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Genetically Induced Subcellular Mislocation of *Neurospora* Mitochondrial Malate Dehydrogenase

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Abstract. Among 60 ultraviolet-induced missense mutations of the structural genes that code for mitochondrial malate dehydrogenase (M-MDH, EC 1.1.1.37) of *Neurospora crassa*, two enzyme phenotypes are observed. In a previously described class (C-mutants), M-MDH is malfunctional because of an abnormal conformation induced by association with mitochondria. We describe here a second class (K-mutants) in which the enzyme is malfunctional because of an altered subcellular location. Thus, although both classes cause lesions in the assimilation of exogenous malate, the nature of the lesions differs. In C-mutants, the enzyme misfunctions because of low affinity for malate but remains mitochondrial-bound as in wild-type. Conversely, K-mutant M-MDH is dispersed throughout the cytoplasm.

Studies of a repressible "glyoxysome" isozyme and a constitutive M-MDH of prototroph and mutants indicate that both isozymes are encoded by the same nuclear structural genes and have polypeptide subunits in common.

The inner space of an eucaryotic cell consists of as many as ten classes of membrane systems, each with a unique complement of enzymes.[1] Of the 1000 or so enzymes,[2] at least 200 may be uniquely localized with the various membranous systems.[1,3] The molecular and genetic principles underlying this remarkably precise phenomenon are not well understood. However, recent studies of two of the more well-defined and amenable organelles, mitochondria and chloroplasts, have begun to formulate the first principles of enzyme locational specificity and membrane biogenesis.[4,5]

Enzymes of mitochondria and chloroplasts are generally encoded by nuclear genes, synthesized on cytoplasmic ribosomes, and then incorporated into the organelles.[4,5] Thus, although these membrane systems have unique genes and are also capable of protein synthesis, neither the subcellular location of the genes nor the site of message translation necessarily determines the final location of enzymes.

Exceptions to the unique location of enzymes in the cell are those enzymes that catalyze the same reaction simultaneously at different subcellular locations and perform different physiological roles.[6] Such a subclass of isozymes will be called "heterotopes." The structural basis of isozymes has been extensively studied;[6] however, the molecular genetics of the most obvious property of heterotopes, their different location, is not well understood.

An extensively investigated pair of heterotopes are the malate dehydrogenases (EC 1.1.1.37) (MDH), characteristically associated with mitochondria (M-MDH) or the cytosol (C-MDH).[7] Although the latter is called the "cytoplasmic" or "soluble" isozyme (S-MDH), recent studies indicate that S-MDH may associate with another membranous organelle called the "glyoxysome" and participate in the glyoxylate cycle.[8] Thus, these heterotopes have distinct physiological roles which are performed at different subcellular locations.

Previous studies[9-20] of *Neurospora* M-MDH indicated that two unlinked nuclear structural genes, *ma*-1 and *ma*-2, encode two polypeptides of different sequence, α and β, which occur in the tetrameric enzyme as $\alpha_3\beta$.[21] Evidence from physiological, genetic, and biochemical experiments indicated that the specificity of location was intrinsic to the genetically-determined structure of both the enzyme and a protein of the mitochondrial membrane called structural protein. On the basis of these studies, a tentative model of enzyme localization was suggested,[16] in extension of a theoretical proposal of Mitchell[32] that a region (or regions) of the enzyme, called locational specificity site, specifically associates with a receptor-site protein in the membrane.[33] Thus, missense mutations of either membrane or enzyme structural gene could cause malfunction of the enzyme *in vivo* as a result of abnormal association with membrane. Abnormal association could, at extremes, result in two enzyme phenotypes: abnormal conformation or gross subcellular dislocation. Analyses of 10 malate-assimilation mutants, selected at random from 60 independently-isolated mutants, revealed the first class, which we call C-mutants. In this paper, we describe the second class, represented by 14 mutants, which are called K-mutants. The remaining 36 are of the C-type or are intermediate between the two classes.

During these studies, the metabolic regulation and properties of a cytoplasmic enzyme (S-MDH) in *Neurospora* (wild-type) were examined.[39-42] S- and M-MDH of the wild-type differ in kinetic, inhibition, immunological, electrophoretic, and metabolic regulation properties.[39-42] In addition, S- and M-MDH of *Neurospora*, porcine, and bovine heart differ in their association with homologous mitochondrial structural protein, as evidenced by inhibition and fluorescence-polarization titration analyses.[43] Since other experiments indicated structural protein may serve as a possible "receptor" protein of M-MDH,[16,18] S-MDH and the M-MDH of K-mutants appeared to be analogous. Therefore, it was of interest to examine the genetic relations of S- and M-MDH. The results of analyses by electrophoresis and isoelectric focusing of S- and M-MDH of wild-type, and C- and K-mutants, indicate that the two heterotopes have the polypeptides, α and β, in common. A tentative mechanism is discussed that would allow S- and M-MDH to differentially associate with glyoxysomal and mitochondrial membranes.

Materials and Methods. Genetic analysis: Malate-assimilation mutants were isolated from a succinate-requiring prototroph (*suc*-46605-21-6A) after ultraviolet irradiation of conidia.[9] Reciprocal backcrosses of K-type malate mutants were made on corn meal agar.[9] Progeny arising from random ascospores from 1-month old crosses were analyzed as previously described.[9] Complementation tests were performed with various sister lines of backcross progeny by the following method. Uniform conidial concentrations in 0.1 ml water were transferred to 3-inch tubes that contained 1 ml of Fries'

minimal medium, supplemented with 2% sucrose and L-malate (200 μg/ml) and DL-tartrate (20 μg/ml), at 30°C. The tartrate reduces the leakiness of the prototroph. Tubes were scored visually for growth at daily intervals up to 5 days when the prototroph control began to grow on minimal medium. Presumptive heterocaryons were isolated from the growth tubes and tested in comparison with the individual components for linear growth rates as previously described.[9]

Mycelial cultures and fractionation: Conidia (5×10^7) were transferred to 20 ml of Fries' minimal medium, containing 2% sucrose and 3.3 mM L-asparagine, in 125 ml Erlenmeyer flasks, and incubated for 2 days at 27°C in stationary culture. Mitochondria and cytosol were prepared by methods previously described.[39,40] Latent M-MDH was released prior to assay by storing mitochondria overnight at 4°C in phosphate buffer plus 0.1 M KCl. This method was preferred to other methods of disruption in that it leads to the least uncertainty about mutant enzyme stability.

Enzyme assays: *Automatic analysis.* Cytosol and mitochondrial fractions of 60 mutants were assayed in duplicate after various growth and preparatory procedures. A basic Technicon autoanalyzer (Technicon Corp., Ardsley, N. Y.) with colorimeter was used in early experiments at a sample rate of 40/hr. Initially, a colorimetric "GOT" (aspartate aminotransferase, EC 2.6.1.1) plattern was adapted to perform colorimetric analysis of oxaloacetate produced from malate and NAD.

Non-automatic assays of MDH. After MDH activities in cytosol and mitochondrial fractions of the 60 malate-mutants were surveyed by the above automatic methods, 14 K-mutants were selected for additional study. Conventional continuous assays measured NADH oxidation or NAD reduction with a Gilford, model 2000, recording spectrophotometer, as previously described.[39,40]

Electrophoresis, isoelectric focusing, culture, and enzyme induction methods were previously described.[39,40]

Results. Subcellular location of MDH: The distribution of M-MDH activity between the cytosol and mitochondrial fraction of the 60 malate-assimilation mutants and the prototroph is summarized in Fig. 1. The distribution coefficient (K), defined as the ratio of M-MDH to (S-MDH + M-MDH), of the prototroph under the conditions of these experiments is 0.50. The frequency

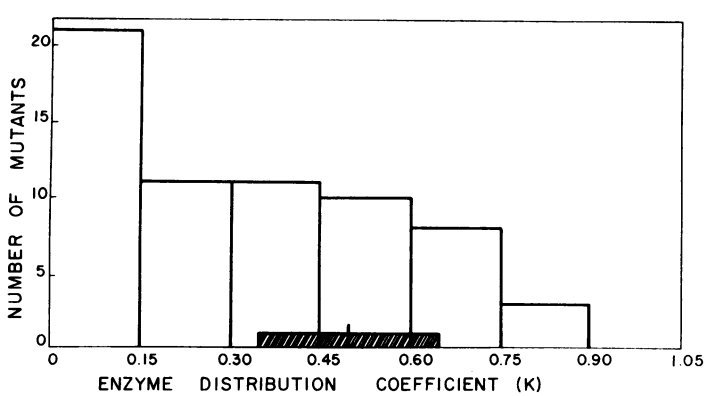

FIG. 1. Distribution of M-MDH activity in cytosolic and mitochondrial fractions of 60 malate assimilation mutants and prototrophic *Neurospora*. The distribution coefficient (K) is defined as the ratio of enzyme activity in the mitochondrial fraction to the sum of activities in cytosolic and mitochondrial fractions. Cross-hatched bar and midpoint indicates the mean and standard deviation of prototroph. K-type mutants are defined as those with K values less than or equal to 0.15.

distribution of mutants about the prototrophic mean is clearly and significantly skewed toward low K values. Fourteen mutants, with a range of K values from 0–0.15, were selected as signficantly different from the prototroph for additional study. These are called K-mutants.

Tables 1 and 2 summarize analyses of enzyme activities. The K values of

TABLE 1. *An analysis of M-MDH activity in K-type malate mutants and a prototroph.*

Enzyme source	Total protein[a]			Sp. act.[b]		Total activity[c]			
	S	M	T	S	M	S	M	T	K[d]
K-mutants[e]	37	9.7	47	570	16	21	0.16	21	0.0076
Prototroph[f]	10	9.7	20	80	65	0.8	0.63	1.4	0.44

[a] mg per g fresh mycelial weight; S, cytosol; M, mitochondrial; T = S + M
[b] International enzyme units per g protein.
[c] International enzyme units per g fresh weight.
[d] K = M/T. K values in this experiment were relatively low due to partial derepression of S-MDH (see text).
[e] Avg. of data from 14 mutants.
[f] Avg. of triplicate cultures of the prototroph, *suc* 46005-21-5A.

the K-mutants were reproducibly and significantly different from the prototroph. The K values are even lower when the cells are partially derepressed for S-MDH.[40]

The increase in cytosolic M-MDH activity in non-derepressed cells is paralleled by a decrease in mitochondrial activity (Table 2) which might reflect a relatively greater susceptibility of K-mutant mitochondria to disruption during isolation; however, that appears unlikely since the average total mitochondrial protein per gram of cells is the same in K- and C-mutants and prototroph (Tables 1 and 2). In addition, the average excess of M-MDH activity in the K-mutant cytosol is six times greater than that of wild-type or C-mutants (Table 2). It

TABLE 2. *Summary of distribution of M-MDH activity in cytosolic and mitochondrial fractions of prototroph and malate mutants.*

Cellular fraction	Total MDH activity*			
	Prototroph (PT)	K-mutants† (KM)	C-mutants‡ (CM)	KM/PT
Cytosolic(S)	0.20	1.2	0.20	6
Mitochondrial(M)	0.22	0.11	0.22	0.5
Total (S + M)	0.42	1.31	0.42	3.1
K = (M/S + M)	0.52 ± 0.15	0.084 ± 0.02	0.52 ± 0.15	

* International units per g fresh mycelial weight; avg. and standard deviation of 3 to 6 experiments.
† Avg. of 14 mutants.
‡ Avg. of 30–40 mutants.

is more likely that this observation reflects an increase in the number of enzyme molecules rather than a mutant enzyme that is intrinsically more active. Hence, the synthesis of M-MDH may be derepressed, perhaps to "saturate" receptor sites in mitochondria, a view that is consistent with the proposals of Mahler *et al.*[48] and Woodward *et al.*[49] concerning "feedback" repression of the synthesis of membrane-bound enzymes.

During the past two years, histochemical methods for the subcellular localization of MDH activity *in situ* by electron microscopy have been developed.[47]

The results of those experiments appear to confirm the results of *in vitro* assays; namely, that M-MDH of K-mutants is scattered throughout the cytoplasm. In addition, the morphology of K-mutant mitochondria *in situ* appears normal; hence, enzyme dislocation is not a consequence of gross mitochondrial disorganization.

Genetic analyses: Analyses of progeny from reciprocal backcrosses of K-mutants to the wild-type indicated that the inheritance is nuclear, with no linkage to the *suc* locus in linkage group I. Identical conclusions were made previously from studies of other alleles of the *ma*-1 and *ma*-2 loci.[9]

To test for functional allelism with the *ma*-1 and *ma*-2 cistrons, complementation tests were performed with previously defined tester mutants: M-20, M-21, and M-24 of the C-class.[9,16] Complementation analyses of C-mutants[9,47] indicate that intercistronic complementation is often not obtained, a result that is attributed to the heterologous polypeptide subunits in the enzyme. Hence, one can only conclude whether an allele is at *either* or *neither* of the two cistrons. The complementation pattern clearly indicated that 7 of the 14 K-mutants are alleles of either one or the other of the two non-linked cistrons. The other seven mutants are "leaky" in that they slowly assimilate malate and are difficult to score unambiguously in these tests.

Electrophoretic and isoelectric properties of MDH: The relative electrophoretic mobilities and isoelectric points of S- and M-MDH, from two heteroallelic C-mutants of each of the two structural genes, after derepression of S-MDH by culture on acetate, are summarized in Table 3. These data indicate

TABLE 3. *Genetic alteration of electrophoretic mobility and isoelectric point of MDH isozymes of C-type mutants of Neurospora.*[a]

Mutant		Relative mobility[b]		Δ isoelectric point[c]		
					M-MDH[d]	
Locus	Allele	S-MDH	M-MDH-1	S-MDH	1	2
ma-1	M-20	+104	+116	−0.37	−0.30	−0.28
	M-46			−0.58	−0.04	−0.05
ma-2	M-24	+105	+125	−0.23	−0.18	−0.10
	M-32			−0.37	−0.23	−0.15

[a] In these experiments, a glucose-repressible isozyme (S-MDH) was derepressed by culture on acetate.[40,41]

[b] Electrophoretic mobility in polyacrylamide gel at pH 7.0 relative to the prototroph at 100. M-MDH-2 (pI = 7.0) does not migrate in prototroph. Standard deviation, ±8%.

[c] In isoelectric focusing polyacrylamide gel (pH 5–8).

[d] M-MDH-1 and 2 are subforms with isoelectric points of 7.50 and 7.05, respectively, in the prototroph. Isoelectric point of prototroph S-MDH is 5.65. Standard deviation of a determination, ±0.016.

that the C-mutant enzymes are more acidic proteins than the prototroph and that mutation at either locus simultaneously alters the net charge of both S- and M-MDH. Previous studies[10] indicated that the amino acid replacement in M-24 is Arg → Trp in the alpha subunit, an observation consistent with the increased acidity.

Analyses of mobilities and isoelectric points of K-mutant MDH are summarized in Table 4. In 9 of 12 mutants, the isozymes are simultaneously altered in charge. Mutants 2, 3, and 8 were not altered in either enzyme. Because

TABLE 4. *Genetic alteration of electrophoretic mobility and isoelectric point of malate dehydrogenases of K-type mutants.**

K-mutant no.	Relative mobility				Δ isoelectric point	
	pH 9.3		pH 7.0		M-MDH	
	S-MDH	M-MDH-1	S-MDH	M-MDH-1	No. 1	No. 2
UW- 1	93	82	93	86	+0.10	+0.05
2	100	100	—	—	—	—
3	100	100	—	—	—	—
4	105	118	—	—	—	—
5	95	81	86	54	+0.16	+0.38
6	91	84	—	—	—	—
7	105	111	—	—	—	—
8	95	100	—	—	—	—
9	94	88	94	97	—	—
10	94	84	69	32	—	—
11	95	86	81	54	+0.17	+0.05
14	—	—	—	—	+0.06	+0.04

* See footnotes, Table 3.

mutants 2 and 3 complement as structural cistrons, the lack of detectable charge alteration may indicate amino acid replacement involving neutral or hydrophobic residues.

The data in Table 4 clarifiy the genetics of the seven leaky mutants that are unclassifiable by complementation. Thus, five of the seven have MDH with altered charge and therefore are probably alleles of the *ma* loci.

Discussion. The present experiments, together with previous analyses of *Neurospora* MDH isozymes, restrict the number of models that could reasonably describe the mechanism of localization. Thus, the K-mutant class of missense mutants demonstrates that the least difference required for localization of MDH can be one amino acid residue. Hence, S- and M- forms in wild-type *Neurospora* could have genetically common polypeptide subunits, as observed, yet differ in at least one amino acid residue. Such a difference could perhaps arise by modification of an amino acid side-chain by the action of other enzymes; phosphorylation of serine or methylation of lysine are possibilities. An apparent precedent for this model is found in the case of *Neurospora* cytochrome *c* which exists in two forms, cytoplasmic and mitochondrial. Complete sequential analysis reveals that they differ only at position No. 72 in lysine and *N*-ε-methyl lysine, respectively.[51] Unfortunately, however, the metabolic significance of the cytoplasmic form is not known. It could perhaps be only a precursor to the mitochondrial form.

In the case of *Neurospora* MDH, there are indications that the cytoplasmic isozyme has a specific function and is not merely a precursor to the mitochondrial form. Recent studies by Kobr *et al.*[8] demonstrate the association of S-MDH with glyoxysomes. In addition, during a five-fold derepression of S-MDH, the level of M-MDH remains relatively constant, suggesting that the amount of M-MDH localized in mitochondria is independent of the pool of S-MDH.[40]

The membrane-binding capabilities of both isozymes redirect attention to the role of membrane proteins in enzyme localization. Previous experiments indicated that purified M-MDH binds mitochondrial structural protein in a 1:1 molar ratio.[17,40] Moreover, a protein of approximately 3000 molecular weight

has been found in a sample of M-MDH purified several hundred times. By an improved polyacrylamide gel electrophoresis (with SDS) method,[50] this protein has been equated with the "miniproteins" that constitute structural protein.[45,50] According to the "miniprotein" hypothesis, there may be six to eight distinct proteins of similar molecular weight which aggregate in varying proportion in different membranes.[45] Thus, the affinity of an enzyme for a specific membrane, e.g. M-MDH for mitochondrial membrane and S-MDH for glyoxysomal membrane, may lie in the relative proportion of each of the "miniproteins" in that membrane.

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Abbreviations: M-MDH, C-MDH, and S-MDH: mitochondrial, cytosolic, and "soluble" (cytoplasmic) malate dehydrogenases.

[1] Roodyn, D. B. (ed.), *Enzyme Cytology* (New York: Academic Press, Inc., 1967).

[2] Dixon, M., and E. C. Webb, *Enzymes* (New York: Academic Press, Inc., 1964).

[3] Goldberger, R. F., and D. E. Green, *Molecular Insights Into the Living Process* (New York: Academic Press, Inc., 1967).

[4] Boardman, N. K., R. M. Smillie, and A. W. Linnane, eds., *Proceedings of an International Symposium On the Autonomy and Biogenesis of Mitochondria and Chloroplasts* (Canberra: Aust. Natl. Acad. Sci., in press).

[5] Wilkie, D. (ed.), *A Symposium on the Autonomy and Biogenesis of Chloroplasts and Mitochondria, Symp. Soc. Exp. Biol.*, **24**, in press.

[6] Weyer, E. M., and E. S. Vesell, "Multiple Molecular Forms of Enzymes, *Ann. N.Y. Acad. Sci.*, **151** 1(1968). Isozymes with distinct physiological roles are also known in procaryotes; however because of the lack of intracellular membranes, differential isozyme localization is generally not observed and apparently is not essential as in eucaryotes.

[7] Kun, E., in *The Enzymes*, ed. Boyer, P. D., H. Lardy, and K. Myrback (New York: Academic Press, Inc., 1963), vol. 7, p. 149. Sund, H., in *Biological Oxidations*, ed. Singer, T. P., (New York: Interscience), p. 461.

[8] Gerhart, B. P., and H. Beevers, *J. Cell Biol.*, **44**, 94 (1970). Kobr, M. J., F. Vanderhaeghe, and G. Combepine, *Biochem. Biophys. Res. Commun.*, **37**, 640 (1969).

[9] Munkres, K. D., N. H. Giles, and M. E. Case, *Arch. Biochem. Biophys.*, **109**, 397 (1965).

[10] Munkres, K. D., and F. M. Richards, *Arch. Biochem. Biophys.*, **109**, 457 (1965).

[11] Munkres, K. D., and F. M. Richards, *Arch. Biochem. Biophys.*, **109**, 465 (1965).

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

[13] Munkres, K. D., *Arch. Biochem. Biophys.*, **112**, 347 (1965).

[14] Munkres, K. D., *Biochemistry*, **4**, 2180 (1965).

[15] Munkres, K. D., *Biochemistry*, **4**, 2186 (1965).

[16] Munkres, K. D., and D. O. Woodward, *Proc. Nat. Acad. Sci. USA*, **55**, 1217 (1966).

[17] Munkres, K. D., and D. O. Woodward, *Biochim. Biophys. Acta*, **133**, 143 (1967).

[18] Munkres, K. D., *Ann. N. Y. Acad. Sci.*, **151**, 294 (1968).

[19] Woodward, D. O., and K. D. Munkres, *Proc. Nat. Acad. Sci. USA*, **55**, 872 (1966).

[20] Woodward, D. O., and K. D. Munkres, in *Organizational Biosynthesis*, ed. Vogel, H. J., J. O. Lampen, and V. Bryson (New York: Academic Press, Inc., 1967), p. 489.

[21] Subsequently, tetrameric forms,[22-24] non-identical subunits,[25-28] and nuclear structural genes[29-31] of MDH from other organisms were described.

[22] Siegel, L., *Biochemistry*, **6**, 2261 (1967).

[23] Shows, T. B., and F. H. Ruddle, *Science*, **160**, 1356 (1968).

[24] Stewart, G. R., and A. D. Sims, *Progress in Phytochemistry* (New York: Interscience, 1967).

[25] Kublick, R. J., and R. W. Barnes, *Biochim. Biophys. Acta*, **167**, 1 (1968).
[26] Zee, D. S., and W. H. Zinkham, *Arch. Biochem. Biophys.*, **126**, 574 (1968).
[27] Mann, K., *Diss. Abstr.*, **28**, 3195 (1968).
[28] Bailey, G. S., G. T. Cocks, and A. C. Wilson, *Biochem. Biophys. Res. Commun.*, **5**, 605 (1969).
[29] Davidson, R. G., and J. A. Cortner, *Nature*, **215**, 761 (1967).
[30] Davidson, R. G., and J. A. Cortner, *Science*, **157**, 1569 (1967).
[31] Longo, G. P., and J. G. Scandalios, *Proc. Nat. Acad. Sci. USA*, **62**, 104 (1969).
[32] Mitchell, P., *Ann. Rev. Microbiol.*, **13**, 407 (1959).
[33] The precise microlocation(s) of MDH, as well as a number of other enzymes, within mitochondria is debatable.[34] Moderating discussions of this problem are given in refs. 35–38.
[34] Korn, E. D., *Ann. Rev. Biochem.*, **38**, 263 (1969).
[35] Smoly, J. M., K. H. Byington, W. C. Tan, and D. E. Green, *Arch. Biochem. Biophys.*, **128**, 762 (1968).
[36] Zahler, W. L., A. Saito, and S. Fleischer, *Biochem. Biophys. Res. Commun.*, **32**, 512 (1968).
[37] Rendon, A., and A. Waksman, *Biochem. Biophys. Res. Commun.*, **35**, 224 (1969).
[38] McKay, R., R. Drugan, G. S. Getiz, and M. Rabinowitz, *Biochem. J.*, **114**, 455 (1969).
[39] Benveniste, K., and K. D. Munkres, *Biochim. Biophys. Acta*, in press.
[40] Munkres, K. D , and K. Benveniste, *Biochim. Biophys. Acta*, in press.
[41] Munkres, K. D., *Biochim. Biophys. Acta*, in press.
[42] Previous studies of M-MDH were with cells cultured in sucrose medium which repressed S-MDH synthesis.[39,40]
[43] A functional rather than chemical definition of structural protein is that protein or class of proteins which differentially associates with and inhibits either wild-type S- and M-MDH or mutant forms of M-MDH.[16,40] Thus, the "allotopy" hypothesis[44] is extended with genetic and physiological considerations. Additional chemical studies of oligopeptides from *Neurospora* and bovine structural protein are reported elsewhere.[45,46]
[44] Racker, E., and A. Bruni, in *Membrane Models*, ed. Bolis, L., and B. A. Pethica (Amsterdam: North Holland Publ. Co., 1968), p. 138.
[45] Munkres, K. D., R. T. Swank, and G. I. Sheir, in *Proceedings of an International Symposium on the Autonomy and Biogenesis of Chloroplasts and Mitochondria* (Canberra: Aust. Natl. Acad. Sci., in press).
[46] Swank, R. T., and K. D. Munkres, *Anal. Biochem.*, in press.
[47] Munkres, K. D., and C. Zuiches, unpublished observations.
[48] Henson, C., P. Perlman, C. Weber, and H. R. Mahler, *Biochemistry*, **7**, 4445 (1968).
[49] Woodward, D. O., D. L. Edwards, and R. B. Flavell, in *Proc. Soc. Exp. Biol.*, **24**, London, in press.
[50] Swank, R. T., and K. D. Munkres, *Anal. Biochem.*, in press.
[51] Scott, W. A., and H. K. Mitchell, *Biochemistry*, **10**, 4282 (1969).