RNA content and growth rate

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Abstract
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This work is licensed under a Creative Commons Attribution-Share Alike 4.0 License.
Blasticidin-S, on inhibitor of protein synthesis in Neurospora.

Cycloheximide has been used widely as an inhibitor of protein synthesis in Neurospora. It is difficult to eliminate the possibility that results obtained with cycloheximide or other antibiotics may be due to secondary effects independent of the inhibition of protein synthesis, which effectively inhibit protein synthesis in Neurospora.

This note concerns a study of the antibiotic, blasticidin-S, on two-day-old mycelial pads of wild type strain ST74A, grown as described previously (Poll 1970 Biochim. Biophys. Acta 203:139). Pads were shaken with blasticidin-S for various periods of time before being shaken with 1 µCi L-3H lysine for 2 minutes. The mycelial pads were then washed, extracted with 5% TCA and the uptake and incorporation into protein (hot TCA insoluble, NaOH soluble fraction) were measured. As shown in Table 1, even a half-minute preincubation with blasticidin-S gives significant inhibition of incorporation into protein. A ten minute preincubation with 50 µg/ml blasticidin-S monohydrochloride gives almost complete inhibition of incorporation. Thus blasticidin-S is rapidly effective in inhibiting lysine incorporation into protein. Cycloheximides inhibits incorporation even more rapidly than do blasticidin-S, a half-minute preincubation with 10 µg/ml cycloheximide inhibiting incorporation by 98%.

Blasticidin-S, under the above condition, shows little effect (<20%) on lysine uptake. The amino acid pool, as measured by cold TCA extractable ninhydrin positive material, shows little (0-30%) increase in the presence of blasticidin-S. Consequently the inhibition of incorporation into protein would be expected to be a good measure of the inhibition of protein synthesis. Other experiments showed that a 10-minute preincubation of pads with 50 µg/ml blasticidin-S monohydrochloride had little or no (0%) effect on the rate of uridine uptake or its incorporation into nucleic acid. The results support the conclusion that blasticidin-S is a rapid, relatively specific inhibitor of protein synthesis in Neurospora.

The blasticidin-S monohydrochloride was manufactured by the Kaken Chemical Co., Ltd., Japan and was a gift of Marubeni-Lida (America), Inc., San Francisco. The author would be happy to supply samples of blasticidin-S monohydrochloride to interested investigators.

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Albghina, F. A. M. and E. Sturani, R N A content and growth rate in Neurospora crassa mycelium.

As shown in Table 1, cultures with quite different growth rates, the medium or the incubation temperature. At any fixed temperature, the RNA content is greater for the faster growing mycelia. A linear relationship may be found between the log of the RNA content and the rate of growth. When the rate of growth is enhanced by increasing the temperature, the RNA content is not affected, or may even slightly decrease.

Table 1. RNA content of N. crassa mycelium in exponential phase of growth.

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>Temperature</th>
<th>Growth rate constant</th>
<th>RNA content</th>
</tr>
</thead>
<tbody>
<tr>
<td>complete + sucrose</td>
<td>25°C</td>
<td>0.32 hr⁻¹</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.38</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>0.44</td>
<td>112</td>
</tr>
<tr>
<td>minimal + sucrose</td>
<td>25</td>
<td>0.27</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.32</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>0.35</td>
<td>98</td>
</tr>
<tr>
<td>minimal + glycerol</td>
<td>25</td>
<td>0.16</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.19</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>0.20</td>
<td>54</td>
</tr>
</tbody>
</table>

Experimental conditions: minimal = Vogel's minimal medium; complete = Vogel's minimal supplemented with 1 g casein hydrolysate (not vitamin-free), 10 mg yeast RNA, 5 mg inositol, 15 mg DL-tryptophan, 15 mg L-asparagin / 100 ml medium. Final conc. of carbon source = 2% (w/v), 200 ml medium / 750 ml flask. Inoculum was 10⁶/ml 7 day old conidio of wild type ST74A, Flasks were shaken in a Dubnoff water bath at 100 rpm. Growth rate constant was determined according to Boag and Hopton (1969 J. Bacteriol. 100:552). RNA content was determined on lyophilized mycelia according to Lurk, Williams and Kennedy (1968 J. Biol. Chem. 243:2618). Data are averages of three independent determinations.
Synaptonemal complexes have been identified in nuclei of *Neurospora*. Synaptonemal complexes are distinctively contrasted. The synaptonemal complex is absent from nuclei which, according to ascus size, should be at early pachytene (Barry 1969 Chromosoma 26: 119).

Unlike in *Neottiella*, the chromatin of the pachytene bivalents of *N. crassa* is poorly contrasted and difficult to distinguish from the nucleoplasm in electron micrographs. However, the components of the synaptonemal complex are distinctly contrasted. The synaptonemal complex is absent from nuclei which, according to ascus size, should be at early pachytene (Barry 1969 Chromosoma 26: 119).

The synaptonemal complex in *N. crassa* consists of two bound lateral components (co. 400 Å in diameter) which are held about 1200 Å apart by a central region containing the ca. 200 Å thick central component. These components seem to contain alternating thin and thick bands with a center spacing of about 170 Å. Thus they are similar to *Neottiella* and other ascomycetes (Westergaard and von Wettstein 1970 Rev. Cytol. et Biol. veg. 33: 1). Occasional local thickenings of the central component into electron dense nodes ca. 500 Å in section are characteristic for the synaptonemal complex of *N. crassa*. These nodes partly fill the space in the central components of the dense granules described by Schrantz (loc. cit.) in the central components of *Postularia cupularis* and *Galactinia plebia*. Institute of Genetics, University of Copenhagen, Øster Farimagsgade 2A, DK-1353, Copenhagen K., Denmark.