Comparison of nuclear DNA with whole cell DNA isolated from Neurospora crassa

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Comparison of nuclear DNA with whole cell DNA isolated from Neurospora crassa

Abstract
Comparison of nuclear DNA with whole cell DNA isolated from Neurospora

Authors

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UV-induced inactivation and mutation-induction in a new two-component heterokaryon (59) homozygous for the excision-repair deficient mutant uvs-2

inactivation and the induction of u-rays and various chemical mutagens (MMR, ICR-170 and others) and the induction of ad-3A and ad-3B mutations.

In heterokaryon 12 which consists of the following two strains: 74-OR60-29A (his-2 ad-3A ad-36 nit-2; ad-2; int) and 74-OR31-16A (al-2; cot-1; pan-2), both point mutations (ad-3A and ad-3B) and multilocus deletions (ad-3A) in the ad-3 region can be recovered (de Serres and Milliny 1971, Chemical Mutagens 2:311). To determine the effect of uvs-2 on the recovery of both of these classes of mutations, a new heterokaryon (59) was constructed that has the same genotype as heterokaryon 12 except that it is homozygous for uvs-2.

The strain numbers and genotypes of this new heterokaryon are as follows: 74-OR276-40A (his-2 ad-3A ad-36 nit-2; ad-2; int; uvs-2; int) and 74-OR244-3A (al-2; cot-1 uvs-2; pan-2). The sensitivity of this new heterokaryon to UV was compared with that of heterokaryon 12 for inactivation of the heterokaryotic fraction as well as the induction of mutations in the ad-3 region. This experiment showed that, whereas heterokaryon 12 has a multihit survival curve with a broad shoulder (de Serres and Kilbey 1971, Mutation Res. 12:221), heterokaryon 59 has greater sensitivity to UV-induced inactivation with a simple exponential survival curve. A comparison of the UV exposure required to give 50% survival, for example, gives a relative biological effect (RBE) for inactivation of 46.7. A comparison of the dose-response curves for the overall induction of ad-3 mutants shows no difference between the slopes of the curves; both curves increase as the square of UV exposure. In a comparison of the UV exposures required to give comparable forward-mutation frequencies (in the range of 10-100 x 10^-5 survivors), the higher yield of ad-3 mutants in heterokaryon 59 results in an RBE of 4.0. Genetic analysis of ad-3 mutants induced in heterokaryon 59 will reveal whether the spectrum of ad-3 mutants is qualitatively different from that found in heterokaryon 12.

These experiments report the development of a new two-component heterokaryon that is excision-repair deficient and demonstrates its expected sensitivity to both inactivation and the induction of specific loci mutations in the ad-3 region. - Office of the Associate Director for Genetics, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, North Carolina 27709


Comparison of nuclear DNA with whole cell DNA isolated from Neurospora crassa.

Huang 1972 Biochem Genetics 6:41. Studies on mycelial whole cell DNA (Dutta 1976 Mycologia 68:388) of numerous Neurospora strains and species exhibited two fractions: a major high G+C (52-56 G:C mol%) DNA fraction which comprised 75-80% of the total genome, and a minor low G+C (32-33 G:C mol%) fraction comprising 20-25% of the genome. It was inferred that most of these low G+C DNA sequences were reiterated and could be partly mitochondrial and/or other non-nuclear origin. The small percentage of repeated sequences in nuclear DNA of Aspergillus nidulans could be due to lack of mitochondrial and/or other non-nuclear DNA. We have thus compared nuclear DNA from mycelial and conidial cells of Neurospora. Purified nuclei from conidial and mycelial cells were isolated by the procedure of Hauatala et al. (1977, J. Bacteriol. 130:704). DNA was isolated by a hydroxyapatite chromatography procedure described previously (1976 Mycologia 68:388).

Unlabeled nuclear DNA and whole cell DNA were first characterized by analysis of hyperchromic shifts using a Gilford spectrophotometer (see Table 1). Whole cell DNA of both N. crassa conidial and mycelial cells showed typically the two fractions mentioned before. Nuclear DNA of these cells contained very little, if any, of the low G+C minor fraction but was comprised almost entirely of the high G+C fraction. This observation suggested that almost all of the low G+C fraction (about 10-25% of total DNA) of N. crassa whole cell DNA was indeed non-nuclear.

Nuclear DNA from conidia was 3H-labeled by nick translation (Krumlauf and Marzluf 1978 Neurospora Newsletter 25:15) and sheared to 400 nucleotide piece size at 50,000 p.s.i. release pressure. These 3H-DNAs were denatured and allowed to reassociate to a Cot of 2.0 followed by SI nuclelease treatment and fractionation with...
TABLE 1

<table>
<thead>
<tr>
<th>Characteristics of Nuclear and Whole Cell DNA</th>
<th>N. crassa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Cell DNA</td>
<td>Minor Fraction</td>
</tr>
<tr>
<td>Tm °C</td>
<td>% of total DNA</td>
</tr>
<tr>
<td>Conidia</td>
<td>80</td>
</tr>
<tr>
<td>Mycelia</td>
<td>81</td>
</tr>
</tbody>
</table>

These data are summarized from optical melting curves at 0.12 M phosphate buffer, pH 6.8. Tm °C (temperature at which 50% of the DNA dissociates) was calculated for each fraction.

G:C content can be calculated by the equation G:C mol% = Tm°C 69.3/0.41.

These nuclear DNA sequences are composed of multiple copies of nuclear rRNA and tRNA genes. (Supported in part by the U.S. Department of Energy) * * * Department of Botany and the Cancer Research Center, Howard University, Washington, D.C. 20059.

Eberhart, B.

A mutant strain of Neurospora crassa has been isolated which is resistant to inhibition by 2-deoxy-D-glucose (2dg) and is characterized by growth rates which are initially faster than wild type strains, when grown on minimal medium supplemented with any one of a number of monosaccharides or disaccharides plus 2dg (saccharide/2dg = 2/1). The strain which has been shown in numerous crosses to segregate as a single gene is designated as dgr. dgr grows more slowly than wild type on standard media. These properties and other experimental results suggest that dgr strains permit the utilization of hexoses in an abnormal manner conferring an increased resistance to 2dg.

The original intention was to screen for mutants with increased cellobiase activity (Hackel and Kahn 1978 Molec. gen. Genet. 164: 295). It was expected that 2dg would inhibit wild type growth on cellobiose, while mutants would grow on such a mixture. To date, no mutants have been found with altered cellobiase levels, but several are clearly resistant to 2dg.

Mutagenesis experiments were carried out using the gluc-2 strain (Eberhart and Beck 1973 J. Bacteriol. 116: 295), which reduces aryl-β-glucosidase activity and permits a clearer nutritional response to cellobiase by putative mutants. Conidia from seven day old gluc-2 cultures were suspended, filtered through glass wool, washed, and diluted to 10⁶/ml in a final volume of 20 ml in a 100 mm diameter round glass dish. Irradiation for 5 min with a U.V. lamp achieved approximately a 50% kill as determined by subsequent growth on complete medium. One ml of irradiated conidia was added to 4 1 of incubation medium in a round 6 1 flask.

The incubation medium included Vogel's minimal medium at 1/4 strength, 0.1% cellobiose, and 0.05% 2dg. Agar (0.1%) was included to decrease the fusion by anastomosis. Sterile air bubbles agitated the suspension for 24 h at 25°C, then aliquots were viewed in petri dishes under a stereo-microscope. Larger colonies were removed, washed with sterile water and placed in tubes of complete agar medium. The restricted growth habitat induced by 2dg (Tatum, Barratt, and Cutter 1949 Science 109: 509) greatly facilitated colony isolation.

Confirmation that the dgr mutant is resistant to 2dg was obtained with solid media in petri plates containing 0.1% cellobiose or fructose, 0.05% 2dg, 1.5% agar and 1/4 strength Vogel's minimal at 25°C. dgr conidia germinated and grew in 24 h at 25°C, whereas wild type initially grew very slowly but eventually adapted by 3-4 days. In 2dg medium the saccharides that showed a greater differential growth between dgr and wild type are cellobiose, trehalose, lactose, fructose and galactose. Saccharides that showed a lesser, but definite, effect are maltose, glucose, and xylose.