# A CLUSTER-GENE: EVIDENCE FOR ONE GENE, ONE POLYPEPTIDE, FIVE ENZYMES*

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SUMMARY: Five enzymes catalyzing consecutive steps in the central pathway leading to the biosynthesis of the aromatic amino acids in Neurospora are physically associated. Previous structural analysis of this enzyme system suggested that the 300,000 dalton native protein consisted of at least four distinct polypeptide chains. Genetic studies conducted in another laboratory indicated that the genes specifying these putative polypeptides are tightly linked. Recently we found that the "subunit" structure observed earlier for this system was created during its isolation and purification by the action of resident proteolytic activity. Here we show that when the enzyme system is purified rapidly by affinity chromatography in the presence of protease inhibitor a single 150,000 dalton band is obtained in dodecyl sulfate gel electrophoresis. Without these precautions, four or more distinct bands with lower molecular weights are obtained. From these results we conclude that the arom enzyme system is a 300,000 dalton dimer consisting of identical 150,000 dalton peptides. Based on the one gene, one polypeptide concept, the arom system appears to represent a case of one gene specifying five enzyme activities rather than five separate genes as previously conceived. The term cluster-gene is proposed to signify a gene encoding a polypeptide with discrete multiple functions.

In Neurospora crassa five of the enzymes which catalyze consecutive reactions in the central pathway leading to the biosynthesis of the aromatic amino acids are known to be physically associated. Giles et al. (1) showed that the "genes" coding for these enzymes were organized in a tightly linked unit which they termed the arom gene cluster. Although the enzyme system has been isolated and purified by two laboratories (2,3), questions remain concerning its structure. Previously it was believed to be a multienzyme complex composed of at least four distinct subunits (3,4). Recently, we have



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shown that the subunits observed earlier were most likely formed *in vitro* during extraction and isolation procedures by the action of resident proteases in *N. crassa* (5). Based on these results we hypothesized that this physically associated enzyme system constituted a multifunctional protein consisting of five enzymes on a single polypeptide chain (5). Here we provide additional data supporting this hypothesis and consider the implications of these results in light of earlier genetic analyses (1,6).

## MATERIALS AND METHODS

Purification of enzyme. The *arom* multienzyme system was isolated and purified from *N. crassa* by ammonium sulfate fractionation, ion exchange, and affinity chromatography, as described earlier (5), with the exceptions that (i) where indicated 1 mM PMSF* was included in the extraction and purification buffers, and (ii) in one example the mycelium was harvested at a very early stage in the growth phase (the yield from 350 l of medium was about 1000 g lyophilized mycelium or approximately 10% of that normally obtained from a late log or stationary phase culture).

Analytical gel electrophoresis. Electrophoresis in polyacrylamide gels was done with and without SDS as described previously (5). For the SDS gel procedure, samples were boiled for 30 min in electrophoresis buffer containing 1% SDS and 1% β-mercaptoethanol.

## RESULTS AND DISCUSSION

Previous evidence suggesting that the five physically associated enzymes of the polyaromatic pathway of *N. crassa* constituted a multienzyme complex was based on the fact that under certain conditions, both with the wild-type organism (2,3) and with mutants (7), four or more distinct subunits could be identified. The results presented here show that if the enzyme system is purified rapidly in the presence of a protease inhibitor the banding pattern obtained by SDS gel electrophoresis is no longer consistent with these earlier conceptions. Rather, a single band with an apparent molecular weight of 150,000 daltons is obtained (Fig. 1A). Moreover, in the absence of protease inhibitor, if the enzyme system is isolated and purified rapidly from cells harvested at a very early stage in the log phase of

*Abbreviations: PMSF, phenylmethanesulfonylfluoride; SDS, sodium dodecyl sulfate; DHS, dehydroshikimate; DHQ, dehydroquinate.

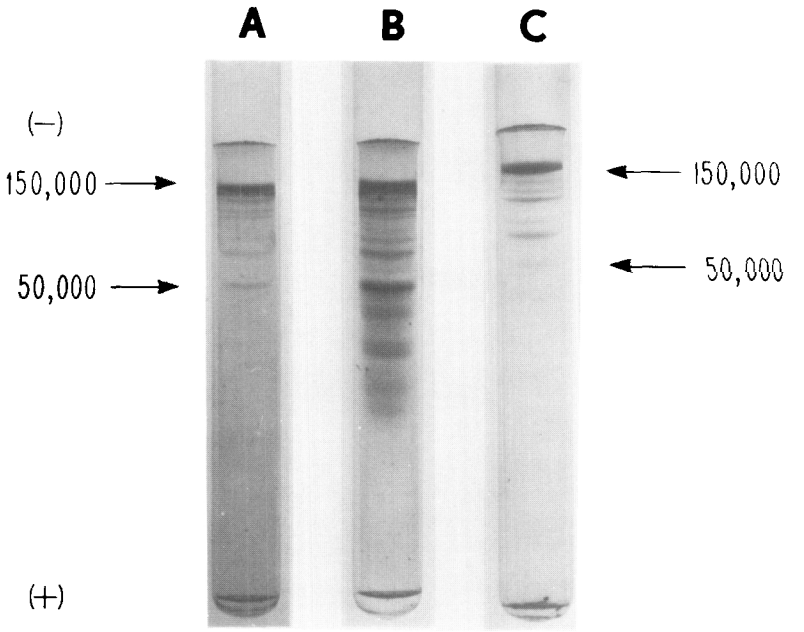


Figure 1. Detergent gel electrophorograms of the arom multienzyme system purified to homogeneity and subjected to SDS gel electrophoresis as described previously (5). (A) late log culture, 1 mM PMSF in all extraction and purification buffers; (B) late log culture, no PMSF in purification buffers; (C) early log culture, no PMSF in purification buffers. The standard proteins used in estimating molecular weights were β-galactosidase (130,000), phosphorylase A (93,000), bovine serum albumin (68,000), ovalbumin dimer (86,000), ovalbumin monomer (43,000), and hemoglobin (15,000).

growth, in contrast to a preparation purified without PMSF from a late log culture (Fig. 1B), over 80% of the protein in SDS gels is in the form of the 150,000 dalton species (Fig. 1C). The latter result apparently is due to decreased levels of intracellular proteases in Neurospora during the early log phase of growth (Spady and Gaertner, unpublished data). It is of interest to note that the appearance of the native enzyme on standard (nondenaturing) gel electrophorograms (Fig. 2) is the same* whether or not the system appears to

*The enzyme preparation purified with 1 mM PMSF exhibits a slightly greater mobility in the standard gel system (Fig. 2) than do the other preparations. PMSF not only inhibits proteolytic activity but also inhibits some of the arom system enzymes (unpublished results). Apparently increased electrophoretic mobility is another indication of the effect of PMSF on the intact arom enzyme system.

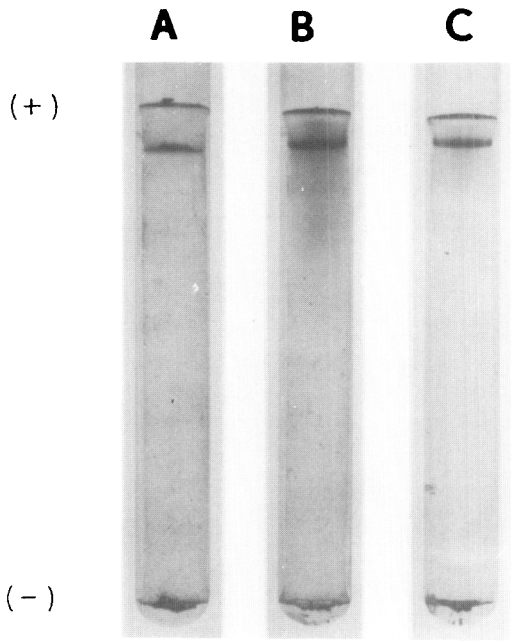


Figure 2. Standard gel electrophorograms of the purified arom enzyme preparations shown in Fig. 1. Electrophoresis under nondenaturing conditions was done as described earlier (5). (A) late log culture, PMSF in purification buffers; (B) late log culture, no PMSF in purification buffers; (C) early log culture, no PMSF in purification buffers.

have multiple subunits by the SDS gel procedure. As we have already suggested (5), such data indicate that the enzyme system can sustain a number of proteolytic clips before it begins to dissociate.

Sedimentation equilibrium analysis of the purified native multienzyme system showed that it has a molecular weight of about 300,000 (3). Therefore, we conclude that this protein consists of a dimer of 150,000 dalton polypeptide chains. Although there appears to be only one band in the 150,000 dalton region of the SDS gels (Fig. 1), we cannot state with certainty from these data alone that the polypeptides are identical. However, from the genetic results of Giles et al. (1) it is clear that the system must consist of identical polypeptide chains. Two of the five enzymes, DHS reductase (shikimate: $NADP^+$ oxidoreductase, EC 1.1.1.25) and DHQ synthase, exhibit allelic complementation. This form of complementation is most readily

explained by partial or complete correction of genetic flaws via intermolecular interaction between identical polypeptide subunits. With the hypothesis that the five enzymes of the arom system are on a single polypeptide, the two enzymes expressing allelic complementation (DHS reductase and DHQ synthase) appear to reside at the carboxy terminus and amino terminus respectively. Rhines et al. (6) demonstrated that the above two enzymes mapped at each end of the arom region, and it was shown from nonsense polarity mutants (8) that DHQ synthase was at the proximal end of the genome. Therefore, this latter enzyme presumably resides at the amino terminal end of the polypeptide chain.

Giles et al. (1) were convinced that the "arom gene region or cluster" was not an example of an operon in a eukaryote. They did not find evidence for operator or other regulatory sites. Moreover, they considered it likely that the primary function of this genetic region was to provide for the synthesis of a five-membered multienzyme complex. In view of the present data we would modify this hypothesis to state that not only is the arom region not an operon, but it is also not a sequence of genes encoding a multienzyme complex. Rather, based on the one gene, one polypeptide concept, we conclude that it is a single gene encoding a multifunctional enzyme. The term "enzyme cluster" may be used to denote all forms of physically associated enzymes including enzyme complexes, membrane-bound enzyme systems, as well as multifunctional enzymes. Here we suggest that the term "cluster-gene" be used to identify a gene producing an enzyme cluster or other such polypeptide with multiple functions.

End-group, peptide, and amino acid sequence analyses will be required to conclusively substantiate the cluster-gene hypothesis in this system. However, evidence is accumulating rapidly that fused genetic units of this type are not unique and in fact may be quite common, especially in eukaryotes (9,10,11 and references cited therin).

## ACKNOWLEDGEMENT

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## REFERENCES

1. Giles, N. W., Case, M. E., Partridge, C.W.H., and Ahmed, S. I. (1967) Proc. Nat. Acad. Sci. USA 58, 1453-1460.

2. Burgoyne, L., Case, M. E., and Giles, N. Y. (1969) Biochim. Biophys. Acta 191, 452-462.

3. Gaertner, F. H. (1972) Arch. Biochem. Biophys. 151, 277-284.

4. Jacobson, J. W., Hart, B. A., Doy, C. H., and Giles, N. Y. (1972) Biochim. Biophys. Acta 289, 1-12.

5. Gaertner, F. H., and Cole, K. W. (1976) Arch. Biochem. Biophys., 177, 566-573.

6. Rines, H. W., Case, M. E., and Giles, N. H. (1969) Genetics 61, 789-800.

7. Case, M. E., and Giles, N. H. (1971) Proc. Nat. Acad. Sci. USA 68, 58-62.

8. Case, M. E., and Giles, N. H. (1968) Genetics 60, 49-58.

9. Stoops, J. K., Arslanian, M. J., Oh, Y. H., Aune, K. C., Vanaman, T. C., and Wakil, S. J. (1975) Proc. Nat. Acad. Sci. USA 72, 1940-1944.

10. Paukert, J. L., Straus, L. D., and Rabinowitz, J. C. (1976) J. Biol. Chem. 251, 5104-5111.

11. Kirschner, K., and Bisswanger, H. (1976) Ann. Rev. Biochem. 45, 143-166.