Conidial germination in scon\textsuperscript{C}

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Abstract
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difficulty in isolating sufficient pure B-peak RNA from the MAK column, due to its overlap with the A and C regions, has prevented us from carrying out hybridization experiments with B-peak RNA. These saturation values are higher than those reported earlier; perhaps difficulties in specific activity determinations of RNAs can account for these variations in hybridization levels.

Separations of rat liver 18S RNA and E. coli 16S RNA into doublet bands byagarose-supported polyacrylamide gels have been reported by Peacock and Dingman (1968 Biochemistry 7: 668). In the present experiments, whole-cell RNA or 18S RNA was run on gels of various concentrations (2.3%, 3.0% and 5.0%), essentially as described by these authors. It was not possible to obtain separation of Neurospora 18S RNA into two distinguishable bands, suggesting a close similarity in molecular weights of A-peak and B-peak 18S RNAs. The molecular weights of 28S and 18S rRNA of Neurospora were estimated from 10 independent runs in 3.0% gel, using rat liver 30S and 18S and E. coli 23S and 16S RNAs as references. These estimated weights are 1.28 ± 0.09 x 10^6 and 0.78 ± 0.03 x 10^6 daltons for 28S and 18S RNAs, respectively.

The present experiments suggest that two types of 18S RNA separable by MAK chromatography differ in base sequence rather than in molecular weight or gross conformation. We also have evidence which suggests that there are two species of 18S RNA are equally methylated. It is still conceivable that the two peaks of 18S RNA are actually due to artifacts inherent in the techniques employed. To truly substantiate rRNA heterogeneity, it seems necessary to undertake sequence analysis of these RNAs.

![Figure 1. MAK column chromatography of purified whale cell RNA and [3H] A-peak RNA.](image1)

Whole-cell RNA was diluted to 1 A260 with 0.3N NaCl, 0.05M sodium phosphate buffer (pH 6.7), mixed with [3H] A-peak RNA, and added to the column. The column was then washed with 50 ml of the same buffer. RNA was eluted from the column with a linear salt gradient made with 130 ml each of 0.3N NaCl and 1.35 N NaCl, 0.05M sodium phosphate buffer (pH 6.7). When the salt concentration of the effluent reached 0.7M NaCl, fractions were collected. A260 measurements were made with a Beckman DB spectrophotometer. To measure radioactivity, fractions were precipitated with 10% cold TCA and filtered onto Boc-T-Flex membrane filters. After drying, IX spectrofluor (Amersham and Searle) was added and counts were read. Counting efficiency was ca. 25%.

A260 (——); [3H] cpm (-----).

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**Schmit, J. C., M. Cohen and S. Brody.**

Conidial germination in sconc.

Developmental mutants that affect conidial germination can be placed in two major classes. The first class includes those mutations that have a defect in the de novo synthesis during conidial germination of some gene product that is specifically necessary for germination. The second class includes those mutations that produce defective conidi during conidiation. These could be of two types: either a gene product necessary for germination is not incorporated into the conidio, or a product is incorporated which is detrimental to germination. These mutants can also be classified as either phase-specific or phase-critical. "Phase specific" mutations are those that affect gene products that are used only in one phase of the life cycle. "Phase critical" mutations are those that affect products needed for many phases but which are more crucial to a particular phase.

![Figure 2. Hybridization of rRNA components with nuclear DNA.](image2)

Nuclear DNA (10µg) was immobilized on nitrocellulose membrane filters. The filters were incubated at 64°C for 22-24 hrs in vials containing various concentrations of labeled RNA in 5 ml of 2XSSC (1XSSC = 0.15M sodium citrate, pH 7.0). A blank filter was added to each vial to correct for background adsorption. After incubation, the filters were rinsed briefly in 2XSSC; incubated for one hour at room temp. in 5 ml 2XSSC containing 50 µg/ml of ribonuclease, and washed on both sides with 50 ml 2XSSC. They were then dried and counted in a liquid scintillation spectrometer. Solid and open points denote independent experiments run with RNAs isolated at different times. Specific radioactivities 28S RNA 3,936 cpm/µg; 18S RNA 3,936 cpm/µg for solid points and 3,846 cpm/µg for open points.
The scon\textsuperscript{C} strain contains a regulatory mutation which results in the constitutive production of the enzymes of sulfur metabolism. It was also reported that scon\textsuperscript{C} could not be recovered efficiently from germinated poorly (Burton and Metzenberg 1972 J. Bacteriol. 109: 140). These observations indicate that the scon\textsuperscript{C} strain has a defect in spore germination, in addition to its metabolic effects during the vegetative phase. This report gives the details of additional studies on conidial germination in this strain.

In these studies, the scon\textsuperscript{C} strain grew as fast as a wild-type strain, RL3-8A, on minimal glucose agar and conidiated abundantly. The conidia produced by this strain had "normal" morphology, but germination was defective. On sorbose plates, only 2 to 8% of the conidio that were plated from the scon\textsuperscript{C} strain formed colonies. The colony forming efficiency of conidio from the wild-type strain was greater than 50% under the same conditions. In liquid shake cultures (Fig. 1), the germination and growth of the scon\textsuperscript{C} strain lagged considerably behind that of the wild-type strain. Thus, the only phase of the asexual cycle that was morphologically defective was conidial germination. In a developmental sense, the scon\textsuperscript{C} strain contains a phase-critical mutation.

The apparent reason for the defective conidial germination in scon\textsuperscript{C} strain is that it forms osmotically fragile conidio. A large amount of UV-absorbing material was released when conidio from scon\textsuperscript{C} were suspended in water (Table 1). In addition, the total amino acid pool dropped from 500 pmol/g residual dry weight in the dry-harvested conidio of the mutant strain to 95 pmol/g in water-washed conidio. Conidio from the wild-type strain lost much less UV-absorbing material (Table 1) and essentially none of its amino acid pool when suspended in water. Sucrose, 20%, prevented the loss of UV-absorbing material from the mutant strain (Table 1). In addition, the colony forming ability of conidio from the scon\textsuperscript{C} strain was improved 4-fold when the conidio were suspended in sucrose rather than water.

Table 1. Release of UV-absorbing material from conidio of scon\textsuperscript{C} and wild type RL3-8A.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Suspending medium</th>
<th>OD 260nm/mg/ml/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>scon\textsuperscript{C}</td>
<td>water</td>
<td>1.715</td>
</tr>
<tr>
<td></td>
<td>20% sucrose</td>
<td>0.344</td>
</tr>
<tr>
<td>RL3-8A</td>
<td>water</td>
<td>0.190</td>
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A about 15 mg of conidio were shaken vigorously in 5 ml of suspension medium at 23°C for 5 min. The conidio were removed by filtration on Millipore filters and optical density was measured.

Conidial germination in scon\textsuperscript{C} apparently was defective because the conidio lost a large proportion of their cytoplasmic material when suspended in water. Thus, the scon\textsuperscript{C} strain has a defect in germination because it produces defective conidio, and not because of a defect in de novo synthesis of a germination-specific product. It is not clear what relationship exists between spore formation and the constitutive sulfur enzyme production. Of the many possibilities, perhaps, excess sulfhydryl production during conidiation alters the structure of the plasma membrane to such an extent that it becomes osmotically fragile.

Many morphological mutant strains of N. crassa have been shown to exhibit a hyphal branching rhythm on solid medium that is characteristically non-circadian. Four of these mutant strains, representing three functionally non-allelic loci, were the subject of this study; namely, \textit{P}_{k-1} (Russell and Srb 1972 Genetics 71: 233) and clock at the peak locus, and 22-214 (allelic with scombo) and 24-310 at two other loci. After 72 hours of growth at 25°C on Vogel's complete medium in petri plates, colony diameter (in mm) were: \textit{P}_{k-1}, 11-13; clock, 71-75, 22-214, 41-52; 24-310, 47-53. The number and type of growth bands at this time were: \textit{P}_{k-1}, two to three, concentric; clock, three, concentric; 22-214, two to four, irregular and non-concentric; and 24-310, th ree, concentric. In addition to the mycelial phenotypes just described, all of the mutants ore abnormal ascus mutants, all being zygote recessives with the exception of \textit{P}_{k-1}, which is zygote dominant. All strains were obtained from A.M. Srb, except clock which was obtained from the FGSC.

Experimentally, we investigated the hypothesis that rhythmical hyphal growth might be characterizedly the result of a particular change in the protein composition of the mycelium. Polyacrylamide gel electrophoresis was chosen as the means of detecting such a change. Only acidic proteins were compared in this study.

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