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Abstract

Effect of various inhibitors on the production of myo-inositol-1-phosphate synthase in Neurospora crassa wild-type strain

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Effect of various inhibitors on the production of myo-inositol-l-phosphate synthase in Neurospora crassa wild type strain.

The synthesis of myo-inositol-l-phosphate synthase (MIPS, E.C.5.5.1.4.) in wild-type Neurospora crassa strains is almost completely repressed by inositol at a concentration of 50 µg/ml (Zsindely et al., 1983 Biochim. Biophys. Acta 741:273).

We studied whether the enzyme was derepressed after removing inositol from the medium. Wild-type Neurospora crassa strain RL-3-8A was grown at 27° C for 22 h in Vogel's culture medium containing 50 µg/ml inositol. Following harvest, the mycelium was washed, suspended in Vogel's minimal medium and growth was tinued for 22 h during which samples were taken at various times. Enzyme activity amount of enzyme protein were determined in the 100,000 g supernatant after disintegration of the mycelium (Table I). Enzyme 'activity was determined according to Barnett et al. (1970, Biochem. J. 119: 183), as described earlier (Zsindely et al., 1977, Acta Biol. Acad. Sci. Hung. 28:281). One unit of activity is 1 nmol Pi released during 1 h incubation. The amount of protein reacting with monovalent immune sera produced against highly purified enzyme was determined by rocket immunoelectrophoresis according to Laurel.1 (1966, Anal. Biochem. 15: 45) in a 1% agrose gel containing 1% immune serum.

Table I shows that MIPS becomes derepressed after removing inositol from the culture medium. Four h later the enzyme activity and the antigen content become similar to those measured in the crude extracts of the wild-type strain cultivated without inositol. No further change in enzyme activity or antigen content was observed up to 22h of cultivation.

TABLE I

Derepression of the effect of inositol upon the synthesis of MIPS in wild-type N. crassa

Growth* ainiral medium (h)	Enzyme activity U/mg protein	Antigen content µg/mg protein	"Specific activity' U/µg antigen
0	16.3	8.7	1.9
0.5	18.4	7.1	2.6
1.0	21.5	9.2	2.3
2.0	33.5	12.8	2.6
4.0	75.5	29.3	2.6
8.0	77.1	31.3	2.5
22.0	72.4	27.8	2.6

*Cultures of strain RL-3-8A were pre-grown in Vogel's medium containing 50 µg inositol/ml, at 27° C for 22 h.

TABLE II

Effect of various inhibitors upon the synthesis of MIPS in wild-type Neurospora crassa

	Enzyme activity u/mg protein	Antigen content µg/mg protein	"Specific activity" U/µg antigen
control° at 0 h	16.3	8.7	1.9
controI*	91.5	35.2	2.6
+ cycloheximide* 2.5 µg/ml	5.0	13.6	0.4
+ edein* 50 µg/ml	23.5	25.3	0.9

°: strain RL-3-8A was grown at 27°C for 22 h in Vogel's medium containing 50 µg/ml inositol
*: the cultures were further grown at 27°C for 5 h in Vogel's medium with and without inhibitors, as shown

We also examined how the MIPS derepression was affected by inhibitors of translation. Cycloheximide and edein were used as inhibitors of translation. The wild-type strain was cultured at 27°C for 22 h with 50 µg/ml inositol and then the growth was continued for 5 h in Vogel's minimal medium without inositol with the addition of the inhibitor. The cultures were then harvested' and enzyme activity and enzyme protein content were measured (Table II).

It was found that enzyme production was significantly diminished in the presence of cycloheximide and edein. Actinomycin D and proflavin, as potential inhibitors of transcription, in concentrations (10, µg/ml) that completely inhibited the growth of our strain did not decrease enzyme synthesis.

We conclude that: a> enzyme production is derepressed when inositol is washed from the culture medium; and b) MIPS production is regulated at the posttranscriptional level. The same type of regulation was observed in MIPS production of yeast (S. Henry et al., 1984' 12th International Conference on Yeast Genetics and Molecular Biology, Edinburgh).

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