn drew attention to the possible occurrence of mutations that would not affect prevailing selective conditions through protein alterations, since a mutation in a given codeword need not lead to a change in the amino acid coded.

In further considering DNA alterations, Freese and Yoshida suggested that base pair transitions may occur preferentially from GC (guanine-cytosine) to AT. There thus may be a trend toward certain amino acids, although such a trend would be blurred by the degeneracy of the code. They also suggested that populations adapt principally through natural selection or genetic drift, but a mutational bias toward useful characters may exist.

A related thought was expressed by Tatum: if there are "hot spots" in DNA, there might be "cold spots." Moreover, some structural regions in genes may not be variable without secondary disturbances. To an extent, therefore, selection may occur on the basis of the structure of DNA itself.

E. K. F. Bautz described model experiments on the contributions of neighboring bases to the mutability as well as functional properties of individual nucleotides of DNA and RNA. He observed that the effect of a non-complementary base upon the interactions between complementary nucleotide sequences is quantitatively different for C, G, and U (uracil).

G. Braunitzer explored proflavine and diaminofluorene as mutagens thought to act through the insertion or deletion of nucleotides. When bacteriophage fd was grown in the presence of either of these mutagens, mutants were isolated containing a protein amino acid not found in the wild type.

Tatum summarized results obtained with two *Neurospora* strains having abnormal characteristics similar to, but more marked than, those in "poky." The abnormal characteristics could be transmitted to other strains by injection of mitochondria from the abnormal strain which has been purified in a sucrose gradient (E. G. Diacumakos, L. Garnjobst, and Tatum). Significantly, D. J. L. Luck and E. Reich have now provided definitive evidence that *Neurospora* mitochrondria contain DNA.

R. Sager reported evidence for recombination of nonchromosomal genes in *Chlamydomonas*. She suggested that organelle DNA may be sequestered from the chromosomes for regulatory purposes.

Variations in RNA

Chromosomal-type regulatory systems of a bacterium were considered in a paper by V. Moses and M. Calvin, who concluded that the messenger RNA for inducible enzymes may be labile, and that for constitutive enzymes stable. Evolutionary changes from inducibility to constitutivity or vice versa were thought to entail corresponding changes in mRNA stability. The lability of the mRNA of inducible enzymes would provide for prompt decay under conditions of noninduction whereas the stability of constitutive-enzyme mRNA would be economical under steady-production conditions.

H. Harris addressed himself to a different aspect of RNA decay. He obtained evidence that much of the RNA made in the nuclei of animal and higher-plant cells is degraded and does not serve as template for protein synthesis. Rather, the part of the genome corresponding to such RNA was regarded as raw material that might evolve so as to produce new template RNA with increased ability to resist degradation.

Evolutionary aspects of soluble RNA were examined by Sueoka. Specificity relations between sRNA's and amino-acyl-sRNA synthetases were studied with preparations from Gram-positive and Gram-negative bacteria and from yeast and rabbit. Among the bacteria, interspecific "cross reactions" are remarkably well conserved despite large differences in the average GC content of the DNA, and hence provide strong evidence for a common evolutionary origin of bacteria. The right kind of cross reactions occur for organisms as far apart as *E. coli* and rabbit. Thus, the present scheme of the code and its translation mechanism probably were established early in evolution.

That the basic machinery for protein synthesis is essentially the same in all present forms of life was concluded in a paper by A. Rich and J. R. Warner, which dealt with protein synthesis on polyribosomes. The ribosome appears to attach at one end of the (mRNA-containing) polyribosomal structure and to be released at the other end after producing a single polypeptide chain. In this process, each ribosome carries only one polypeptide chain but two sRNA molecules. The role of sRNA in facilitating the movement of ribosomes along the mRNA was analyzed. It was suggested that the present type of translation mechanism from polynucleotides to polypeptides is relatively ancient; however, an evolutionary precursor of modern ribosomal RNA may have played a role not seen today.

On Proteinoids and Proteins

Early evolutionary events were pictured by Horowitz, who reviewed the theory of retro-evolution which he had proposed, in the light of results then emerging in the laboratory of G. W. Beadle and Tatum, nearly 20 years ago. According to this theory, the evolution of biosynthetic sequences proceeded backward, one step at a time, from an end product that had been exhausted from the supply of organic matter produced nonbiologically, as contemplated by A. I. Oparin. Horowitz discussed environmental features relating to retro-evolution, and pointed to space exploration as a potential major source of information on factors relevant to primitive-earth conditions.

S. Granick concerned himself with the problem of power supply for organic syntheses during the primitive-earth era. He proposed a photovoltaic mineral model for the conversion of radiant energy to chemical energy. Uroporphyrin may have been the earliest organic photochemical catalyst. S. Fox described experiments leading to the thermal production of proteinoids and to their spontaneous organization into proteinoid microspheres. The proteinoids contained dicarboxylic amino acids in relatively high proportions. In the microspheres, septate division could be induced by elevation of *p*H. Electron micrographs of microsphere sections stained with osmic acid showed bilamellar boundaries.

Zuckerkandl and Pauling suggested that synthetic proteinoids, possibly with weak enzymatic activities, may be particularly relevant to the early stages of evolution. They presented arguments, however, why enzymes of relatively weak activity, in general, need not be expected to evolve into enzymes with relatively strong corresponding activity.

A wealth of material was presented on the evolutionary variability of proteins. Explicitly or implicitly, it was considered that the number of protein species has tended to increase, in line with the evolution of duplicated genes. In their detailed discussion of amino acid sequences in mammalian hemoglobins, Zuckerkandl and Pauling reflected on evolutionary convergence (that is, movement from different ancestral structures to similar descendent structures) at the molecular level. Since "convergence" may have connotations as to selective conditions, they propose use of the less biased term "coincidence." In general, it is thought that coincidence is not likely to complicate the interpretation of homology, as judged by sequence analysis, among proteins.

A paper by Buettner-Janusch and R. L. Hill dealt with hemoglobins from several taxonomically representative primates: α-chains were found to vary slightly, but β- (or β-like) chains vary considerably. Certain segments of the hemoglobin molecule appear to be invariant throughout the primate order. The differences and similarities among primate hemoglobins harmonize with present classifications and notions of phylogeny, with some noteworthy exceptions (which are deemed to encourage further research, not new classifications).

"Sequence gaps" in a number of hemoglobins were studied by Braunitzer. Such gaps are noted when some amino acids of an α-chain have no corresponding partners in the β-chain or vice versa. It was suggested that the gaps correspond to nucleotide deletions or insertions that have affected hemoglobin genes in the course of evolution from a common genic ancestor. Insect hemoglobin chains are appreciably shorter than vertebrate ones. The hemoglobin of a primitive vertebrate, the lamprey, consists of one peptide chain, apparently without sequence gaps.

P. Handler presented a paper by J. G. Joshi, T. Hashimoto, K. Hanabusa, H. W. Dougherty, and himself on a comparison of phosphoglucomutases from a series of diverse organisms. The data obtained indicate a similarity in action mechanism among phosphoglucomutases from rabbit, flounder, yeast, and *E. coli*, but a different mechanism for the enzymes from *Micrococcus lysodeikticus* and *Bacillus cereus*; the enzymes from the first group, but not from the second, can be labeled with substrate-derived phosphate. Identical pentapeptide sequences were found at the active sites of the *E. coli* and rabbit muscle enzymes which, however, differ grossly in other respects.

The primary structure of cytochrome *c* in the context of evolution was discussed in a presentation by E. Margoliash and Smith. They concluded that the cytochrome *c*'s from several mammals, a marsupial, a bird, a reptile, a teleost, an invertebrate, a higher plant, and fungi (but not from bacteria) are derived from a common ancestor. Approximately 50 percent of the amino acid residues of the homologous molecules are invariant, including an 11-amino-acid sequence, which may not have changed in more than one billion years. Further instances of evolutionary constancy among these molecules are seen in the arrangement of hydrophobic and basic segments. At the presumed active site, variations have occurred, the longest invariant sequence containing five residues.

N. O. Kaplan investigated lactic dehydrogenases from a variety of sources. Although considerable variations in these enzymes were discovered among vertebrates, identical active-site peptides were demonstrated in such contrasting organisms as rabbit and yeast. With the aid of immunological, coenzyme analogue, and electrophoretic techniques, Kaplan was able to arrive at conclusions on evolutionary relations among dehydrogenases from fish, reptiles, birds, and mammals. C. A. Price found two lactic dehydrogenases in *Euglena*, one of them mitochondrion-linked, and he mentioned possible evolutionary implications of separate metabolic systems in organelles.

Three dehydrogenases (lactic, malic, and alanine) from the same organism, *B. subtilis*, were examined by Freese and Yoshida. The crystalline enzymes were compared with respect to their amino acid and peptide composition, molecular weight, and immunological specificity. It was concluded that these three dehydrogenases may have evolved from a common enzymic ancestor.

W. J. Rutter studied a number of enzymes catalyzing aldolase-type reactions. Such enzymes may exhibit one of two fundamental active-site regions, and may thus have evolved from two primitive systems.

Among various considerations regarding the evolution of heme enzymes, Granick described one such protein believed to have emerged, along with its controls, when animals progressed from water to land.

Abelson described research on the shell proteins of mollusks, which have left an excellent fossil record. These proteins occur in layers that bond the mineral crystals of the shell. The composition of proteins of closely related species occasionally show some remarkable differences but, in general, prove to be quite similar. Representatives of very old families were found to exhibit relatively small differences. Proteins having the same function can thus remain essentially unchanged for long periods of time; in this sense, evolution is not inevitable (Abelson). The general problem of relating a time scale to rates of change in some proteins was discussed by several participants (Abelson, Margoliash and Smith, and Mayr).

W. D. McElroy, H. H. Seliger, and M. De Luca explored the catalytic properties of firefly luciferases and concluded that subtle differences in protein structure can, without change in the catalytic center, lead to different colors of light and thus have selective significance. They found that conformational changes are important not only for catalysis, but also for the resonance energy levels of the excited states. A wide range of spectral variations was observed when the emission spectra of 20 species of firefly were measured in vivo. The possibility of isozymes was indicated by the presence of two different luciferases in a single organism, the "automobile bug." In flight, this firefly emits a bright yellow light; when resting, the unconventional insect shows green "parking lights."

The role of specific selective pressures in the evolution of specific proteins was stressed by S. E. Luria. *E. coli* and *Shigella dysenteriae* have immunologically very different β-galactosidases, although the corresponding genes are homologous by genetic test. Unlike *E. coli, Sh. dysenteriae* lacks galactoside permease and, consequently, shows impaired galactoside utilization. It therefore seemed significant that the *Sh. dysenteriae* enzyme was rather ineffective. On the other hand, this organism's *i* gene (whose regulatory action tends to prevent formation of such ineffective enzyme) functions as well as the *i* gene of the *E. coli* strain tested.

In the evolution of proteins, even amino acid residues without known functional significance must have high selective value, and there must be strong selection pressures constantly, in view of the remarkable uniformity of the amino acid sequence for a particular protein in individuals of a given species (Mayr). Protein regions of unknown functional significance may, for example, be concerned with the attachment of the protein to appropriate structures (P. Siekevitz).

Evolution of Pathways: Conservation of Function

The evolutionary fate of a biosynthetic pathway can be regarded as reflecting the fate of the participating enzymes. Clearly, through the eons, extensive changes tend to accumulate in the enzymes, the translation and transcription machinery, and the genes themselves. In contrast, the catalytic function of enzymes—and hence the pathways in which they act—often seems to persist with impressive tenacity. What is conserved in this context, therefore, is function, not structure.

In organismal evolution, the appearance of some key compounds and, presumably, of the means for producing them must have been decisive events in diversification. K. Bloch concluded that among such key compounds are certain lipids (which function as components of cytoplasmic and intracellular membranes). Thus, sterols occur in all eucaryotic cells, but not in the procaryotes examined. Polyunsaturated fatty acids are found in eucaryotes and in the photosynthetic apparatus of blue-green algae, but otherwise are absent from procaryotes. Evidence justifying a distinction betwen animal- and plant-type polyunsaturated fatty acids was also presented; organisms such as chrysomonads showed dual traits in this respect. Steps for the introduction of double bonds into fatty acids and into intermediates of sterol synthesis require molecular oxygen as a direct electron acceptor. Bloch suggested that the advent of molecular oxygen in the atmosphere during evolution not only facilitated the development of a vastly superior system for energy metabolism, but also provided a unique reagent for biosynthetic innovations of pivotal evolutionary importance. Accordingly, the occurrence of special lipids and of the facilities for their production are very well correlated with phylogenetic considerations.

A tracer study on the distribution of two different lysine pathways over a range of organisms was described by H. J. Vogel. One of the paths (via α, ε-diaminopimelic acid), which may be the more ancient, was found in bacteria, blue-green and green algae, nonvascular and vascular higher plants, and certain lower fungi distinguishable by type of spore flagellation. Other lower fungi, as well as higher fungi and euglenids, exhibited the second lysine path (via α-aminoadipic acid). The consistency in the distribution of the two paths indicates a high phylogenetic significance for the character, lysine synthesis. A common ancestor of organisms having the α-aminoadipic acid path was postulated. The inferred ancestor was viewed as an organism, not producing lysine, in a line that had veered from the main stream of evolution of the plant kingdom in the direction of animality.

In trying to contemplate, as a whole, the variety of findings reported at this symposium, one gains a palpable impression of the molecular raw material of evolution. A range of evolutionary constancy and variability in the nature and workings of nucleic acids and proteins is now becoming discernible. In other contexts, structure is often associated with sameness, and function with change. At this meeting, we have seen many fascinating examples of macromolecular variation and of metabolic constancy. In the domain of evolution, have Parmenides and Heraclitus reversed their roles?

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Military Problems in Cold Regions

Examples of failure to prepare adequately for military operations in cold regions can be found in history from the time of Hannibal to the Korean conflict. Cold, darkness, wind, snow, ice, muskeg, glaciers, vast unpopulated areas, insects, ice fog, and whiteouts are well-known examples of factors which characterize the harsh and hostile arctic environment. It is depressingly true that many of the major military problems faced by our troops in Korea more than 10 years ago are still with us today. A periodic reminder of this fact, an assessment of the current status of research that is nibbling at the edges of military operational problems, is not only enlightening but serves as a stimulus for a more concerted effort toward their resolution.