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Repeated DNA sequences in different Neurospora species.

The significance of the role of repeated DNA sequences has been emphasized by several workers in studies of gene regulation of higher organisms (Britten and Davidson 1969 Science 165:349). Brooks and Huang (1972 Biochem. Genet. 6: 41) reported the existence of 20% repeated DNA sequences in the species N. crassa. We have obtained only 10-12% repeated DNA sequences in N. crassa (74A) and in strains of two other species, N. sitophila (56.1a and 10B A) and N. tetrasperma (85A). Procedures for isolation of ^{32}P-labeled DNAs, unlabeled DNAs, shearing of DNAs to 400 nucleotide pairs, DNA:DNA reassociation kinetics studies and thermal stability studies of homo- and heteroduplexes are described by Dutta and Ojha (1972 Mol. Gen. Genet. 114:232). The procedure followed for isolation of repeated DNA sequences was as follows: ^{32}P-labeled sheared DNA was denatured by heating at 100°C for 3 minutes in a medium containing 0.14 M phosphate buffer (PB), 0.4% sodium lauryl sulphate (SLS) and 10^{-4} M ethylenediaminetetraacetate-disodium (EDTA) abbreviated as PB-SLS-EDTA. After denaturation the DNA solution was rapidly cooled to 60°C and incubated at 60°C for sufficient time to obtain a $C_o t$ of 2 ($C_o t$ = moles x sec/liter). The mixture was then passed through a hydroxyapatite (HA) column equilibrated at 60°C with 0.14 M PB-0.4%SLS. Approximately 90% of the DNA did not bind to HA and was found to represent non-repeated DNA as indicated by DNA:DNA reassociation. The same proportion of repeated DNA sequences was obtained in all of the three Neurospora species studied. The discrepancy of our results with that of Brooks and Huang (1972) could be due to experimental conditions such as DNA piece size and reaction mixtures.

The $T_e 50$ (temperature at which 50% elution from HA takes place) of thermal stability profiles of reassociated homoduplexes of ^{32}P-labeled repeated DNA alone was 5-6°C lower than the $T_e 50$ of ^{32}P-labeled non-repeated (unique) DNA sequences. Kinetics of DNA:DNA reassociation of repeated DNA sequences of two species, N. crassa and N. sitophila showed a 1/2 $C_o t$ (i.e., the $C_o t$ value at which 50% of the single strand DNAs reassociate) of 0.058 and 0.060, respectively, which indicated that there were approximately 50 copies of these repeated DNA sequences in each species. The typical second order reactions (Britten and Kohne 1968 Science 161:529) obtained in DNA:DNA reassociation kinetics studies indicated that these repeated DNA sequences were similar in each species. In preliminary studies of transcription (by DNA:RNA hybridization in liquid buffers) of repeated and non-repeated DNAs using a RNA $C_o t$ of 12,000, none of the repeated DNAs reacted with the unfractionated RNA, whereas non-repeated DNA showed 10% DNA:RNA hybridization under similar conditions.

This research was supported in part by National Science Foundation Grant No. GY10452. We appreciate the laboratory assistance of T. Osgood in these studies. - - - Department of Botany, Howard University, Washington, D.C. 20001.