Short Communication

PARALLEL INDUCTION OF A CYTOSOLIC MALATE-DEHYDROGENASE ISOZYME (c-MDH₂) AND CONIDIATION IN *NEUROSPORA CRASSA*

GILBERT TURIAN, DONATELLA BERNARDINI, AND RAFFAELE PEDUZZI

*Laboratoire de Microbiologie générale, Département de Biologie végétale,
University of Geneva, CH-1211 Geneva 4, Switzerland*

(Received August 4, 1986)

The differentiation of macroconidia on the tips of aerial hyphae implies a metabolic switch from aerobic glycolysis to gluconeogenesis (1), a conclusion originally based on the finding that the growth of *N. crassa* on acetate as the single source of carbon leads to premature conidiation (2). That the glyoxylate cycle induced under such conditions (3) was not obligatory for that conidiogenous switch was shown by the fact that the non-acetate-using (acetate-useless or acu-) mutants of FLAWELL (4) were still able to conidiate on sugar media by opening the gluconeogenic pathway directly from the Krebs cycle through an immunochemically detected (5) malate-dehydrogenase (MDH). This was presumed to be the required soluble isozyme because of the positioning of its MDH-positive arc similar to that obtained from acetate-grown, conidiating cultures (6). Such a cytosolic isozyme (c-MDH) should therefore be the soluble, derepressible counterpart of the constitutive mitochondrial isozyme (m-MDH) with which it forms a heterotope pair (7). We now present evidence confirming our immunochemical postulate by relating the MDH-positive precipitin arcs to isozymic MDH proteins separated from a known cellular compartment of the alternative cytosol (c) versus mitochondria (m). This enables us to demonstrate that it is indeed the cytosolic component of the MDH pair which is induced in parallel with conidiation.

We have found that from lysed mitochondria of both the sterily developing "amyc" (sucrose medium) and young vegetatively growing "acu-3" mutant, only the 1-MDH band could be stained with the tetrazolium technique (8) at an average *R_f* of 49.5 on columns of polyacrylamide gel; such an m-MDH isozyme should then correspond to the MDH₁ arc shown to be absent from immunochemical electrophoretograms produced with supernatant extracts (Fig. 1b, f) but visualized as a

Address reprint requests to: Prof. G. Turian, Laboratoire de Microbiologie générale, Département de Biologie végétale, University of Geneva, CH-1211 Geneva 4, Switzerland.

Fig. 1. Immuno-electrophoretic patterns of precipitin arcs stained with thiazine red T (a-b) and tetrazolium (c-e) to locate arcs enzymatically positive for MDH activities^a.

a, Proteins of total extracts of conidiating (4 days) wild type *N. crassa*; MDH_{1,2,3} arrowed in correspondance with the enzymatically detected arcs in e). b, Proteins of the supernatant of same cultures after removal of mitochondria; absence (*) of the arc MDH₁. c, One-arc pattern visualized from extracts of aconidial, sucrose-grown cultures of 6 days for the "amycelial" strain and 3 days for the "acu-3": it is similar in position to the arc MDH₁ absent in the supernatant b). d, Two-arcs pattern visualized from the total extract of the "amycelial" strain induced to conidiate (6 days) on the acetate medium or the "acu-3" starting to conidiate after 6 days on sucrose medium; this induced 2nd arc corresponds to the cathodic MDH₂ also arrowed on the wild type pattern e). e, The three MDH-positive arcs (MDH_{1,2,3}) from a total extract of conidiating (4 days) wild type *N. crassa*. Polyacrylamide-gel electrophoretic patterns of protein bands (top and front of migration arrowed) positive for MDH activity. f, Single MDH-positive band on the column of polyacrylamide gel (at *Rf* 49.5) identified as m-MDH, corresponding to the arc MDH₁ by its *Rf* which is the same as the *Rf* obtained with electrophorezed proteins isolated from

single arc (Fig. 1c) on the immunoelectrophoretic slides prepared from total extracts of either non-conidiating sucrose-grown "amycelial" or "acu-3" mutants. Such an m-MDH band was accompanied by a 2nd band, at an average *Rf* of 46, in total extracts of both an "amycelial" mutant induced to conidiate on the acetate medium (Fig. 1g) and older conidiating cultures of "acu-3"; that 2nd MDH band was revealed even with supernatant extracts deprived of their mitochondria and would therefore be the soluble or c-MDH isozyme corresponding to the MDH₂ cathodic arc immunochemically detected in extracts of the conidiating wild type (Fig. 1a) and induced in parallel with conidiation in acetate-grown "amyc" cultures (Fig. 1d).

The electrophoretic immunochemical pattern of wild type extracts showed a 3rd anodic arc positive to MDH staining (Fig. 1a, e) and described as MDH₃ (6); this is in conformity with the 3-band pattern previously observed in extracts of 4-days old wild type cultures of *N. crassa* (9). We noticed its presence as a 2nd soluble MDH band at *Rf* 42 on electrophoretic gels of supernatant extracts of wild type (data not shown). It was the only MDH arc obtained from the stringently aconidial

mitochondria. g. Two-bands pattern obtained from extracts of conidiating cultures; the second, inducible c-MDH corresponding to the MDH₂ arc appears as a cathodic band (*Rf* 46) on the PAGE-column.

^a *Neurospora crassa* wild type Lindegren (FGSC 354 A) and the mutants amycelial "amyc" (FGSC 350 A) and acetate non-utilizer "acu-3" (FGSC 1732 A) were grown at 25°C in WESTERGAARD and MITCHELL (15) liquid media with sucrose 2% or Na-acetate 2% as C sources. Mycelia were homogenized at 4°C with quartz sand in Sørensen phosphate buffer (pH 7.2) and 0.018 M NaCl before centrifugation 10 min at 10,000 × *g* to obtain total extracts—soluble proteins of both cytosol and leaked mitochondria—used as antigens or for electrophoretic analyses. To separate mitochondria from the supernatant—cytosolic proteins—mycelia were homogenized in the same phosphate buffer but enriched with sucrose (0.2 M) only. The homogenate was centrifuged 10 min at 1,500 × *g* to remove hyphal debris before two centrifugations of 15 min each at 17,000 and, after resuspension 15,000 × *g*. The supernatants were combined (cytosolic fraction) and the mitochondria resuspended in phosphate buffer in which they were lysed 3 hr by Triton X-100 (1% final) to liberate their proteins. Immunoelectrophoresis according to an adapted technique (6) of GRABAR and WILLIAMS (16) was made on agarized slides with punched central wells receiving the antigens described in a–e and side wells filled with antisera against wild type extracts produced in rabbits (6). All precipitin arcs of the total extracts and the supernatant were visualized by thiazine red T staining and the MDH isozymes by the standard tetrazolium system (L-malate-MDH-phenazine metho-sulfate and NBT). Polyacrylamide gel vertical electrophoresis according to ORNSTEIN (17) and DAVIS (18) was performed on the isozymes by use of 7% acrylamide separation gels and a buffer pH 9.0. The gels were stained for MDH activity by the tetrazolium technique outlined by Canalco. All gels were measured and the *Rf* values calculated by dividing the distance the enzyme traveled from the top of the gel (cathode) by the distance the tracking dye (bromophenol blue) traveled.

Controls of the efficiency of our separations have been made on fractionated extracts of the 4th-day cultures which had been resolved into a 3-band pattern by polyacrylamide electrophoresis (9). Densitometric measurements of total soluble extracts at 465 μm on a Gilford (2410S) spectrophotometer have confirmed the pattern and showed that 2 more cathodic bands remained in the cytosolic fraction (MDH₂–MDH₃) while isolated mitochondria contained the single anodic band (MDH₁).

mutant "fluffy", not only when grown on sucrose but also on acetate (6) in which it would necessarily, as soluble MDH, "open" gluconeogenesis. In contrast, as shown in the conditionally aconidial "amyc" and in the "acu-3" mutant, c-MDH₂, being the only derepressible, soluble partner of the heterotope pair, would have to act not only gluconeogenetically but as the developmentally-regulated isozyme associated with the induction of *Neurospora* conidiation.

The functional significance of the c-MDH, isozyme could derive from the known fact (10, 11) that cytosolic and mitochondrial isozymes of malate dehydrogenase (L-malate: NAD oxidoreductase, EC 1.1.1.37.) and aspartate-glutamate transaminase (aspartate amino-transferase, L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1.) cyclically cooperate in a malate-aspartate shuttle transferring reducing equivalents to extramitochondrial NAD⁺ (NADH itself cannot pass into the cytosol directly); the reducing power of NADH would then be implicated in the gluconeogenic synthesis of glucose from oxaloacetate produced by the activities of both c-MDH₂ and MDH₃ from the mitochondrially extruded malate. It should then be expected that, through the malate-aspartate shuttle, a part of the reducing power of cytosolic malate would be driven ultimately by c-MDH₂ to glutamate, the crucial metabolite having to be, teleologically, accumulated in the macroconidia as a source of reducing power necessary to insure their further germination (12). This proposal would explain the need and role of the c-MDH₂ heterotopic isozyme for macroconidiogenesis—developmentally derepressed in the wild type (7) and acetate-induced in the conditionally aconidial "amycelial" mutant—while its absence in the sterile "fluffy" mutant would be correlated with its stringent a (macro)conidiation. However, as conidiogenetically-regulated enzymatic articulation, the privileged interaction between the cytosolic MDH₂ and aspartate transferase will need another explanation, possibly based on close cytology, to replace that disproved (13, 14).

REFERENCES

- 1) G. TURIAN and D. E. BIANCHI, *Bot. Rev.*, **38**, 119 (1972).
- 2) G. TURIAN, *C. R. Acad. Sci.*, **252**, 1374 (1961).
- 3) G. TURIAN, *Pathol. Microbiol.*, **24**, 819 (1961).
- 4) R. B. FLAWELL and J. S. R. FINCHAM, *J. Bacteriol.*, **95**, 1063 (1968).
- 5) D. RIATSCH, R. PEDUZZI, and G. TURIAN, *Pathol. Microbiol.*, **40**, 235 (1974).
- 6) R. PEDUZZI and G. TURIAN, *Ann. Inst. Pasteur Lille*, **122**, 1081 (1972).
- 7) K. D. MUNKRES, K. BENVENISTE, J. GORSKI, and C. A. ZUICHES, *Proc. Natl. Acad. Sci. U.S.A.*, **67**, 263 (1970).
- 8) J. URIEL, *Ann. N. Y. Acad. Sci.*, **103**, 936 (1963).
- 9) G. COMBÉPINE and G. TURIAN, *Arch. Microbiol.*, **72**, 36 (1970).
- 10) K. BENVENISTE and K. D. MUNKRES, *Biochem. Biophys. Acta*, **220**, 161 (1970).
- 11) A. L. LEHNINGER, *Biochemistry*, 2nd ed., Worth Publ. Inc. New York (1975).
- 12) S. BRODY, *In The Fungal Spore: morphogenetic controls*, ed. by G. TURIAN and H. R. HOHL, Academic Press, New York and London, (1981), p. 605.
- 13) G. B. KITTO, M. E. KOTTKE, L. H. BERTLAND, W. H. MURPHY, and N. O. KAPLAN, *Arch. Biochem. Biophys.*, **121**, 224 (1967).

- 14) K. D. MUNKRES, *Ann. N. Y. Acad. Sci.*, **151**, 294 (1968).
- 15) M. WESTERGAARD and H. K. MITCHELL, *Am. J. Bot.*, **34**, 573 (1947).
- 16) P. GRABAR and C. A. WILLIAMS, *Biochim. Biophys. Acta*, **10**, 193 (1953).
- 17) L. ORNSTEIN, *Ann. N. Y. Acad. Sci.*, **121**, 321 (1964).
- 18) B. J. DAVIS, *Ann. N. Y. Acad. Sci.*, **121**, 404 (1964).