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

Abstract

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Timberlake (1978 Science 202:973) reported only 2-3 percent reiterated sequences in DNA isolated from nuclei of Aspergillus nidulans. When whole cell DNA from Neurospora crassa was studied, 10-20 percent repetitive DNA sequences were observed (Dutta 1974 Nucleic Acid Res. 1:1411; Brooks and Huang 1972 Biochem. Genetics 6:41). Studies on mycelial whole cell DNA (Outta 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 percent of the total genome, and a minor low G:C (32-33 mol %) fraction comprising 20-25 percent 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 could be due to lack of mitochondrial and/or other non-nuclear DNAs. We have thus compared nuclear and whole cell DNA isolated from N. crassa. Purified nuclei from conidial and mycelial cells were isolated by the procedure of Hautala et al. (1977, J. Bacteriol 130:704). DNA was isolated by a hydroxyapatite chromatography procedure described previously (1976 Mycolagia 68:388).

Unlabeled nuclear DNA and whole cell DNA were first characterized by analysis of hyperchromic shifts using a Gilfard spectrophotometer (see Table 1). Whole cell DNA of both N. crassa conidial and the mycelial cells showed typically the two fractions mentioned before. Nuclear DNA from all 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 IO-25% of total DNA) of N. crassa whole cell DNA was indeed non-nuclear.

Nuclear DNA from conidia was ^{3}H-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 ^{3}H-DNAs were denatured and allowed to reassociate to a C_0t of 2.0 followed by S1 nuclease treatment and fractionation with

TABLE 1

Characteristics of *N. crassa* nuclear and whole cell DNA

	Whole Cell DNA				Nuclear DNA	
	Minor Fraction		Major Fraction		Minor Fraction	Major Fraction
	Tm °C	% of total DNA	Tm °C	% of total UNA		Tm °C
Conidia	80	24	93	76	0	97
Mycelia	81	25	92	75		

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.

hydroxyapatite. At this C t, only 2-3% of the CNA behaved as repeated DNA. These studies confirm that the nuclear DNA of *N. crassa* has a very small fraction (3-4%) of repeated sequences as was reported for *A. nidulans*. These nuclear repeated 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.