Meetings

Neurospora

In the area of genetic recombination, *Neurospora*, together with other Ascomycetes, has provided important data on meiosis. The results stem from the use of ordered meiotic products in the sexual structures, combined with efficient materials and techniques for resolving very short genetic intervals. In the past several years, the phenomena of high frequencies of recombination in short intervals, gene conversion, and "polarized" recombination (giving asymmetrical frequencies of outside marker combinations among selected intragenic recombinants) have been subjected to intense experimental study and theoretical consideration. These phenomena, in fact, may eventually be embraced by a unitary mechanism of recombination, at least that aspect of recombination which may be understood in terms of pairs of DNA duplexes. Various implications of Neurospora research were discussed at the third Neurospora Information Conference, held in Oak Ridge, Tennessee, 12–14 May 1966. Approximately 120 North American investigators and seven foreign scientists attended.

Molecular models of recombination were introduced by Whitehouse (University of Cambridge), who discussed his and Hastings' model in comparison to that of Holliday. The two models are similar in assuming that the chromatids, at meiosis, can be regarded as single DNA molecules (or a number of such molecules joined end-to-end) whose nucleotide chains may break and make interchromatid pairings. Where the association covers the site of a mutation, heterozygous duplexes may result, leading to postmeiotic segregation, or, if there is enzymic correction of the mispairing, to conversion. Polarized recombination patterns are attributed to fixed primary breakage points, which appear to be at the ends of genes, and negative interference to conversion occurring within the hybrid DNA segments of a crossover.

The two models differ in the polarity of the chains broken initially, in the requirement for synthesis of new DNA, in the nature of the mispairing in the two chromatids, in the explanation of regular nonreciprocal recombination between alleles, and in the explanation of conversion associated with a parental arrangement of outside marker genes. The latter provides the best means of distinction, but demands study of recombination in neighboring genes. One point of interest mentioned by other workers was that, with the proper experimental system, it is possible to observe reversal of polarized recombination (Murray, University of Cambridge). Such a result indicates that the polarities of strands broken or the direction of repair is not always fixed with respect to a given set of outside markers. A second point of interest was the question of whether polarized recombination could extend beyond a single gene. No clear answer is available on this point yet. However, the several systems developed by Murray and by Stadler (University of Washington), in which mutants can be found in adjacent genes, represent suitable experimental material.

Whitehouse pointed out that the data of Case and Giles [*Genetics* **49**, 529 (1964)] for *pan*-2 indicated an association between postmeiotic segregation and multistranded recombination within the gene or in its vicinity. He suggested that correction of mispairing might require an enzyme to move along the DNA molecule and that multistranded recombination might prevent such movement.

He concluded by suggesting that the patterns of intragenic polarity in recombination could be accounted for if one assumed that for any particular gene the hybrid DNA of a crossover always arose from one specific end and could extend through that gene and into the next. Thus the pattern for any given gene also depended on the orientation of the neighbor. The similarity of the initial steps in crossing-over to those of messenger RNA formation appeared significant.

The study of DNA "repair" after mutagenic treatment will also come to occupy an important role in interpreting any recombination mechanisms in terms of repair. Few studies of mutagenesis related to repair are well developed in *Neurospora*, but it has clearly become of great interest as bacterial work along these lines has progressed. In Neurospora, the study of photoreactivation (Terry, University of California, La Jolla; Kilbey, Oak Ridge National Laboratory; and Howe, University of Georgia) and of factors leading to unusually low frequencies of one-strand mutational events ("mosaics") (Baylis, Florida State University) indicate that repair either does or could prevail after mutation. The exploitation of such systems will lead to an increasing unity of work in recombination and mutation. A final aspect of recombination studies, introduced by Catcheside (Australian National University), is the genetic control of recombination frequencies. Several workers, including Catcheside, reported recessive genes which lead to high frequencies of recombination in specific genes or in specific intervals. Such behavior might conceivably point to the action of the dominant alleles as regulators of synapsis or exchange in meiosis. Whatever the recombination mechanisms, mutants affecting them are a valuable material for study.

One of the dominant themes of the conference was the interactions among polypeptides and proteins, and the "locational" specificity of enzymes. The simplest level of polypeptide interaction is in allelic complementation, where homologous polypeptides, altered in different ways by mutation, hybridize to form a partially active enzyme (Fincham, John Innes Institute). Since interaction of polypeptides specified by different genes to form complex proteins or enzyme aggregates can be understood in similar terms, allelic complementation serves as a fundamental model. It is quite clear that monomers are quite capable of assembling themselves into multimers. Thus the study of conditions affecting the assembly process in vitro, while not duplicating those in the cell, are valuable in specifying the environment or the chemical and conformational aspects of the proteins necessary for interaction.

Many workers reported on clear or suspected cases of multienzyme aggregates, some involving most of the enzymes of certain biosynthetic pathways. The inferred integrity of these

aggregates in vivo has several implications. First, the coordination of sequential metabolic steps may be achieved by spatial arrangement such that intermediates of a pathway do not have to achieve general distribution in the cell to yield optimal flux through the pathway. Second, the quantitative coordination of enzyme activities may be achieved wherever two or more catalytic activities share a common polypeptide, or where one polypeptide governs the aggregation of others. It is surprising, to many workers, that much of the gene-enzyme work in Neurospora could be understood previously without consideration of aggregation, if this phenomenon is indeed important to function. In particular, the effects of an altered or missing enzyme upon the function of an aggregate, if any, is not sufficiently severe to complicate non-allelic complementation tests. Such results also suggest that enzymes may aggregate without reference to their origin from one nucleus or another of a complementing heterokaryon. Several cases of closely linked genes, however, such as the *arom* cluster (Case and Partridge, Yale University) which governs the pathway leading to aromatic amino acids, suggest that the synthesis of the enzymes, the aggregation of the enzymes, or both, might be a very closely coordinated process. The expression of the cluster is such that it is reminiscent of an operon of bacteria. The "polarized" complementation observed in this system suggests that mutations of one gene may affect the action of other genes in the cluster. However, some departures from an ideal polarized pattern, as well as enzyme analyses, lead to the alternate possibility that the aggregation of the enzymes, rather than merely their synthesis, is an important factor in the complementation pattern. It may well be that gene clusters—of which more examples are being recognized among previously "simple" loci—are exactly those in which proper or efficient polypeptide aggregation is impossible to achieve in the absence of controlling factors dependent upon tight genetic linkage. The existence of aggregates also may explain the unexpected inability of certain mutants to use intermediates of a deficient pathway. This would be expected in cases where mutation disrupts not only the catalytic action of one enzyme, but also the organization of others following it in the pathway.

The presentations of D. Woodward and Munkres (Stanford University) were interesting in connection with location and organization of enzymes in the cell. They have recently reported that classic cytoplasmically inherited respiratory deficiencies such as "poky" have a mitochondrial structural protein with altered composition of amino acids. Aside from the important implications regarding cytoplasmic inheritance, the effect of the alteration upon the ratios and localization of respiratory enzymes reflects the importance of assembly, as well as the catalytic function, of enzymes. The relation of catalytic activity and localization of malate dehydrogenase was discussed by Munkres. He noted that enzyme from certain mutants for this step showed no aberrant catalytic properties unless the enzyme was bound to mitochondrial components. He suggested in other cases that the binding itself might be an independently mutable property of the enzyme. There is no doubt that biochemical, cytological, and regulatory work will be increasingly influenced by the "organizational' aspects of metabolism now being uncovered.

Enzyme regulation is an important area in which Neurospora serves as a model, eucaryotic system. Because of its mutants, its easy culture, and its accessibility to biochemical analysis, it is used to make experimental comparisons with and evaluations of regulatory theories originating in bacterial work. While it is very clear that mechanisms of enzyme regulation prevail in Neurospora (and there is no doubt that allosteric behavior of proteins is common to both bacteria and *Neurospora*), the vocabulary of bacterial work tends at times to be a hindrance in interpreting variations in enzyme activities. Structural gene mutations and strictly regulatory mutations are difficult to distinguish; structural mutations may have simple, though indirect, regulatory effects by influencing the metabolic milieu to which regulatory systems respond. At the same time, there are no clear equivalents of "regulatory" or "operator" mutations in *Neurospora*, though several might be interpreted in such terms. Gross's (Duke University) observations that leucine intermediates may have inductive effects upon leucine enzymes, and that quasi-coordinate regulation, within limits, may be achieved with unlinked genes indicate that novel mechanisms of regulating enzyme synthesis are to be found in further study of *Neurospora* and higher organisms.

Enzymes of long-standing interest in regulatory work are those appearing upon the presentation or exhaustion of nutrients, the last being dubbed "hard times" enzymes by Horowitz (California Institute of Technology) and Metzenberg (University of Wisconsin). Horowitz suggested that regulation of tyrosinase might depend upon a rapidly turning-over repressor. The rapid turnover of the repressor, itself a protein, would make it more sensitive to "hard times" (and inhibitors such as cyclohexamide) than the enzymes it repressed. It could therefore act in the negative-controlling role postulated for it.

It is entirely possible that the organizational features of enzyme pathways in the cell represent an alternative to differential enzyme synthesis in allowing economical variations of metabolic rates or distributions of metabolites in *Neurospora*. Mora (University of Michigan), for instance, gave evidence for the segregation of endogenous arginine from enzymes of arginine breakdown under certain conditions. Quantitative studies of the actual flux of metabolites in vivo, such as those developed by Kacser's group (University of Edinburgh) for arginine, will be necessary before important generalizations can be made on regulation of metabolite flow.

A related aspect of regulation is development. There is hope that eventually *Neurospora* will provide material for analysis of simple developmental steps, such as branching, ascus formation, and the like. Aside, however, from the association of a glucose-6-phosphate dehydrogenase alteration with a morphological, "colonial" mutation (Brody, Rockefeller University), and of an invertase deficiency with a "periodic" mutant (Sargent, Stanford University), rather few clear causal connections between enzymatic and morphological behavior have been seen.

Considerable discussion was devoted to metabolite uptake and metabolic pools. Here, as in regulation work, bacterial analogies at times become a hindrance. Mutations at a number of loci are now known which affect uptake. They are not mutations of clearly separate systems, but overlap in their effects and can be described only in the most specific operational terms. This reflects our poor understanding of the phenomena of uptake and accumulation, and it is hoped that the mutants now becoming available through new selection techniques (Stad-

ler) will aid considerably in the description of these phenomena in eucaryotes in general. Kappy (University of Wisconsin) noted that an "uptake" mutant resistant to ethionine had a weaker cell membrane, as indicated by osmotic treatments of protoplasts. DeBusk (Florida State University) reported that conidia normally contain transport systems for all amino acids except proline. Several workers were able to lend confirmation to Wiley's (Batelle-Northwest Laboratory) suggestion that the maintenance of active uptake systems is strongly dependent upon continued protein synthesis.

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A more definitive report of the conference has been published in *Neurospora Newsletter*, Number 9, June 1966.

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Forthcoming Events

October

23. **Research in Medical Education,** 5th annual conf., Assoc. of American Medical Colleges, San Francisco, Calif. (P. J. Sanazaro, Div. of Education, Assoc. of American Medical Colleges, 2530 Ridge Ave., Evanston, Ill. 60201)

23–25. **Vacuum Microbalance Techniques,** 6th informal conf., Newport Beach, Calif. (7500 Jefferson St., Paramount, Calif. 90723)

23–29. **Cancer** 9th intern. congr., Tokyo, Japan. (Secretariat, Cancer Inst., Nishisugamo, Toshima-ku, Tokyo)

24. American Assoc. of **Poison Control Centers,** 9th annual mtg., Chicago, Ill. (M. S. McIntire, The Association, 44th and Dewey Ave., Omaha, Nebr. 68105)

24–26. Canadian Assoc. for **Applied Spectroscopy,** natl. mtg., Montreal, Que. (S. Barabas, Research Center, 240 Hymus Blvd., Pointe Claire, Que.)

24–26. **Medical Education,** symp., Beirut, Lebanon. (B. Thurston, American Univ. of Beirut, Beirut)

24–27. **Instrument** Soc. of America, 21st annual conf., New York, N.Y. (ISA, 530 William Penn Pl., Pittsburgh, Pa.)