Factor(s) in the culture medium of a slime strain which stimulate DNA uptake.

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Factor(s) in the culture medium of a slime strain which stimulate DNA uptake.

Abstract
Factor(s) in the culture medium of a slime strain which stimulate DNA uptake.

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When extracts of celllobiose-grown myelia were assayed at pH 6, both \(\beta\)-galactosidase and \(\beta\)-glucosidase activities were detected. The extracts were then subjected to AS fractionation. Very little of the enzyme activities (1% of the \(\beta\)-galactosidase and 4% of the \(\beta\)-glucosidase present in the crude extract) was precipitated by 0.50% AS; whereas, much more (75% of the \(\beta\)-galactosidase and 40% of the \(\beta\)-glucosidase) was precipitated by 50-75% AS. The 50.75% AS-fraction was then separated by chromatography on DEAE-cellulose. The fractions obtained were assayed for both activities (see Fig. 1). \(\beta\)-glucosidase activity appeared in two well-defined peaks (I and II), while \(\beta\)-galactosidase activity appeared in only one peak, which closely coincided with peak I of the \(\beta\)-glucosidase activity. There was, essentially, total recovery of both activities applied to the column. When activities were normalized to the peak maxima, the \(\beta\)-galactosidase peak and the \(\beta\)-glucosidase peak I were superimposable.

Fractions corresponding to the two peaks were used to characterize further the \(\beta\)-galactosidase and \(\beta\)-glucosidase enzyme activities. Effects of pH on these activities are shown in Fig. 2. The pH optima of 5 and 6 for peak-II and peak-1 \(\beta\)-glucosidase activities are similar to the optima reported for aryl-\(\beta\)-glucosidase (Mahadevan and Eberhart 1964 Arch. Biochem. Biophys. 108: 22) and celllobiose (Eberhart and Beck 1970), respectively. The peak-1 \(\beta\)-galactosidase and \(\beta\)-glucosidase cannot be distinguished by the effect of pH upon their activities nor by the use of inhibitors: D-celllobiose or mercuric chloride. Finally, the peak-1 activities were tested for their thermal stabilities. At 50°C, \(\beta\)-galactosidase and \(\beta\)-glucosidase activities showed very similar kinetics of inactivation, with half-lives of 3 to 4 min.

In summary, these data show that the celllobiose-induced, pH 6-\(\beta\)-galactosidase of Neurospora is associated with a \(\beta\)-glucosidase activity, possibly celllobiose. It is not known at this time whether this is because Neurospora possesses a single enzyme activity that has dual specificity for \(\beta\)-galactoside and \(\beta\)-glucoside substrates, or whether there are two separate enzymes that copurify in the procedures used.

**Figure 2.** Effect of pH on DEAE-cellulose peak-1 \(\beta\)-galactosidase activity (●), peak-1 \(\beta\)-glucosidase activity (○), and peak-II \(\beta\)-glucosidase activity (x). The number of units used were: peak-1 \(\beta\)-galactosidase, 1.30; peak-1 \(\beta\)-glucosidase, 1.30; and peak-II \(\beta\)-glucosidase, 1.84. Activities were measured at pH optima.

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**Schablik, M. B. Yocsar and G. Szabo.**

Factor(s) in the culture medium of a slime strain which stimulate DNA uptake.

We reported earlier (Schablik et al. 1977 Acta biol. Acad. Sci. hung. 28: 273) that the Neurospora crassa ragged mutant strain rgi-1(a R2506-5-101) incorporates a substantial amount of \(3^H\)-labelled DNA under optimal experimental conditions.

Since DNA accumulation was found to be inversely proportional to the age of the culture, we suspected that in older cultures the thickening of the cell wall might interfere with the attachment of DNA molecules to cell membrane receptors.

We report here studies of DNA uptake by slime cells, and present results which suggest: (1) enhanced DNA accumulation at the early stationary phase of growth; and (2) the presence of heat-sensitive factor(s) in the culture medium of 48 h slime cells which stimulates DNA uptake.

The slime strain (FGSC 111118) was obtained from the Fungal Genetics Stock Center. DNA was extracted from wild type strain (RL-3-8 A) obtained from Rockefeller University, New York, utilizing a modified Marmur's method (Aradi et al. 1978 Acta Biochim Biophys. Acad. Sci. Hung. 13: 259).

Slime cells were maintained and grown on Nelson B medium (Nelson et al. 1975 Neurospora News 1. 22: 15) containing 1.5% saccharose, 7.5% L-sorbose, 1X Vogel's salts with or without 1.5% agar. Liquid medium (80 ml) in 500 ml flasks was inoculated with 2-3 x 105 cells from 6 to 7 day old agar slants and cultures were grown in a New Brunswick Incubator at 27°C with shaking (100 rev/min). The isolation of \(3^H\)-labelled, high molecular weight
Figure 1. -- DNA uptake by *N. crassa* slime strain (FGSC #1118). 3H DNA taken up by cells of different ages during 120 min incubation (symbol . - .).

DNA, and optimal conditions for its uptake by the *N. crassa* strain have been described (Aradi et al. 1978 Acta Biochim. Biophys. Acad. Sci. Hung. 13: 259).

In the present experiments slime cells harvested by centrifugation at 1500-2000 g for 15 min at 27°C. Two ml samples (5 x 106 cells/ml) were treated with 6 to 6.6 µg DNA dissolved in 100 µl liquid medium and incubated at 27°C in a shaking water bath. After appropriate times, samples were digested with 1 ml of DNase I solution (77,000 Bormase units) at pH 7.0 for five min at 27°C, cooled to 0°C, washed three times with 0.5 N NaCl and, then once with 0.5 N HClO4 at 4°C. The remaining radioactivity was extracted from the samples with 0.5 N HClO4 at 100°C for 15 min and measured by scintillation counting (Schablik et al. 1979 Neurospora News 26: 17).

Fig 1 shows DNA uptake by slime cells different phases of the life cycle. Cells from the early stationary phase (48 h) accumulated significantly more exogenous DNA than cells from the exponential or late stationary phase of growth. DNA uptake was influenced not only by the age of the cells, but also by the amount of washing which they received. Successful washings of 48 h cells resulted in a gradual loss of their capacity to take up DNA (Table 1) as was found earlier in the case of the *N. crassa* strain (Schablik et al. 1977 Acta biol. Acad. Sci. hung. 28: 273). These results suggested the presence of a factor in the medium which enhances DNA uptake.

### Table 1

<table>
<thead>
<tr>
<th>Time of incubation (min)</th>
<th>DNA Uptake in medium</th>
<th>DNA Uptake after washing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>exhausted</td>
<td>fresh</td>
</tr>
<tr>
<td></td>
<td>cpm x 10^-14</td>
<td>cpm x 10^-14</td>
</tr>
<tr>
<td></td>
<td>g/cell</td>
<td>g/cell</td>
</tr>
<tr>
<td>0</td>
<td>39</td>
<td>38</td>
</tr>
<tr>
<td>60</td>
<td>403</td>
<td>118</td>
</tr>
<tr>
<td>120</td>
<td>973</td>
<td>201</td>
</tr>
</tbody>
</table>

CaCl2: 50 mM
3H DNA concentration: 60 x 10^-14 g/cell
Adenine: 50 µg/ml

### Table 2

Effect of treatment with DUSF and antibiotics upon DNA uptake

<table>
<thead>
<tr>
<th>Cultivation</th>
<th>Incubation time (hours)</th>
<th>Treatment</th>
<th>DNA Uptake at 0 time of incubation</th>
<th>DNA Uptake at 120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 µg/ml</td>
<td>dpm x 10^-14</td>
<td>dpm x 10^-14</td>
</tr>
<tr>
<td>Fresh</td>
<td></td>
<td>Fresh</td>
<td>20</td>
<td>300</td>
</tr>
<tr>
<td>DUSF</td>
<td></td>
<td>DUSF</td>
<td>32</td>
<td>2644</td>
</tr>
<tr>
<td>Cycloheximide</td>
<td></td>
<td>Ethidium</td>
<td>62</td>
<td>1205</td>
</tr>
<tr>
<td>CUSF</td>
<td></td>
<td>Brongide</td>
<td>68</td>
<td>2748</td>
</tr>
<tr>
<td>Culture fluid</td>
<td></td>
<td></td>
<td>56</td>
<td>1361</td>
</tr>
</tbody>
</table>

Calcium: 50 mM
3H DNA concentration: 55 x 10^-14 g/cell
Adenine: 50 µg/ml
In the following experiments cells were harvested at 24 h and resuspended in fresh medium and in medium from a 48 h culture, which supposedly contained the stimulatory factor(s). The results given in Table 2 show that DNA accumulation (i.e. binding of DNA in form resistant to pancreatic DNase and non-extractable by high ionic strength) results from treatment with the 48 h medium. Addition of ethidium bromide (10 μg/ml) to the stimulated cells did not influence DNA accumulation, although Cyclomethide (10 μg/ml) was inhibitory. These results suggest that continuous DNA uptake requires de novo protein synthesis.

To learn about the nature of the DNA uptake stimulating factor(s), we examined its heat tolerance. Treatment of the stimulating culture medium for 5 min at 60°C caused a 50% loss of activity. These preliminary results suggest that some N. crassa strains synthesize a phase-specific substance resembling the bacterial competence factor. **Institute of Biology, University Medical School, H-4012 Debrecen, Hungary.**

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Sturani, L., E. Martegani and M. Francavilla.

Effects of Glucagon on the Growth of Neurospora cc.

Adenosine 3'-5' monophosphate (cAMP) seems to be involved in the control of morphology and differentiation of Neurospora crassa; some morphological mutants of Neurospora show altered adenylyl cyclase activity and cAMP levels (Scott 1976 Ann. Rev. Microbiol. 30: 85). However, it is still unclear whether cAMP is involved in the control of growth of Neurospora, as it is in other eukaryotes (Pastan and Johnson 1975 Ann. Rev. Biochem. 44: 493).

Membrane-bound adenylyl cyclase activity isolated from the slime mutant has been shown to be activated by glucagon, and incubation of slime cells with this hormone led to an increase in glycogenolysis (Torres 1972 Proc. Natl. Acad. Sci., USA 69: 2870). To determine if conditions that lead to an increase in endogenous cAMP levels also induce changes in growth rate, we studied the effects of adding glucagon to cultures of wild type (St. Lawrence 74A).

Glucagon (at concentrations up to 1 x 10^-5M) had no effect on the growth rate of young cultures, growing exponentially (A450 = 0.2) in Vogel's medium with 0.05% glucose. However, when the concentration of Vogel's medium was lowered to 1/20 of the normal amount, glucagon above 5 x 10^-6M had a paramorphogenetic effect; after 30-60 min, hyphae aggregated and formed small balls of mycelium. In control cultures lacking glucagon, growth was normal at least up to A450 of 0.6. Attempts to find which component(s) of Vogel's medium prevented the paramorphogenetic effect of glucagon suggested that the ionic strength (and/or the buffering capacity) of the medium must be reduced to demonstrate the effects of glucagon. An analogous morphogenetic effect was found by Mishra (Naturwissenschaften 1976 63: 485) upon the addition of cAMP to liquid cultures. In the presence of 9 x 10^-6M glucagon, both RNA and protein accumulation was inhibited by 30-40%, while 7 x 10^-6M glucagon completely blocked accumulation of these macromolecules. Accumulation of RNA and protein was monitored by incorporation of radioactive precursors ([^3]H-uridine and [^14]C-leucine). Glucagon (9 x 10^-5M) had no effect on the rate of protein degradation, which was negligible in the presence or absence of glucagon (Martegani and Alberghina 1979 J. Biol. Chem. 254: 7047).

Glucagon also affected glycogen metabolism. In early exponential growth of slime cells, glycogen level (measured according to Stewart (1975 Methods in Cell Biol. 12: 111)) was ca. 55 μg/A450 unit of culture. Glucagon addition (9 x 10^-6M) inhibited glycogen accumulation by about 50% in 90-120 min.

Therefore adding glucagon to Neurospora wild type cultures causes: a) morphological changes similar to those caused by addition of cAMP, b) inhibition of glycogen accumulation, and c) inhibition of growth. It seems likely that these effects are related to an increase of the endogenous cAMP level.

In considering these results, it is interesting to note that a protein hormone is produced during the sexual cycle of Neurospora, which induces both the aggregation of hyphae for the formation of protoperithecia and the synthesis of tyrosinase, which is also induced by cAMP (Scott 1976 Ann. Rev. Microbiol. 30: 85). It has recently been proposed (Trevillian and Pall 1979 J. Bacteriol. 138: 397) that elevated cAMP levels, induced in Neurospora by depolarization of plasma membrane, stimulate cell wall biosynthesis, suggesting a biochemical basis for the morphogenetic effect induced by glucagon. **Centro del C.N.R. per la Biologia Cellulare e Molecolare delle Piante, Istituto di Scienze Botaniche, e Cattedra di Biochimica Comparata, Universita di Milano, Italia.**