Fungal Genetics Reports

Volume 11 Article 18

RNA and protein synthesis in Neurospora crassa conidia

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Abstract

RNA and protein synthesis in conidia

Hitchcock, S. E. RNA and protein synthesis in Neurospora crassa conidio.

In a study of the germination of conidio of wild type strain Em 5297a (ATCC# 10816), the synthetic capacities of conidia incubated in minimal medium with and without a carbon source were investigated. Conidio were grown on Vogel's medium N + 1% glucose and 2% agar. Conidio were harvested at 7 days, washed, and incubated in Vogel's liquid medium N with (called "germinating") or without (called "non-germinating") 2 % glucose at a spore concentration of 10 mg FW/ml.

Figure 1 shows RNA synthesis, as measured by umcil-2-C^{14} incorporation into hot alcohol-extracted, cold TCA-insoluble material, under these two conditions. There is no difference until 30 minutes, when there is a marked stimulation of incorporation into the conidio incubated in glucose-supplemented medium. This is about the time germ tube formation begins. The difference is nearly fifty-fold after 3 hours and represents a large increase in specific activity as well as in total activity, which is shown here. This stimulation is inhibited by cycloheximide (100 µg/ml).

Protein synthesis, as measured by phenylolonins-U-C^{14} incorporation into hot alcohol-extracted, cold TCA-insoluble material, seems to be different from RNA synthesis (Figure 2). While the label incorporated into conidio incubated in supplemented medium takes on immediote lead over those incubated in mineral salts only, the difference is only two-fold after 5 hours. Since the protein in "germinating" conidia has about doubled during this time, there is little, if any, increase in specific activity.

Fractionation of cells into hot alcohol-soluble fractions (containing free amino acids, nucleotides, neutral compounds, and organic acids), RNA, and protein fractions gave interesting results which influence the interpretation of Figures 1 and 2.

Table 1. Incorporation of uracil and phenylalanine.

Glucose (%)		Uracil 0	Uracil 2		Phenylalanine 0	Phenylalanine 2
	t (hr)			t hr)		
Alcohol fractions cpm x 10^{-3}	1	30.8	48.5	2	888.4	238.2
	2	49.8	313.4	4	877.7	251.8
% of cell activity*	1	77.1	81.9	2	91.7	40.8
	2	67.0	56.4	4	74.2	28.6
RNA (protein)** cpm x 10^{-3}	1	9.1	24.5	2	70.8	338.9
	2	10.7	214.3	4	292.9	616.2
% of cell activity	1	22.8	32.9	2	7.3	58. 1
	2	18.0	43.5	4	24.8	70.1

Flasks contained 1 µc uracil-2-C^{14}, 2.100 x 10^6 cpm, final conc. 0.261 µM; 1 µc L-phenylalanine-U-C^{14}, 2.160 x 10^6 cpm, final conc. 0.77 µM (10 ml). * Cell activity = total counts recovered in cell fractionation. Supernatant and washings not included. ** RNA = uracil-2-C^{14} incorporated into hot TCA-soluble material; protein = phenylalanine-U-C^{14} incorporated into hot NaOH-soluble material.

Figure 1. Incorporation of uracil-2-C^{14} 5 µc, 0.075 µmolar, 10 ml) into RNA in "germinating" (G) and "non-germinating' (U) conidia. SG = spore germination.

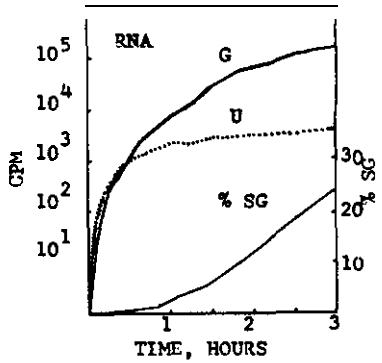

Figure 2. Incorporation of phenylalanine-U-C^{14} (1 µc, 1.27 µmolar, 10 m!) into protein. Symbols some as for Figure 1.

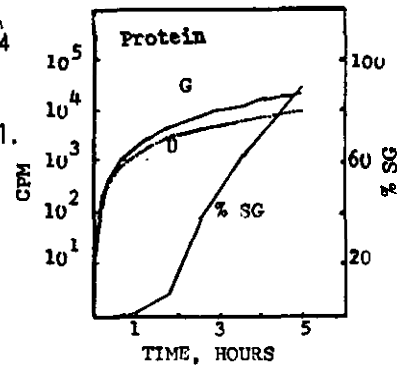

Table 1 shows that the large difference in uracil incorporated into RNA shown in Figure 1 reflects a difference in the abilities of the cells to take umcil into the cell, rather than gross differences in synthetic ability. In 2 hours there is a 6-fold difference in uptake of uracil by conidio incubated in minimal plus glucose, while there is only a slightly increased uptake by the conidio incubated in minimal salts only. In the "non-germinating" conidio, much of the uracil token up is incorporated, indicating that there cells ore capable of RNA synthesis. On the other hand, phenylalanine is token up more readily by the "non-germinating" conidia than by the "germinating" conidio. A considerable fraction of this is incorporated into protein (24.8 % at 4 hours), but not as much as in the "germinating" conidia (70.1% at 4 hours).

There results show that conidio incubated either in mineral salts or in mineral salts plus glucose ore capable of RNA and protein synthesis and stress the importance of looking at uptake of the precursor as well as its incorporation into RNA or protein.

This research was supported by National Science Foundation Grant No. GB 1401. - - - Deportment of Biology, Wesleyan University, Middletown, Connecticut 06457.