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Repeated DNA sequences in fungi

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ABSTRACT

Several fungal species, representatives of all broad groups like basidiomycetes, ascomycetes and phycomycetes, were examined for the nature of repeated DNA sequences by DNA:DNA reassociation studies using hydroxyapatite chromatography. All of the fungal species tested contained 10-20% repeated DNA sequences. There are approximately 100-110 copies of repeated DNA sequences of approximately 4×10^7 daltons piece size of each. Repeated DNA sequence homoduplexes showed on average $5^{\circ}C$ difference of T_e50 (temperature at which 50% duplexes dissociate) values from the corresponding homoduplexes of unfractionated whole DNA. It is suggested that a part of repetitive sequences in fungi constitutes mitochondrial DNA and a part of it constitutes nuclear DNA.

INTRODUCTION

The existence of a fast recombining fraction of repeated base sequences in the DNA molecules of higher organisms were reported as early as 1964 (1). Its presence has since then been established for a large number of higher organisms. This subject has been extensively reviewed in which the need for knowledge of its biological role has been emphasized (2,3). The following facts have been established from investigations in this field: a) some of the base sequences in the DNA molecules of higher organisms repeat themselves several times, b) these multiple copies reassociate at a much faster rate than the rest of the sequences in the DNA molecule, c) the amount of repeated DNA sequences in various species of higher organisms range from 20-80% of the DNA and d) repetitive DNA fraction is made up of many families of similar nucleotide sequences. The generality of their occurrence in eukaryotes, with increasing complexity

of repetitive sequences in higher organisms, indicates that repeated DNA sequences play a role in evolution (2,4,5). Occurrence of repeated DNA sequences in cell differentiation has been the subject of great interest recently (3,6). To date experimental data (2) show the presence of repetitious DNA sequences in eukaryotes above the level of fungi, while none in prokaryotes. However, many more organisms will have to be investigated before the boundary between those life forms, which do and those, which do not possess repetitious DNA sequences can be ascertained. It is of interest to know how repeated DNA sequences in fungi are organized in the chromosome and whether these sequences control gene regulation as has been shown in other eukaryotic organisms recently (5,7). Fungal organisms are primitive eukaryotes which show remarkable variation of cell differentiation and thus prove to be excellent experimental systems for genetic studies in evolution. Very little information on occurrence of repeated DNA sequences are, however, available in fungi.

Preliminary reports regarding the existence of repeated DNA sequences in two fungal genera have appeared from this and other laboratories earlier (8,9,10). In this paper, results of extensive studies on occurrence and characteristics of repeated DNA sequences in representative genera of all major groups of fungi are given. The genera Neurospora and Coprinus have, however, been studied more thoroughly.

MATERIALS AND METHODS

Strains: their maintenance and culturing: Table 1 gives a comprehensive list of all strains used in this study. Maintenance of Neurospora and and Coprinus species is described by us elsewhere (11,12). Mucor azygospora and Rhizopus stolonifer were maintained on potato dextrose agar. Details of culturing Neurospora, Coprinus, Mucor and Rhizopus species are described by Dutta and Ojha (13).

Procedures of isolation and purification of unlabeled and ^{32}P-labeled DNAs,

Table 1. Summary of occurrence of repeated DNA sequences in fungi

Name	Source	Percentage of Repeated DNA sequence based on		$\frac{1}{2}$ $C_o t$ values from DNA:DNA reassociation kinetics	Calculated molecular weights in daltons	No. of copies
		^{32}P-labeled DNA	Optical absorbency at 260nm			
Basidiomycetes						
Coprinus lagopus $H_2A_6B_5$	PD	12-15	15-18	0.060	3.6×10^7	112
Ascomycetes						
Neurospora crassa 74A	FGSC 987	10-12	12-15	0.055	3.2×10^7	100
N. intermedia A	DDP 420P	10-12	12-15	0.055	3.2×10^7	100
N. tetrasperma 35A	FGSC 1270	10-12	12-15	0.058	3.2×10^7	100
Phycomycetes						
Mucor azygospora	ATCC 1105	-	15-18	0.058	3.4×10^7	103
Rhizopus stolonifer	ATCC 6204	-	15-18	-	-	-
Allomyces arbuscula*		15-20	-	-	-	-

The estimation of percent repeated DNA sequences was made giving a $C_o t$ of 2.0 in the incubation mixture of sheared (approx. 400 nucleotides piece-size) single strand DNAs as described in the text. Molecular weights were calculated by measuring relative rates of reassociation of repeated DNAs using E. coli ^{14}C-DNA as internal standard (15). PD = Peter Day of Connecticut Agricultural Experiment Station, New Haven, Conn., DDP = David D. Perkins of Stanford University, Stanford, California, FGSC = Fungal Genetics Stock Center, California State University at Humboldt, California, ATCC = American Type Culture Center, Rockville, Md.

Confirmed data on $\frac{1}{2}$ $C_o t$ values of Rhizopus and Allomyces could not be obtained due to lack of material. However preliminary data show that the values are close to Mucor, within the range of 0.058 $\pm$ 0.005.

*Taken from unpublished data of Ojha and Dutta (1973). Procedures for isolation and characterization of Allomyces DNA have been described by Ojha and Turian (16).

shearing of DNAs, tests of purity of DNAs, hybridization and thermal elution are described by Dutta and Ojha (13). Unsheared ^{14}C-labeled E. coli DNA was received as gift from Dr. Roy J. Britten, formerly of the Carnegie Institution, Washington, D.C.

Fractionation of ^{32}P-labeled and unlabeled repeated DNAs: Purified ^{32}P-labeled and unlabeled DNAs of different fungi were fractionated into unique and repeated segments by hydroxyapatite (HAP) chromatography. To do this ^{32}P-labeled sheared (50,000 p.s.i) DNA was denatured by heating at 100°C for 3 minutes in a medium containing 0.14 M sodium phosphate buffer (PB) pH 6.8, 0.4% sodium lauryl sulphate (SLS), ethylene diaminetetraacetate disodium salt (EDTA), abbreviated as PB-SLS-EDTA. After denaturation, the solution was rapidly cooled to 60°C and incubated at 60°C for sufficient time to obtain a C_0t (Moles. Liter^{-1}. Seconds) value of 2.0. The mixture was then passed through HAP column equilibrated at 60°C with 0.14 M PB, 0.4% SLS. An aliquot of each DNA sample was tested for non-specific zero-time binding (14) before fractionation, which was found to be less than 1.0 percent with an average value of 0.4 to 0.6 percent.

Procedures of DNA:DNA hybridization and kinetics studies of DNA:DNA reassociation are as described elsewhere (13,15).

RESULTS

It was necessary to routinely discard the zero-time reassociating fractions of DNAs in all experiments to avoid confusions in interpretation of DNA reassociation results.

Identification of Repeated DNAs: Table 1 summarizes data on occurrence of repeated DNA sequences in each of the fungal species studied. In general DNAs from fungal species, including primitive Phycomycetes fungi, Rhizopus, Mucor and Allomyces, possess 10-20% repeated DNA sequences.

Reassociation kinetics of Repeated DNAs: Fig. 1 illustrates the pattern of DNA:DNA reassociation studies of the C. lagopus repeated, non-repeated

and whole DNAs. E. coli ^{14}C-DNA and C. lagopus ^{32}P-labeled DNAs were mixed and sheared together. Kinetics of whole and non-repeated ^{32}P-labeled DNAs were studied separately under identical conditions. Similar studies were made with DNAs of other fungal species as summarized in Table 1. DNA:DNA reassociation kinetics studies of repeated DNAs show typical second order reactions having $\frac{1}{2}$ C_0t (the C_0t value at which 50 percent of single stranded DNAs reassociate) values of 0.05 to 0.06 approximately, indicating homogeneous nature of these repeated DNA sequences studied. This compares differently with kinetics of unfractionated (whole) DNAs which do not show second order reactions. Non-repeated DNAs of all fungi tested, however, also show typical second order reactions, as expected indicating homogeneity.

Thermal stability profiles of repeated DNAs: Fig. 2 gives a comprehensive picture of thermal stability of different DNA fractions of C. lagopus. T_e50 (temperature at which 50% of hybrids dissociate) values of homoduplexes of unfractionated and non-repeated DNAs did not differ significantly but T_e50 values of these repeated DNA homoduplexes showed great differences (5°C difference) compared to homoduplexes of non-repeated and whole DNAs. Similar pattern of thermal stability profiles is also obtained (17) from N. crassa repeated DNA.

DISCUSSION

Data presented in this paper have clearly established the presence of repeated DNA sequences in all fungal species tested. Since these represent a broad group of fungi, it is expected that perhaps all fungal DNAs have repeated DNAs. It is evident (Figures 1 and 2) that the separation of repeated DNAs from whole DNAs was complete and also that the isolated repeated DNA sequences were free from non-repeated sequences. This is supported by the following observations: (a) DNA:DNA reassociation kinetics of all repeated DNAs, studied with several fungal DNAs like the C. lagopus DNA (Fig. 1) and the N. crassa DNA (17) showed second order reactions, which indicate

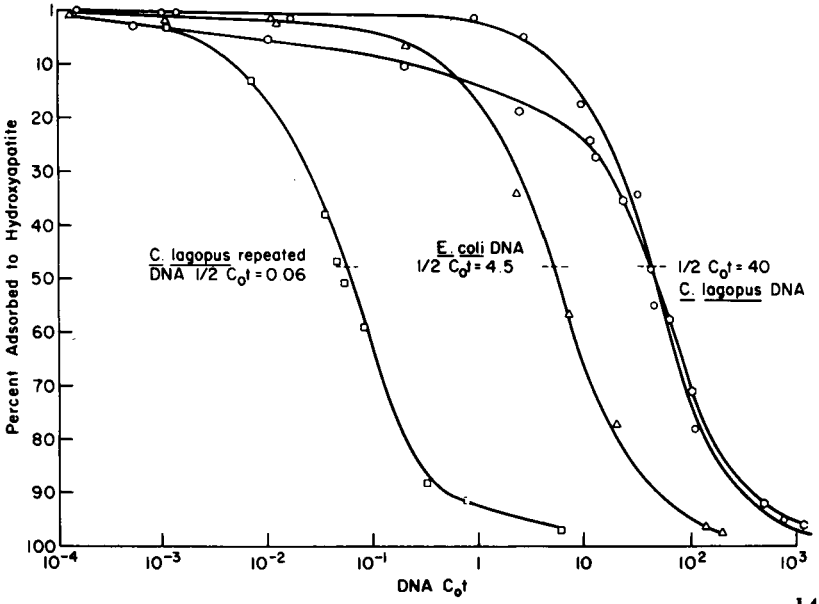

Figure 1. Summary of DNA:DNA reassociation kinetics studies with ^{14}C-labeled E. coli (Δ) DNA (1000 μg), ^{32}P-labeled C. lagopus whole (○) DNA (9,000 μg), non-repeated (o) DNA (8,500 μg) and repeated (□) DNA (120 μg).

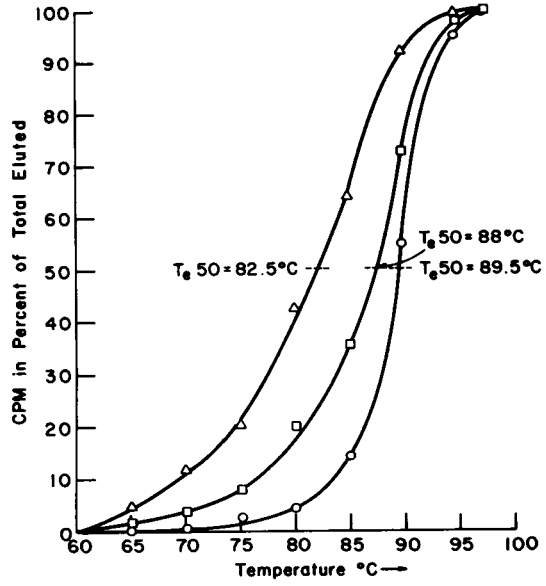

Figure 2. Thermal stability profiles of the homoduplexes of ^{32}P-labeled repeated (Δ) DNA (210,000 cpm/μg DNA), unfractionated (□) DNA and non-repeated (o) DNA of C. lagopus. Procedures of these studies are described elsewhere (13).

homogeneous nature of these DNAs and (b) thermal stability curves of repeated DNA homoduplexes showed characteristically 5-6°C lower T_e50 (i.e. when 50% reassociated DNA dissociate) than that of homoduplexes of unique or whole DNAs of C. lagopus (Fig. 2) and as has been observed in N. crassa (17) as well.

DNA:DNA reassociation kinetics studies indicated that the average size of repeated DNAs of all fungi studied was approximately $3\text{-}4\times10^7$ daltons. The basis of the calculation is as follows. For example, in Figure 1 the haploid complexity of C. lagopus (using both whole and unique) DNA is 2.4×10^{10} daltons i.e. 8.8 times the E. coli genome size of 2.7×10^9 daltons. The repeated DNAs reassociated 75 (i.e. $\frac{1}{2}$ C_0t 4.5/0.06) times faster than DNA of E. coli indicating the size of each copy of repeated DNA as 3.2×10^7 ($2.7\times10^9/75$) daltons. If C. lagopus has 15% repeated DNAs (i.e. 3.6×10^9 daltons), then there are about 112 ($3.6\times10^9/3.2\times10^7$) copies of repeated DNA sequences. As stated before most of these copies of repeated DNAs are similar, if not identical. The haploid complexities of Neurospora and Mucor genomes (13) were 2.2×10^{10} and 2×10^{10} daltons respectively which figures were used as the bases of calculation of complexity of repeated DNA sequences for these fungi.

Detailed studies of dispersion of repeated DNA sequences in higher organisms (5,7) by several workers have given insights for understanding molecular structures and organization of chromosomes. The spectrophotometric thermal denaturation curves of N. crassa DNAs (13) are bimodal indicating one low GC (32 moles %), and another high GC (52 moles %) fractions. These two fractions are not separable in bio-gel column chromatography or by CsCl density gradient centrifugation (Nelson and Dutta, unpublished 1974). This suggests, although not conclusive, that high AT rich regions may be interdispersed in high GC rich regions. Since fungal repeated DNA sequences are AT rich, it is apparent that large fractions of repetitive and single copy DNA sequences are interdispersed throughout the genome.

Unlike most other higher eukaryotes (2) fungal repetitive DNA sequences are apparently mostly similar. This is based on the consistent second order reaction of repeated DNA:DNA reassociation kinetics obtained using DNAs of several fungal species.

Dutta and Kohne (18) calculated that the haploid complexity of N. crassa mitochondrial DNA was approximately $6\text{-}7 \times 10^7$ daltons, which is close to haploid complexity of repetitive DNAs as shown in Table 1. We have run RNA driven DNA:RNA reactions with repeated (Dutta and Chaudhuri, unpublished) DNA of N. crassa at this laboratory. RNA that hybridized to the repetitive DNA apparently constituted a very very small fraction (typical for mitochondrial RNA) of the total cellular RNA because approximately 60,000 $C_o t$ (RNA $C_o t$) was required for completion of reactions. On the other hand Brooks and Huang (10) reported using pulse labeled RNAs that N. crassa repeated DNAs are transcribed.

We (Dutta and Chaudhuri, unpublished) have fractionated mitochondrial DNA from whole cell lysate using poly L-lysine kieselguhr method (19) indicating that N. crassa mitochondrial DNA compromises less than 3-4% of N. crassa total DNA. Hence it is evident that part of the repetitive DNA in N. crassa is of nuclear origin and part could be mitochondrial DNA.

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REFERENCES

1. Britten, R. J. and Waring, M. (1965) Carnegie Inst. Washington Year Book 64, 316-317.
2. Britten, R. J. and Kohne, D. E. (1968) Science 161, 529-540.
3. Britten, R. J. and Davidson, E. H. (1969) Science 165, 349-357.
4. Hough, B. R. and Davidson, E. H. (1972) J. Mol. Biol. 70, 491-509.
5. Crippa, M., Meza, I. and Dina, D. (1973) Cold Spring Harb. Symp. Quant. Biol. XXXVIII 933-942.
6. Brown, D. D., Wensink, P. C. and Jordan, E. (1972) J. Mol. Biol. 63, 57-73.

7. Lodish, H. F., Firtel, R. A. and Jacobson, Allan (1973) Cold Spring Harb. Symp. Quant. Biol. XXXVIII, 899-914.
8. Dutta, S. K. and Schwartz, R. (1973) Neurospora Newsletter 20, 19-20.
9. Williams, W. K. and Dutta, S. K. (1972) Genetics 71, (abs).
10. Brooks, R. R. and Huang, P. C. (1972) Biochem. Genetics 6, 41-49.
11. Dutta, S. K., Richman, N., Woodward, V. W. and Mandel, M. (1967) Genetics 57, 719-727.
12. Dutta, S. K., Penn, S. R., Knight, A. R. and Ojha, M. (1972) Experimentia 28, 582-584.
13. Dutta, S. K. and Ojha, M. (1972) Mol. Gen. Genetics 114, 232-240.
14. Britten, R. J., Graham, D. E. and Neufeld, B. R. (1974) Methods in Enzymology Vol. XXIX part E. Edtd. by Grossman and Moldave pages 363-418.
15. Chattopadhayay, S. K., Kohne, D. E. and Dutta, S. K. (1972) Proc. Natl. Aca. Sci. 69, 3256-3259.
16. Ojha, M. and Turian, G. (1971) Mol. Gen. Genetics 112, 49-59.
17. Dutta, S. K. (1973) Biochem. Biophys. Acta. 324, 482-487.
18. Dutta, S. K. and Kohne, D. E. (1969) Proc. XI Bot. Cong. Seattle, Washington, abs. page 50.
19. Finkelstein, D. B., Blamire, J. and Marmur, J. (1972) Biochemistry 11, 4853-4858.