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Protein synthesis during growth in Neurospora crassa

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Abstract

Protein synthesis during growth

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Fungal spores have a low metabolic activity compared to mature hyphae and this physiological characteristic separates, in part, the spore phase from the actively growing phase. Spores contain all the metabolic machinery for germination and once they are incubated in a suitable medium they start germinating and form a new differentiated thallus. We earlier reported (Bhagwat and Mahadevan 1973 J. Bacteriol. 113: 572) differential gene activity in Neurospora. In order to substantiate these findings, the pattern of protein synthesis was also studied during similar growth periods. The data presented in this report show that the rates at which certain proteins are synthesized during the different growth stages are different.

Neurospora crassa RL3-8 A (Rockefeller wild type) was grown in 100 ml of 2% sucrose Vogels minimal medium in 250 ml flasks kept well aerated in a rotary shaker. At 8, 16 and 24 hrs of growth cultures were labelled with 0.2 u ci/ml ^{14}C-leucine (Sp. Act. 45.6 mCi/m mole); after 5 min of labelling, the cultures were filtered through a layer of "Mira cloth" (Chicopee Mills N.Y.), to separate ungerminated and germinated conidia. The resulting mycelial pad was washed with distilled water, and disrupted by manual grinding with an equal weight of acid-washed sand. After grinding, an equal amount (w/v) of 0.1 M sodium phosphate (pH 7.0) buffer was added and the homogenate was centrifuged at 16,000 rev/min for 30 minutes in the SS34 rotor of a Sorval RC-2B centrifuge. The supernatant was dialyzed for 24 hrs against three changes of 0.1 M sodium phosphate buffer (pH 7.0). Acrylamide gel electrophoresis was done according to the method of Davis (1964, N.Y. Acad. Sci. 121: 404) using 7% standard gels. Enzyme staining was done as follows: glutamic dehydrogenases (Sanwal and Lata 1964 B.B.R.C. 6: 404), malic dehydrogenase (Shaw and Koen 1969 J. Histochem. and Cytochem. 13: 451), isocitrate dehydrogenase (Henderson 1965 J. Expt. Food Sci. 158: 263), alkaline phosphatase (Hudson et al. 1962 Clinica Chemica Acta. 1: 255). To quantitate the amount of enzyme activity present in each band, densitometric tracings were recorded using a Joyce-Loebl chromoscan; the integrated readings which represent total area of each enzyme peak is represented numerically. Radioactivity in each band was counted according to the method of Tishler et al. (1968 Anal. Biochem. 22: 89). Specific activity was calculated as counts per minute per integral number.

Table 1

Changes in the enzyme synthesis pattern at various growth periods

	Growth period in hours		
	8	16	24
Enzyme	Counts per minute in the band / Densitometric reading		
Isocitrate dehydrogenase	464/81	510/83	430/87
NAD-Glutamate dehydrogenase	1700/81	1148/180	1683/86
NADP-Glutamate dehydrogenase	1516/401	808/646	1800/808
Alkaline phosphatase	2880/265	1300/266	1401/241
Malate dehydrogenase			
I	2580/10	4370/15	2590/10
II	2990/440	4350/280	4730/370
III	2790/120	4690/60	3420/98
IV	4620/20	4150/25	4290/24

In each case the amount of protein loaded onto the gel was 50 µg.

The separation of total soluble proteins labelled with ^{14}C-leucine showed that there was differential incorporation of radioactive label into proteins and some labelled bands appeared only at the later stages of growth. It can be seen from the data presented in Table 1 that the specific activity of isocitrate dehydrogenase was the same at the three stages of growth. In contrast, NADP-glutamic dehydrogenase showed a high ^{14}C-leucine incorporation per unit of enzyme activity at the 8 hr growth period (Table 1). This perhaps means that the enzyme was synthesized at a faster rate at 8 hr. At 16 hr, the total enzyme activity level was higher as compared to 8 hr, but the radioactive incorporation was considerably lower. This suggests that there was little synthesis of the enzyme at this stage. NAD-dependent glutamic dehydrogenase followed the same pattern as NADP-GDH. Alkaline phosphatase showed a single band at 8 and 16 hrs growth periods but showed an additional band at 24 hrs. The ^{14}C-leucine incorporation pattern was identical with that of NADP-GDH described earlier. Malate dehydrogenase showed four isoenzymes and interestingly in 3 of 4 isoenzymes radioactive incorporation was much higher at the 16 hr growth period as compared to 8 hr. The total amount of enzyme activity found at 16 hrs was lower than at 8 hr.

These observations suggest that there are differential rates of synthesis of proteins during Neurospora growth. It may be argued that the total leucine pool may be different at 16 and 24 hrs of growth, resulting in the decreased incorporation of the labelled leucine; however, this possibility cannot explain the differential synthesis observed since in the case of isocitrate dehydrogenase and malate dehydrogenase one does not observe the same pattern as found with the other enzymes. Although the possibility exists that the radioactivity associated with each of the enzyme bands could also come from another protein moving at the same rate, the conclusion that at different stages of growth there is differential rate of synthesis of different proteins is still valid. - - - Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay 400 085, India.