Fungal Genetics Reports

Volume 27

Article 8

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Abstract
DNA homologies of ribosomal RNA genes of Neurospora species.
Neurospora is known to contain glycogen, but no reports indicate the presence of amylose (linear starch) even though Wescodyne stains the mycelia blue-black. Since Wescodyne contains iodine and a blue-black response with iodine is a positive test for starch, we stained Neurospora with iodine solution (0.2% I₂-2.0% KI). As a blue-black stain we used sodium salicylate, 1 N NaOH, or boiling water, all of which will dissolve starch from algae and higher plants. None of these solubilized Neurospora starch.

Earlier work (McCracken 1974 Plant Physiol. 54:414) showed the existence of an amylase precipitating factor in fungi. This factor has now been isolated from Neurospora and characterized (unpublished results). In our attempts to extract starch, we found that the blue-black staining material co-precipitated with the amylase precipitating factor. This factor binds only to amylose and not to amylpectin, glycogen, cellulose, dextrans, inulin, or a variety of simple sugars. Moreover, in this case the iodine stain was blue. Thus we conclude that Neurospora does indeed contain starch in the form of amylose. Furthermore, since the blue stain is associated with cell walls, this amylose may be a cell wall component. *Department of Biological Sciences, Illinois state University, Normal, Illinois 61761.


DNA homologies of ribosomal RNA genes of Neurospora species.

Ribosomal RNA genes (rDNAs) of Neurospora crassa contain DNA sequences which code for 17S, 5.8S, and 26S rRNAs, in addition to internal and external spacers (Free, Rice, and Metzenberg 1979 J. Bacte. 137:1219). As has been reported for many eukaryotes, the DNA sequences which code for 17S, 5.8S, and 26S rRNAs in Neurospora species are probably conserved while the internal and external spacer regions are probably variable sequences. Extensive electron microscopic studies (Schibler et al. 1975 J. Molec. Biol. 94:503) of 45S precursor rRNA of several cold and warm blooded animals confirm that spacer regions vary extensively from species to species.

It was desirable to know whether such differences in rDNA sequences exist between Neurospora species. Any such difference should be detectable using standard procedures for DNA homology studies (Dutta 1976 Mycologia 68:388). rDNA sequences were isolated from N. crassa mycelial cells using the procedure described previously (Chattopadhyay et al. 1972 Proc. Natl. Acad. Sci. 69:3256). The purified rDNA was P-labeled (by nick translation) and reassociated with total DNA isolated from the heterothallic species N. crassa and from three homothallic
N. lineolata, and N. africana. In addition, 32P-labeled total DNA of N. crassa was re-annealed with unlabeled bulk DNA from N. crassa, N. dodgei, and N. lineolata.

The purified 3H-rDNAs (nick translated) of N. crassa, sheared to 400 nucleotide fragments had 1 x 106 cpm (counts per minute) per microgram of DNA. The 3H-rDNA Cot used for these reactions was 2 x 10^-3, at which there was no detectable self reaction. The 32P-DNA of N. crassa DNA, sheared to 400 nucleotides had 20,000 cpm/ug DNA. 32P-DNA Cot used was 0.05 and the 1-2 percent reaction obtained with 32P-total was routinely deducted from the total DNA:DNA reassociation. In all reactions unlabeled DNA Cot was at least 700. Te50 (50% dissociation) was determined from thermal stability curves.

The results of various DNA:DNA and rDNA:DNA reactions are summarized in Table 1. With total 32P-DNA of N. crassa, it was impossible to detect DNA sequence differences among the three homothallic species, although differences between heterothallic and homothallic species were obvious. However, 2 to 5 percent differences in nucleotide sequence were observed when purified rDNA of N. crassa was reacted with the three homothallic species. These observations suggest the existence of non-identical rDNA sequences among different species of Neurospora. Whether these differences are in the spacer regions is now being investigated. (Supported in part by the U.S. Department of Energy). - Department of Botany and the Cancer Research Center, Howard University, Washington, D.C. 20059.

An initial survey of the FGSC collection of N. intermedia natural isolates revealed two variants with abnormal growth patterns. When the wild isolates are grown in race tubes, the majority show linear growth at rates close to that of N. crassa. However, two variants from Kauai, Hawaii, showed 'stop-start' behavior. Further isolates from Kauai were obtained from the collection of D. D. Perkins (designated 594, 608, 804); three of these also showed stop-start patterns. The growth curves are shown in Fig. 1, together with a control. All variants stop well before reaching the end of the growth tube, and any subsequent growth varies in time of initiation and in duration.

Strains of Hanalei-1f (FGSC 2360) and Xekaha-1 (FGSC 2363) were crossed to normal Kauai strains, using both as male and female parents. Hanalei-1f is mostly female-sterile, but one apparently successful cross yielded 2 isolates out of 79 which showed growth patterns similar to Hanalei-1f. Two additional isolates grew slowly, taking 27 days to reach the end of the 500 mm tube. When used as a male parent, Hanalei-1f never produced stop-start

### TABLE 1

<table>
<thead>
<tr>
<th>Unlabeled DNA Fragments</th>
<th>with 3H-rDNA</th>
<th>with 32P-total DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterothallic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. crassa 74A (FGSC #987)</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>Homothallic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. dodgei (FGSC #1692)</td>
<td>97</td>
<td>86</td>
</tr>
<tr>
<td>N. lineolata (FGSC #1910)</td>
<td>95</td>
<td>63</td>
</tr>
<tr>
<td>N. africana (FGSC #1740)</td>
<td>92</td>
<td>82</td>
</tr>
</tbody>
</table>

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**Figure 1.** -- Representative growth curves of abnormal variants of N. intermedia in growth tubes containing minimal medium. Hanalei-1f (FGSC 2361) is a normal control. FGSC 2360, 2363, and 2361 were obtained from FGSC; the remaining cultures were donated by D. D. Perkins.


Possible natural cytoplasmic variants of N. intermedia.