RNA and protein synthesis in Neurospora crassa conidia

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RNA and protein synthesis in Neurospora crassa conidia

Abstract
RNA and protein synthesis in conidia
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 investi-gated. 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-C14 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 phenylalanine-U-C14 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 immediate 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.

<table>
<thead>
<tr>
<th>Glucose (%)</th>
<th>Uracil</th>
<th>Phenylalanine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t (hr)</td>
<td>t (hr)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>cpm x 10^-3</td>
<td>1</td>
<td>30.8 48.5 2 888.4 238.2</td>
</tr>
<tr>
<td>% of cell activity*</td>
<td>1</td>
<td>77.1 81.9 2 91.7 40.8</td>
</tr>
<tr>
<td>RNA (protein)**</td>
<td>1</td>
<td>9.1 24.5 2 70.8 338.9</td>
</tr>
<tr>
<td>cpm x 10^-3</td>
<td>2</td>
<td>10.7 214.3 4 292.9 616.2</td>
</tr>
<tr>
<td>% of cell activity</td>
<td>1</td>
<td>22.8 32.9 2 7.3 58.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>18.0 43.5 4 24.8 70.1</td>
</tr>
</tbody>
</table>

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 mineral plus glucose, while there is only a slightly increased uptake by the conidio 
incubated in mineral salts only. In the "non-germinating" conidio, much of the uracil taken up is incorporated, indicating that 
there cells are capable of RNA synthesis. On the other hand, phenylalanine is taken up more readily by the "non-germinating" conidio 
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" conidio (70.1% at 4 hours).

There results show that conidio incubated either in mineral salts or in mineral salts plus glucose are 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.

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Flasks contained 1 μg uracil-2-C14, 2.100 x 10^6 cpm, final conc. 
0.261 μM; 1 μg L-phenylalanine-U-C14, 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-C14 incorporated into hot TCA-soluble material; protein = phenylalanine-U-C14 incorporated into hot NaOH-
soluble material.

Figure 1. Incorporation of uracil-2-C14 (5 μc, 
0.075 μmolar, 10 ml) into RNA in "germinating" (G) and "non-
germinating" (U) conidio. SG = spore germination.

Figure 2. Incorporation of phenylalanine-U-C14 
(1 μc, 1.27 μmolar, 10 ml) into protein. Symbols some as for Figure 1.