FINAL TECHNICAL REPORT

AEC CONTRACT (11-1)-3016 (FORMERLY (30-1)-3098)
WITH YALE UNIVERSITY - (1963-1972)

Administered by Norman H. Giles

"STUDIES ON MECHANISMS OF MUTATION, RECOMBINATION,
AND GENE ACTION IN *NEUROSPORA*"

This report will attempt to summarize the results of research performed under the designated AEC contract during the period indicated. Since annual progress reports have been submitted previously, the present report will only provide a summary of the major accomplishments of the investigations performed under this contract. In addition, it will include a listing of publications appearing during this period and of personnel associated in whole or in part with the research program.

The initial research carried out under this contract was concerned with continuing studies of the mechanisms of recombination in the ascomycete, *Neurospora crassa*. Principal attention was devoted to allelic recombination mechanisms at the pan-2 locus. These studies provided clear evidence for "gene conversion" in which atypical segregations lead to aberrant Mendelian ratios always involving the same two alleles which entered a given cross. In addition, in three-point crosses the use of double pan-2 mutants crossed to heteroallelic single pan-2 mutants provided the first evidence for simultaneous "conversion" of a considerable segment of DNA, often essentially equivalent to the entire pan-2 locus. These observations were initially interpreted on a "copy-choice" model, but now appear to be more easily accounted for by a "hybrid DNA model" such as the ones proposed by Whitehouse and by Holiday.

More recently research emphasis has been shifted to mechanisms of gene action. In particular these studies have centered on the organization and significance of gene clusters in *N. crassa* having operon-like characteristics. Major attention has been devoted to his-3 and the arom gene clusters. In both instances these gene clusters encode multienzyme complexes (aggregates), and this may, in fact, be a primary basis for the evolution and/or survival of the two gene clusters.

Major recent attention has centered on the arom gene cluster and the arom multienzyme complex which it encodes. This complex catalyzes steps two through six in the prechorismic acid part of the aromatic synthetic pathway. Two distinct categories of mutants have been detected within this arom cluster: (1) five types of single gene (presumably missense) mutants which lack only single activities (one mutant class was obtained by indirect means) and form aggregates of normal molecular weight, and (2) pleiotropic mutants which lack two or more activities. A major class of pleiotropic mutants consists of non-complementary types which lack all five activities, map within the proximal (arom-2 - DHQ synthetase) structural gene in the cluster and are (in at least some cases) suppressed by nonsense suppressors. These mutants provide the major evidence that the arom cluster is normally transcribed in a polarized fashion as a single m-RNA which is subsequently translated also in a polarized fashion into

RECEIVED BY TIC MAY 29 1973

RECEIVED BY TIC MAY 29 1973

MASTER

04220

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

the multienzyme aggregate. Dissociation studies with purified aggregate protein from wild type, combined with both *in vivo* and *in vitro* allelic complementation tests, have led to the following hypothesis concerning the organization of the arom multienzyme complex: this aggregate (M.W. ca. 230,000) consists of two identical sub-aggregates (M.W. ca. 115,000) which can rather easily dissociate from each other. Additional evidence suggests that these sub-aggregates contain five different polypeptide chains (possibly associated initially in a 2 + 3 arrangement) each carrying one of the five different catalytic sites and that only two of the five pairs of chains are physically juxtaposed in the "mature" aggregate. Additional recent experiments with wild type arom aggregate material purified by a new procedure indicate that the complex can dissociate into still smaller subunits (some as low as M. W. 20,000) with retention of detectable levels of individual activities. Attempts are now being made to isolate the various polypeptides present in the aggregate in order to verify the presence of five unique polypeptides as is suggested by the genetic data. These polypeptides will be compared to each other and to polypeptides isolated from partial aggregates found in several classes of pleiotropic mutants and to homologous enzymes isolated from other organisms which lack arom aggregates. Such comparisons should establish the organization of the arom aggregate and may reveal evolutionary homologies among the polypeptides involved in the aggregate.

All available evidence continues to support the hypothesis that at least one function of the arom multienzyme complex is to segregate two potentially competitive aromatic metabolic pathways in Neurospora - the constitutive synthetic pathway and the inducible quinic (shikimic) acid biodegradative pathway.

The initial steps in this latter pathway (first detected in Neurospora in the course of these investigations) have now been shown to be controlled by a newly-discovered gene cluster (the ga cluster). The ga system consists of a closely linked cluster of four genes in linkage group VII controlling the three inducible enzymes catalyzing the first three reactions in the catabolism of quinic acid. Three of these genes appear to be the structural genes for the three enzymes -- ga-2 (catabolic dehydroquinase), ga-3 (quinic dehydrogenase), and ga-4 (dehydroshikimic dehydrase). In addition, the protein encoded in the ga-3 gene is able to catalyze the dehydrogenation of shikimic acid to dehydroshikimic acid -- an activity referred to as shikimic acid dehydrogenase. Mutants in the fourth gene, ga-1, are pleiotropic types, non-inducible for all three enzymes, and form heterocaryons which complement mutants in each of the structural genes. Evidence concerning the role of the ga-1 gene and the nature of its product has been sought in several ways: complementation studies (both inter- and intragenic); attempts to detect non-sense suppressors of several ga-1 mutants; the detection of temperature-sensitive ga-1 mutants; the recovery by reversion and directly from WT of constitutive mutants, ga-1^C, (some of which are temperature-sensitive) in the ga-1 gene; the preliminary biochemical characterization of the catabolic dehydroquinase produced by ga-1 mutants; additional genetic fine structure mapping of various types of ga-1 mutants. These studies support the hypothesis that the ga-1 gene produces a multimeric (oligomeric) protein which plays only a positive regulatory role in initiating synthesis of the three structural gene products. The results also suggest that the ga-1 protein is divisible into at least two distinct functional regions - an amino acid sequence involved in interacting (directly or indirectly) with a DNA promoter region adjacent to the three

structural genes for the quinate catabolic enzymes, and a second sequence involved in interacting with inducers such as quinic acid. Preliminary experiments encourage the belief that it may be possible to isolate this protein by its binding to radioactive quinic acid. Successful isolation of such a protein would apparently provide the first such case in a Eucaryote.

Although these results indicate many similarities between the ga gene cluster and bacterial operons, the evidence is not yet complete. To date, no clear indication has been obtained of promoter mutants or of pleiotropic polar mutants. Indeed, certain assay data suggest that the synthesis of the three enzymes controlled by the ga-1 gene may not be coordinate. For example, considerable variation is observed in the activity ratios for the three enzymes in different ga-1 mutants, suggesting the possibility that a separate promoter may be associated with each of the three structural genes. Clearly, additional biochemical and genetical data will be required to elucidate in more detail the organization and functional relationships of the ga region in N. crassa. However, the substantial progress made to date encourages us to believe that much more can be accomplished with the ga system, which we feel is a particularly favorable one for investigating genetic regulatory mechanisms in a eucaryotic organism.

Personnel Associated with this Contract

Two Research Associates have been directly associated with this Contract - Drs. Mary Case and C. W. H. Partridge.

No other research personnel have received their major support from this Contract, which has provided no specific funds for post-doctorals or graduate students. However, during the period covered by this report the following personnel have received indirect support of their research activities through provision of supplies and/or of multiple-use equipment provided by this Contract.

1. Visiting Professor:

Colin H. Doy, Research School of Biological Sciences,
Australian National University

2. Research Associates and Post-doctoral Fellows:

S. I. Ahmed, Department of Microbiology, University of British
Columbia, Vancouver, B.C.

Mary Berlyn, Department of Biology, Yale University

L. Burgoyne, Department of Biology, Flinders University
Adelaide, Australia

M. Collins, Department of Biology, Yale University

Jean Foley (Deceased)

Beth Hart, Department of Microbiology, School of Medicine,
University of Vermont

J. Jacobson, Department of Biology, Yale University

H. Papazian, Department of Biology, Yale University

C. F. Roberts, Department of Genetics, University of Leicester,
England

3. Graduate Students awarded Ph.D. degrees:

K. D. Munkres	1963
B. Z. Dorfman	1964
G. J. Sorger	1964
G. R. Fink	1965
M. B. Berlyn	1966
P. Engel	1966
L. Larison	1966
A. de Leeuw	1968
A. El-Kryani	1968
L. R. Livingston	1968
H. W. Rines	1969
R. S. Chaleff	1972 (candidate)
R. Gravel	1972 (candidate)

Publications Resulting from Research

Supported by AT(11-1)-3016

Giles, Norman H. 1963. Gene conversion studies based on tetrad analysis at the pan-2 locus in Neurospora crassa. (In discussion of paper by Herschel Roman) in Methodology in Basic Genetics, pp. 221-225 (Walter J. Burdette, ed.), Holden-Day, Inc.

Giles, Norman H. 1963. Interallelic complementation. (In discussion of paper by Charles Yanofsky) in Methodology in Basic Genetics, pp. 317-322 (Walter J. Burdette, Ed.), Holden-Day, Inc.

Partridge, C. W. H. and N. H. Giles. 1963. Sedimentation behavior of adenylosuccinase formed by interallelic complementation in Neurospora crassa. Nature 199: 304-305.

Ahmed, A., M. E. Case, and N. H. Giles. 1964. The nature of complementation among mutants in the histidine-3 region of Neurospora crassa. Brookhaven Symposia in Biology 17 17: 53-65.

Case, M. E. and N. H. Giles. 1964. Allelic recombination in Neurospora. Tetrad analysis of a three-point cross within the pan-2 locus. Genetics 49: 529-540.

Giles, Norman H. 1964. Genetic fine structure in relation to function in Neurospora. Genetics Today Symposium 1: 17-30. Proc. XI Internat. Cong. of Genetics. (S. J. Geerts, Ed.), Pergamon Press, Oxford.

Giles, Norman H. 1965. Symposium on Genes and Chromosomes: A Summary. Proc. of the Symp. on Genes and Chromosomes, Buenos Aires, Dec., 1964. Nat. Cancer Inst. Monograph 18: 341-354.

- Munkres, K. D. 1965. Simultaneous genetic alteration of Neurospora malate dehydrogenase and aspartate aminotransferase. Arch. Biochem. and Biophys. 112: 340-346.
- Munkres, K. D., N. H. Giles, and M. E. Case. 1965. The genetic control of Neurospora malate dehydrogenase and aspartate aminotransferase. I. Mutant selection, linkage, and complementation studies. Arch. Biochem. and Biophys. 199: 397-403.
- Sorger, George J. and Norman H. Giles. 1965. Genetic control of nitrate reductase in Neurospora crassa. Genetics 52: 777-708.
- Giles, N. H., M. E. Case, C. W. H. Partridge and S. I. Ahmed. 1967. A gene cluster in Neurospora crassa coding for an aggregate of five aromatic synthetic enzymes. Proc. Nat. Acad. Sci., 58: 1453-1460.
- Giles, N. H., C. W. H. Partridge, S. I. Ahmed and M. E. Case. 1967. The occurrence of two dehydroquinases in Neurospora crassa - one constitutive and one inducible. Proc. Nat. Acad. Sci., 58: 1930-1937.
- Case, M. E. and N. H. Giles. 1968. Evidence for nonsense mutations in the arom gene cluster of Neurospora crassa. Genetics 60: 49-58.
- Burgoyne, L., M. E. Case, and N. H. Giles. 1969. Purification and properties of the aromatic (arom) synthetic enzyme aggregate of Neurospora crassa. Biochem. and Biophys. Acta 191: 452-462.
- Case, Mary E., L. Burgoyne, and N. H. Giles. 1969. In vivo and in vitro complementation between DHQ synthetase mutants in the arom gene cluster of Neurospora crassa. Genetics 63: 581-583.
- Rines, H. W., M. E. Case, and N. H. Giles. 1969. Mutants in the arom gene cluster of Neurospora crassa specific for biosynthetic dehydroquinase. Genetics 61: 789-800.
- Case, Mary E. and N. H. Giles. 1971. Partial enzyme aggregates formed by pleiotropic mutants in the arom gene cluster of Neurospora crassa. Proc. Nat. Acad. Sci., 68: 58-62.
- Valone, James A. Jr., M. E. Case, and N. H. Giles. 1971. Constitutive mutants in a regulatory gene exerting positive control of quinic acid catabolism in Neurospora crassa. Proc. Nat. Acad. Sci. 68: 1555-1559.
- Case, Mary E., Norman H. Giles, and Colin H. Doy. 1972. Genetical and biochemical evidence for further interrelationships between the polyaromatic synthetic and the quinate-shikimate catabolic pathways in Neurospora crassa. Genetics (in press).
- Jacobson, James W., Beth A. Hart, Colin H. Doy, and Norman H. Giles. 1972. Purification and stability of the multienzyme complex encoded in the arom gene cluster of Neurospora crassa. MS. submitted to Biochem. and Biophys. Acta.

Partridge, C. W. H., Mary E. Case, and Norman H. Giles. 1972. Direct induction in wild type *Neurospora crassa* of mutants (*ga-1^c*) constitutive for the catabolism of quinate and shikimate. Genetics (in press).