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Abstract

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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, 5.85, 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 ^{3}H-labeled (by nick translation) and reassociated with total DNA isolated from the heterothallic species N. crassa and from three homothallic

species: N. dodgei, N. lineolata, and N. africana. In addition, ^{32}P-labeled total DNA of N. crassa was re-annealed with unlabeled bulk DNA from N. crassa, N. dodgei, and N. lineolata.

TABLE 1

Summary of DNA:DNA Reassociation of N. crassa total ^{32}P-DNA and of ^{3}H-rDNA with total DNA of Neurospora species

Unlabeled DNA Fragments	with ^{3}H-rDNA		with ^{32}P-total DNA	
	Percent Reassociation (Normalized)*	Te50 in °C	Percent Reassociation (Normalized)	Te50 in °C
Heterothallic				
N. crassa 74A (FGSC #987)	100	88	100	86
Homothallic				
dodgei (FGSC #1692)	97	86	65	82
N. lineolata (FGSC #1910)	95		63	81
N. africana (FGSC #1740)	92	84	64	82

The purified ^{3}H-rDNAs (nick translated) of N. crassa, sheared to 400 nucleotide fragments had 1 x 106 cpm (counts per minute) per microgram of DNA. The ^{3}H-rDNA C_0t used for these reactions was 2 x 10^{-3}, at which there was no detectable self reaction. The ^{32}P-DNA of N. crassa DNA, sheared to 400 nucleotides had 20,000 cpm/µg DNA. ^{32}P-DNA C_0t used was 0.05 and the 1-2 percent reaction obtained with ^{32}P-total was routinely deducted from the total DNA:DNA reassociation. In all reactions unlabeled DNA C_0t 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 ^{32}P-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.