*A GENE CLUSTER IN NEUROSPORA CRASSA CODING FOR AN AGGREGATE OF FIVE AROMATIC SYNTHETIC ENZYMES**

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The widespread occurrence of functionally related clusters of genes, the operon of Jacob and Monod,[1] in various bacteria (procaryotes) is now well established,[2] and several distinctive characteristics of these genetic systems have been defined.[3] By contrast, comparative genetic evidence has appeared to indicate that such systems do not occur in eucaryotes. Recently, however, studies with histidine-requiring mutants in *Neurospora*, yeast, and *Aspergillus* have established the existence of a cluster of three genes having a number of properties characteristic of bacterial operons.[4] These results prompted the present studies of a gene cluster, first detected in *Neurospora* by Gross and Fein,[5] in which mutation produces polyaromatic auxotrophs. Comparative investigations of almost 500 newly induced mutants, employing combined complementation, recombination, and biochemical techniques, indicate that this cluster contains five structural genes coding for the enzymes controlling steps two through six in the polyaromatic pathway prior to chorismic acid.

Evidence that this gene cluster exhibits properties characteristic of bacterial operons has been obtained, such as biochemical pleiotropy, polarity effects, and the asymmetrical genetic localization of completely noncomplementing mutants, some of which are suppressible by an apparent nonsense suppressor.[6] However, certain mutants in the cluster have properties which distinguish them from mutants in typical bacterial operons. These differences appear to result primarily from the fact that the five enzymes coded for by this gene cluster remain associated as a multienzyme aggregate. Since this genetic system has a number of features not characteristic of typical bacterial operons, and since there is, as yet, no evidence for operator or regulator genes affecting these activities, it seems best, at the present stage in these investigations, to refer to this system by the more noncommittal designation of *arom* gene cluster (or *arom* region), rather than as the *arom* operon.[7] Indeed, the present studies suggest that the primary function of the cluster may not be related to gene regulation, but may well be to code for an enzyme aggregate which provides a channeling mechanism effecting an intracellular separation of two potentially competing pathways in aromatic metabolism.

Materials and Methods.—(1) *Origin and initial classification of mutants:* Approximately 500 aromatic amino acid-requiring mutants were isolated, principally by filtration-concentration in strains closely related to wild-type 74A, following treatments with ultraviolet or with the chemical mutagens ethyl methanesulfonate or N-methyl-N-nitro-N-nitrosoguanidine. The mutants studied are polyaromatic auxotrophs which typically require for growth tryptophan, phenylalanine, tyrosine, and para-aminobenzoic acid. The following levels of supplements (amounts per ml) were used throughout, unless otherwise noted: 40 μg/ml each of L-tryptophan, L-phenylalanine, and L-tyrosine, plus 0.25 μg/ml para-aminobenzoic acid. Complementation tests to classify mutants employed mixed conidial suspensions on Fries minimal agar plates without sorbose.

(2) *Crossing procedures:* Sterility in crosses has proved to be a considerable problem with many of the *arom* mutants. However, adequate data have been obtained from two factor crosses,

sometimes employing either of two adjacent proximal markers, *pe* (peach) or *arg-12*, indicating the relative positions of mutants within the *arom* cluster. Subsequent use in three-point crosses of double *arom* mutants induced in an *arom-1* strain has served to check the two-point cross data.

(3) *Biochemical methods:* (a) *Enzyme preparations:* Mycelium was routinely grown for 72 hr at 25° in standing culture. Fries minimal was supplemented with limiting tryptophan (16 μg/ml) and double the usual concentration of other required supplements. Harvested mycelium was freeze-dried, powdered, extracted in 0.1 *M* KPO_4 buffer, pH 7.4, containing 0.1 mM α-thioglycerol, treated with excess protamine sulfate, and supernate used to obtain a precipitate in the 30–50% $(NH_4)_2SO_4$ saturation range. Protein determinations were made using biuret reagent.[8]

(b) *Enzyme assays:* Spectrophotometric assays were carried out with a total volume of 0.6 ml, fluorometric assays with 0.4 ml. All assays were performed at 37°C.

DHQ synthetase was measured by the production of DHS in the presence of excess dehydroquinase. The reaction mixture contained 0.20 mM DAHP, 50 mM pH 7.4, KPO_4 buffer, 0.05 mM α-thioglycerol, 0.13 mM NAD, 0.17 mM $CoCl_2$, $^1/_{60}$ vol of an *arom-2* enzyme preparation (source of excess dehydroquinase), and $^1/_{30}$ vol of the enzyme preparation being assayed. The change in absorption at 240 mμ was followed over the initial linear reaction period.

Dehydroquinase was measured by a modification of the method of Gross and Fein.[5] The reaction mixture contained approximately 0.3 mM crude DHQ, 50 mM pH 7.4 KPO_4 buffer, 0.2 mM EDTA, and $^1/_{30}$ vol of enzyme preparation.

DHS reductase was measured by a modification of the method of Gross and Fein.[5] The reaction mixture contained 4.2 mM shikimic acid, 1.67 mM NADP, 42 mM pH 10.6 glycine buffer, and $^1/_{12}$ vol of dialyzed enzyme preparation.

Shikimic acid kinase was measured either by (*a*) loss of shikimic acid or (*b*) production of anthranilic acid in the presence of excess *arom-5* extract. In method (*a*), a modification of the method of Fewster,[9] the reaction mixture contained 2.5 mM shikimic acid, 5 mM ATP, 5 mM $MgCl_2$, 50 mM pH 8.0 Tris HCl buffer, and 0.03 ml of enzyme preparation in a total of 0.4 ml. In method (*b*), the reaction mixture contained 2.5 mM shikimic acid, 5 mM ATP, 5 mM $MgCl_2$, 5 mM L-glutamine, 1 mM NADP, 1 mM PEP, 37 mM pH 8.0 Tris HCl, 8 mM β-mercaptoethanol, $^3/_{40}$ vol of an *arom-5* enzyme preparation, and $^1/_{20}$ vol of the enzyme preparation being assayed. The reaction was stopped with $^1/_4$ vol of 1 *M* HCl, and the anthranilic acid extracted for fluorescence measurement.

EPSP synthetase was measured either by (*a*) loss of SAP or (*b*) by the above fluorometric method with the substitution of *arom-4* mutants (or of *E. coli* mutant, 83-24). In (*a*), a modification of the method of Levin and Sprinson,[10] *E. coli* strain 83-3 was used. The incubation mixture contained 1 mM PEP, 1 mM SAP, 50 mM citrate buffer, pH 5.6, 0.1 mM DTT, and enzyme fraction in a total of 1.5 ml. In (*b*), with mutant *E. coli* extract, the reaction mixture was supplemented with 1 mM NADH and 7.5 mM L-phenylalanine, and SAP (1.8 mM) was substituted for shikimic acid.

(c) *Abbreviations used:* EDTA, ethylenediaminetetraacetic acid; ATP, adenosine 5′-triphosphate; NAD, nicotinamide-adenine dinucleotide; NADH, reduced nicotinamide-adenine dinucleotide; NADP, nicotinamide-adenine dinucleotide phosphate; PEP, phosphoenolpyruvic acid; DTT, dithiothreitol; Tris HCl, tris(hydroxymethyl)aminomethane hydrochloride; E-4-P, D-erythrose-4-PO_4; DAHP, 3-deoxy-D-arabinoheptulosonic acid-7-PO_4; DHQ, 5-dehydroquinic acid; DHS, 5-dehydroshikimic acid; SA, shikimic acid; SAP, shikimic acid-5-PO_4; EPSP, 3-enolpyruvylshikimic acid-5-PO_4; and CA, chorismic acid.

Results.—(1) *Classification of mutants by complementation and biochemical analyses:* The polyaromatic auxotrophic mutants were first classified on the basis of complementation analysis and then examined for their biochemical defects by *in vitro* complementation with bacterial mutants carrying known defects and by direct enzymatic assays. The mutants have all been shown to be defective for one or more of the last six enzymatic reactions in aromatic biosynthesis prior to CA (Fig. 1). Mutants in one group were found to be equivalent to the *arom*-3 mutants of Gross and Fein[5] and lack CA synthetase activity.

The second group of mutants (the *arom* gene cluster or region) is complex both

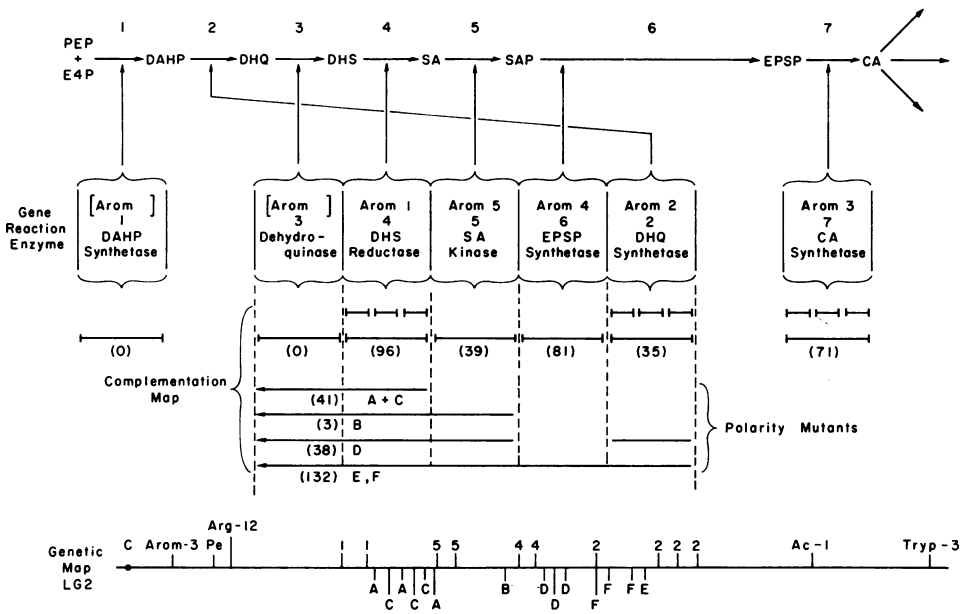

FIG. 1.—The organization of the *arom* gene cluster in *Neurospora crassa* on the basis of complementation, biochemical, and genetic recombination data. Reactions in the polyaromatic pathway prior to chorismic acid, indicated in detail by Giles,[7] are shown at the top of the figure (abbreviations are explained in the text). Designated below are the structural genes which code for the indicated enzymes catalyzing specific reactions. (The presumptive gene(s) controlling the first reaction and the identified (*arom-3*) gene controlling the seventh reaction, neither of which is within the *arom* cluster, are also included in the figure.) On the complementation map, numerals in parentheses indicate the number of mutants of a particular type. The short bars on the complementation maps of the *arom*-1, *-2*, and *-3* genes serve only to indicate the occurrence, but not the detailed pattern, of allelic complementation for these mutants. Categories of polarity mutants are indicated by the letters *A* through *F*. The symbols on the genetic map are as follows: *L.G. II* (linkage group II), *Cen* (centromere), *Pe* (peach), *Arg-12* (arginine-12), *Ac-1* (acetate-1), *Tryp-3* (tryptophan-3). On the genetic map, the approximate positions of single gene mutants within the *arom* clusters are indicated above the line, those of polarity mutants, below.

genetically and biochemically and consists of two major categories. The first major category, designated single gene mutants, consists of four clear-cut classes of complementing mutants each defective for a single activity but possessing, in general, high levels of the other four activities, either equivalent to or higher than the wild-type level (Table 1). These include classes equivalent to the *arom-1* (deficient for DHS reductase) and *arom-4* (deficient for EPSP synthetase) groups previously identified,[5] plus two newly identified groups, *arom-2* (deficient for DHQ synthetase) and *arom-5* (deficient for shikimic acid kinase).

The second major category of mutants within the *arom* region, designated polarity mutants, differs in certain notable respects from the first. These mutants either fail to complement with mutants in two, three, or all four of the single gene groups, or exhibit marked quantitative reductions in complementation responses (based on growth tests) with two or more of the mutant groups (e.g., the distinction between A and C types and *arom-1* mutants is based on this latter characteristic). In addition, assay data (Table 1) indicate that these mutants are biochemically pleiotropic, either lacking or having markedly reduced activities for all five enzymes.

TABLE 1

SPECIFIC ACTIVITIES AS A PER CENT OF WILD TYPE IN VARIOUS *arom* MUTANTS FOR THE FIVE ENZYMES CODED FOR BY THE *arom* GENE CLUSTER IN *Neurospora crassa*, PLUS THE SPECIFIC ACTIVITY FOR AN INDEPENDENTLY CONTROLLED RELATED ENZYME*

	Enzyme Activities					
	Dehydroquinase		DHS	SA	EPSP	DHQ
Mutant category	Induced*	Constitutive	Reductase	Kinase	Synthetase	Synthetase
Single gene mutants						
arom-1 (1183)	5.5	165	4	107	107	153
(80)	14.6	148	1	110	81	133
arom-2 (81)	0	118	176	115	104	0
(82)	0	163	168	130	99	0
arom-4 (49)	16.5	251	225	260	0	243
(1050)	19.0	305	251	270	0–2	325
arom-5 (56)	16.0	128	105	0	82	119
(1146)	14.0	156	93	2	84	126
arom-3 (47)	10.6	208	248	297	252	300
(87)	15.0	297	285	313	360	300
Polarity mutants						
A (58)	30.0	0	5	13	25	0
A (1131)	16.5	0–1	2	4	7	0
C (63)	8.8	0	2	0	7	0
C (1148)	30.8	0	3	0	4	0
B (1136)	8.1	0–1	4	8	20	0
B (14)	29.0	0	7	1	35	0
D (75)	17.7	1	2	1	2	0
D (1199)	18.3	0	2	0	9	0
E (54)	4.7	0	3	3	1–2	0
E (34)	0.4	0	3	0	1	0
F (25)	0	0	3	0–1	0–1	0

All cultures were grown in the absence of quinic acid. Assays were performed on material precipitated by ammonium sulfate solution between 30 and 50% saturated, except in the case of dehydroquinase where the material precipitated below 30% saturation was also assayed. The wild-type specific activities expressed as mμmoles/min/mg protein were as follows: DHS reductase, 25; dehydroquinase, 13; DHQ synthetase, 5; SA kinase, 21; EPSP synthetase, 46. SA kinase was assayed by method (*a*), and EPSP synthetase by method (*a*) (see *Methods* section). E_{240} for DHS was taken as 12,000. Assays of crude extracts indicated no significant differences in relative activities from the values in the above table.

* Since no induced dehydroquinase (see text) was detected in wild type grown without exogenous inducer, the specific activities of this enzyme are given directly based on protein values of 0–50% saturated $(NH_4)_2SO_4$ samples compared with constitutive dehydroquinase for which specific activities are based on protein values from 30–50% saturated samples. The induced form of the activity is defined as that portion of the total which is stable at 71° for 10 min in 100 mMpH 8.0 Tris HCl plus 1 mM EDTA. The constitutive form is defined as that portion lost under these conditions.

Extreme mutants of the F type fail to complement with all other *arom* region mutants and essentially lack all five activities. E mutants are also noncomplementing, but differ in having dehydroquinase activity. The complementation responses of other mutants, when represented on a complementation map (Fig. 1), tend, in general, to exhibit a marked polarity, and for this and other reasons these mutants have been designated polarity mutants. It should be noted that polarity mutants, even those classified within the same category, exhibit considerable diversity in their complementation responses.

One initially puzzling result of these studies was the absence of an anticipated class of single gene mutants, i.e., one lacking dehydroquinase activity alone. In all mutants studied to date a loss of, or marked reduction in, dehydroquinase activity is accompanied by a loss of, or marked reduction in, the other four enzyme activities coded for by the *arom* region. Recent biochemical evidence indicates the probable explanation, i.e., that *Neurospora crassa* has two distinct dehydroquinase activities, one constitutive and one inducible, the latter apparently coded for by a gene not in the *arom* cluster. Detailed evidence for this distinction, based in part on thermolability (Table 1) and zone centrifugation studies, will be presented in a later paper.[11]

(2) *Genetic mapping of mutants:* Crossing data confirm previous evidence[5] that the two groups of polyaromatic mutants occur in the right arm of linkage group II (Fig. 1). These data indicate the order of the four identified genes—each of which maps in a distinct, localized part of the region—and establish the relative positions of certain polarity mutants (Fig. 1). The polarity mutants map at various positions in the *arom* region, their locations corresponding, in general, to those predicted on the basis of their polarized complementation patterns. The completely noncomplementing mutants map asymmetrically at one end of the region among the *arom-2* mutants. The precise localization of other types of polarity mutants relative to the other three structural genes is not yet clear.

(3) *Density gradient centrifugation studies:* Representative comparative results from zone centrifugation[12] (Fig. 2 and Table 2) indicate that in wild type and all single gene mutants examined to date, the activities coded for by the *arom* region are associated in an enzyme aggregate with a molecular weight of *ca.* 200,000. By contrast, at least three of the five activities in wild-type *Salmonella* and *E. coli* are easily separable by this same procedure, and all three have much lower molecular weights than those determined for similar activities in *Neurospora.*

The results of comparable studies with polarity mutants are quite different from those with wild-type and single gene mutants. The only constitutive activities so far examined in gradients, EPSP synthetase, and SA kinase have been found to have much lower molecular weights (less than 100,000).

Discussion.—On the basis of the previously summarized characteristics of the polyaromatic auxotrophic mutants, the organization and functioning of the *arom* region in wild-type *Neurospora crassa* is interpreted as follows: This region is considered to be a functionally integrated cluster of five contiguous structural genes

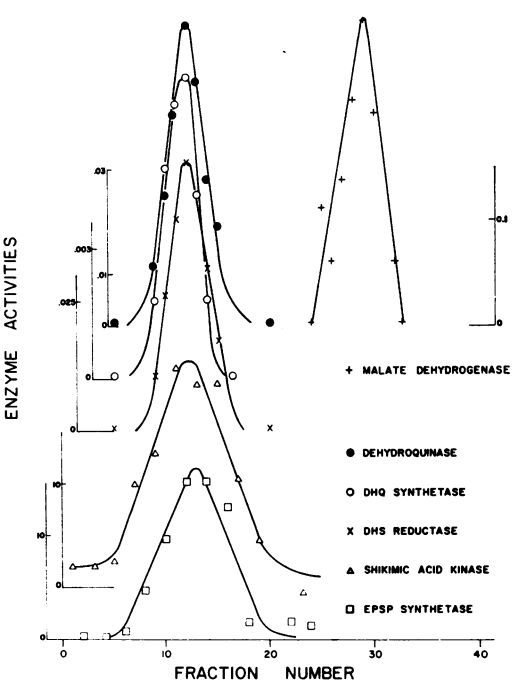

FIG. 2.—Distribution, after centrifugation in a sucrose density gradient, of activities of the five aromatic synthetic enzymes coded for by the *arom* gene cluster in *Neurospora crassa* (and of the reference activity—malate dehydrogenase) from an *arom-3* mutant (lacking CA synthetase). Conditions as described in Table 2. Assays of shikimate kinase and EPSP synthetase were performed by method (*b*) (see *Methods* section). Centrifugation of a crude extract and of an alcohol-precipitated subfraction from the ammonium sulfate fraction indicated no significant shift in distribution.

TABLE 2

SEDIMENTATION CONSTANTS OF ENZYME ACTIVITIES OF WILD TYPE (74A), *arom* GENE CLUSTER MUTANTS LACKING SINGLE ACTIVITIES ONLY, AND AN *arom-3* MUTANT (LACKING CA SYNTHETASE)

Mutant category	No. of strains	Enzymic Activity				
		DHQase	DHS Reductase	EPSP Synthetase	SA Kinase	DHQ Synthetase
arom-1	3	11.5	(none)	10.9	10.9	11.6
arom-2	2	11.3	11.4	11.1	11.1	(none)
arom-4	2	11.1	11.1	(none)	11.1	11.1
arom-5	4	11.3	11.2	11.2	(none)	11.3
arom-3	1	11.3	11.3	11.4	11.3	11.3
Wild type	1	11.1	11.3	11.4	11.1	11.6

Samples were precipitated by ammonium sulfate solution between 32 and 48%, saturated, and applied in a volume of 0.4- to 4.6-ml gradients of 5–20% sucrose in 100 mM, pH 8.0 Tris HCl, and in pH 7.4 KPO_4, each containing 0.1 mM EDTA and 0.1 mM DTT. Centrifugations were carried out for 16–17 hr at 35,000 rpm in a Spinco model L2 ultracentrifuge using an SW50 rotor, at 3.5°.

Procedures and computations followed Martin and Ames.[12] Endogenous *Neurospora* malate dehydrogenase was used as the standard (*S* 4.77)[13] after comparison with several standard purified enzymes ranging throughout the gradient. Assay methods are described in the *Methods* section. EPSP synthetase and shikimate kinase were assayed by method (*b*). Average values are given.

coding for five different polypeptides, each carrying the active site for one of the five enzymes catalyzing steps two through six in the prechorismic acid part of the polyaromatic pathway. The cluster is transcribed in a polarized fashion via a single messenger RNA. The initiation of transcription occurs in, or adjacent to, the *arom-2* gene, which is the most proximal gene in the cluster (the most distal in the chromosome arm) and codes for the polypeptide carrying the active site for DHQ synthetase. Transcription proceeds distally within the cluster, involving, in order, the four other structural genes, as shown in Figure 1. Normally, after translation of the messenger RNA (which presumably starts with the codons specifying the DHQ synthetase polypeptide), all five different polypeptides remain associated (at least two apparently as multimers on the basis of intragroup complementation evidence) in a single aggregate.

The four known classes of single gene mutants are interpreted as resulting from missense mutations in four of the five structural genes in the *arom* cluster, the resulting single amino acid substitutions giving polypeptides each lacking a normal specific active site. However, these polypeptides are retained as part of an intact aggregate in which the other four activities are, in general, comparable to those in the wild type.

Polarity mutants are clearly very different in a number of important characteristics from single gene mutants. The most significant polar mutants are the noncomplementing ones essentially lacking all five activities coded for by the *arom* gene cluster, since these mutants provide the most cogent evidence that the cluster constitutes a supragenic functional unit. The evidence that these mutants map at one end of the cluster (within the *arom-2* gene), that some are suppressible by a presumptive nonsense suppressor,[6] and that one mutant yields both complete revertants indistinguishable from wild type and other revertants equivalent to certain *arom-2* mutants,[6] leads to the interpretation of these mutants as nonsense (polarity) mutants in the first (proximal) structural gene of the cluster.

The A, B, and C polarity mutants are interpreted as nonsense mutants occurring within any one of the structural genes located within the cluster, distal to the *arom-2* gene. Whether D mutants are located within or distal to the *arom-2* gene is not yet clear. The biochemical evidence that all five enzyme activities are either

absent or markedly reduced, and that any remaining detectable activities are associated with proteins having molecular weights much less than that of the aggregate present in wild-type and in missense mutants is taken to indicate that the nonsense (chain-terminating) mutations in these polarity mutants prevent the formation of a normal aggregate. The marked "reverse polarity" effect characteristic of these mutants (e.g., in A and C mutants, the strong reduction or essential absence, on the basis of direct assay, of activities coded for by genes located proximal to A and C mutant sites) presumably results from the fact that the *arom* region normally codes for an enzyme aggregate. Thus, the normal expression of any of the five enzyme activities apparently depends upon the presence in a complete aggregate of a specific number and arrangement of the postulated five kinds of complete polypeptide chains. However, the possibility also exists that some of these mutants result from frame shifts, or from missense mutations which in some way affect aggregation. In particular, additional evidence will be required to intrepret the D mutants, which constitute an exceptional class in having an interrupted rather than a continuous complementation pattern when ordered in accord with the genetic map.

The organization of the *arom* gene cluster as discussed here differs somewhat from that inferred from initial studies,[7] since at that time no definitive recombination data were available, nor had the evidence been obtained for two different dehydroquinase activities, with polarity mutants possessing only the inducible activity. Further evidence concerning the physical organization of the enzyme aggregate controlled by the *arom* gene cluster must await current attempts at purification and direct disruption by physical and/or chemical treatment of the wild-type aggregate.

There remains the question as to the functional significance of this supragenic unit. It is evident that this gene cluster has many features characteristic of bacterial operons. However, certain other features, in particular the presence of an enzyme aggregate, serve to distinguish this cluster from most, although not all,[14] bacterial operons. In addition, there is at present no genetic evidence for regulatory genes or for an operator gene in this system. Thus, a possible regulatory significance of the cluster remains to be elucidated. However, the present studies do suggest at least one functional role for this gene cluster based on the evidence that *Neurospora crassa* possesses two distinct dehydroquinases: a constitutive form, which is part of the aromatic enzyme aggregate in the biosynthetic pathway, and an inducible form, which is part of an aromatic degradative pathway.[11, 15] It appears that the *arom* enzyme aggregate in *Neurospora* may provide a channeling mechanism for segregating these two potentially competing pathways in aromatic metabolism—one synthetic and one degradative—which have one step in common, namely, the conversion of DHQ to DHS. Other authors have recently proposed a role of enzyme aggregates in providing channeling mechanisms.[16] A gene cluster transcribed via a single messenger would appear to provide a potentially efficient method of coding for an enzyme aggregate, since such a system should facilitate the juxtaposed synthesis, both in time and space, of the several different polypeptide chains forming an aggregate.

Summary.—Genetical and biochemical evidence is presented for the existence in *Neurospora crassa* of a functionally integrated cluster of five contiguous structural genes coding, apparently via a single polycistronic mRNA, for an enzyme aggregate

catalyzing reactions two through six in the aromatic biosynthetic pathway prior to chorismic acid. Although this gene cluster has many features characteristic of bacterial operons, no clear evidence has yet been obtained that it plays a role in gene regulation. However, one function of the cluster may be to facilitate the formation of the enzyme aggregate which apparently acts as a channeling mechanism to effect the separation of two potentially competing pathways in aromatic metabolism, one synthetic and one degradative. Evidence is presented for the occurrence in *Neurospora* of two dehydroquinases, one constitutive and one inducible, catalyzing the one step common to both pathways.

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[1] Jacob, F., and J. Monod, *J. Mol. Biol.*, **3**, 318 (1961).

[2] Demerec, M., these PROCEEDINGS, **51**, 1057 (1964).

[3] Ames, B. N., and R. G. Martin, *Ann. Rev. Biochem.*, **33**, 235 (1964).

[4] Fink, G. R., *Genetics*, **53**, 445 (1966).

[5] Gross, S. R., and A. Fein, *Genetics*, **45**, 885 (1960).

[6] Seale, T., *Genetics*, in press; Case, M. E., N. H. Giles, and C. W. H. Partridge (Abstr.) *Genetics*, **56**, 548 (1967).

[7] Giles, N. H., *Natl. Cancer Inst. Monograph*, **18**, 341 (1965).

[8] Munkres, K. D., and F. M. Richards, *Arch. Biochem. Biophys.*, **109**, 466 (1965).

[9] Fewster, J. A., *Biochem. J.*, **85**, 388 (1962).

[10] Levin, J. G., and D. M. Sprinson, *J. Biol. Chem.*, **239**, 1142 (1964).

[11] Giles, N. H., C. W. H. Partridge, S. I. Ahmed, and M. E. Case, these PROCEEDINGS, in press.

[12] Martin, R. G., and B. N. Ames, *J. Biol. Chem.*, **236**, 1372 (1961).

[13] Munkres, K. D., *Arch. Biochem. Biophys.*, **112**, 340 (1965).

[14] Henning, U., G. Dennert, R. Hertel, and W. S. Shipp, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 31 (1966), p. 227; Bauerle, R. H., and P. Margolin, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 31 (1966), p. 203.

[15] Tatum, E. L., S. R. Gross, G. Ehrensväld, and L. Garnjobst, these PROCEEDINGS, **40**, 271 (1954).

[16] Lynen, F., *Angew. Chem.*, **77**, 929 (1965); Davis, R. H., in *Organizational Biosynthesis*, ed. H. J. Vogel, J. O. Lampen, and V. Bryson (New York: Academic Press, in press).