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Sequence Complexities of the Nuclear and Mitochondrial Genomes of the Take-all Fungus, *Gaeumannomyces graminis* var. *tritici*

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The nuclear (n) and mitochondrial (mt) genomes of the take-all fungus, *Gaeumannomyces graminis* var. *tritici*, were examined by reassociation kinetics with *Escherichia coli* DNA as internal standard. Only one kinetic component was detected in each DNA, with second-order rate constants of 0·022 M^{-1} s^{-1} for nDNA and 10·9 M^{-1} s^{-1} for mtDNA, corresponding to sequence complexities of 29 × 10^6 base pairs and 60 × 10^3 base pairs respectively.

INTRODUCTION

Gaeumannomyces graminis var. *tritici* (hereafter called *G. graminis*), which causes take-all, an important root-rot disease of wheat and barley, is a homothallic ascomycete fungus (Asher & Shipton, 1981). Although *G. graminis* has been much studied as a plant pathogen there have been few investigations on the genetics or molecular biology of the fungus. In the present communication we report measurements of the sequence complexities of the nuclear (n) and mitochondrial (mt) genomes of *G. graminis* by DNA reassociation kinetics and compare them with those of other ascomycete fungi.

METHODS

DNA extraction and characterization. Gaeumannomyces graminis clone 3b1a conE/A1 was grown in shaken liquid culture to stationary phase as described previously (McFadden *et al.*, 1983). Isolated nuclei were obtained, and nuclear and total DNA were extracted, by the methods described by Timberlake (1978) for *Aspergillus nidulans*. Separation of nDNA and mtDNA was achieved by centrifugation in CsCl density gradients (starting density 1·62 g cm^{-3}), containing 1 mg ethidium bromide ml^{-1}, at 45000 r.p.m. for 24 h in a Beckman VTi rotor. Thermal denaturation of DNA was carried out in a Varian Cary 219 spectrophotometer. DNA to be sized was electrophoresed in alkaline agarose gels with *Hin*dIII and *Eco*RI restriction endonuclease digests of phage λ DNA and φX174 DNA as standards as described by McDonell *et al.* (1977).

DNA reassociation kinetics. Denaturation and reassociation of DNA were carried out essentially as described by Britten *et al.* (1974) except that 10 mM-Tris/HCl, 2 mM-Na_2EDTA, pH 7·6 buffer (for denaturation) and 10 mM-Tris/HCl, 0·15 M-NaCl, 2 mM-Na_2EDTA, pH 7·6 buffer (for reassociation) were used and the fraction of DNA remaining single-stranded was assayed with S1 nuclease (Hastie *et al.*, 1978). The *G. graminis* DNA was end-labelled (after sonication) with ^{32}P using [γ-^{32}P]ATP and polynucleotide kinase (Szekely & Sanger, 1969), and *Escherichia coli* DNA, which was used as an internal standard, was labelled with ^3H, by nick translation (Rigby *et al.*, 1977). Unlabelled and nick-translated DNA were sonicated to an average size of 500 nucleotides as determined by alkaline agarose gel electrophoresis. It has been shown that when S1 nuclease is used to measure renaturation of randomly sheared DNA, the reaction does not follow ideal second-order kinetics, but follows the form $S/C_0 = \{1/(1 + kC_0t)\}^{0.45}$, where S is the concentration of totally single-stranded (ss) DNA, C_0 is the concentration of ssDNA at the start of the reaction, t is time and k is the second-order rate constant (Hastie *et al.*, 1978). This equation rearranges to $1 + kC_0t = (C_0/S)^{2.22}$, so that k can be determined from plots of $(C_0/S)^{2.22}$ against C_0t.

RESULTS AND DISCUSSION

The total cellular DNA of *G. graminis* had an $A_{260}:A_{280}$ ratio of 2·0 and a hyperchromicity on thermal denaturation of 40%; it migrated in agarose gel electrophoresis with an apparent size of

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25 × 10^3 base pairs (bp). After isopycnic centrifugation in caesium chloride density gradients two clear bands near the middle of the gradient were detected. When DNA was prepared from isolated nuclei, only the lower of the two bands was obtained. The lower band therefore represents nDNA and the upper band mtDNA. A similar separation of nDNA and mtDNA in caesium chloride gradients has been reported for another ascomycete fungus, *Podospora anserina* (Stahl *et al.*, 1978).

The sequence complexities of *G. graminis* nDNA and mtDNA were determined by reassociation kinetics with ^{32}P-labelled DNA and ^{3}H-labelled *E. coli* DNA as internal standard as described in Methods. Least squares analysis of the data indicated the presence of only one kinetic component for each DNA with second-order rate constants 0·022 $M^{-1} s^{-1}$ for *G. graminis* nDNA, 10·9 $M^{-1} s^{-1}$ for *G. graminis* mtDNA and 0·14 $M^{-1} s^{-1}$ for *E. coli* DNA. Reciprocal C_0t plots are shown in Fig. 1. Taking the genome size for *E. coli* to be 4·6 × 10^6 bp (Zimmerman & Goldberg, 1977), the sequence complexity of *G. graminis* nDNA was calculated to be 29 × 10^6 bp. This value is similar to the nuclear genome sizes of two other ascomycete fungi, *Aspergillus nidulans* (26 × 10^6 bp, Timberlake, 1978) and *Neurospora crassa* (27 × 10^6 bp, Krumlauf & Marzluf, 1980). Furthermore, the detection of only one kinetic component indicates the absence of substantial amounts of repetitive DNA. In this respect it is significant that Timberlake (1978) found only 2 to 3% of reiterated sequences (probably the genes for ribosomal RNA) in the *A. nidulans* nuclear genome and these sequences could only be detected after a hydroxyapatite enrichment procedure. Our results support Timberlake's suggestion that the ascomycete fungi do not contain interspersed reiterated DNA sequences as found in the higher eukaryotes.

The sequence complexity of *G. graminis* mtDNA was calculated to be 60 × 10^3 bp. This is within the range of mitochondrial genome sizes found for other ascomycetes (cf. *A. nidulans*, 32 × 10^3 bp, Grisi *et al.*, 1982; *N. crassa*, 62 × 10^3 bp, Bernard *et al.*, 1975; *Saccharomyces cerevisiae*, 70 × 10^3 bp, Caron *et al.*, 1979; *P. anserina*, 95 × 10^3 bp, Cummings *et al.*, 1979). Since mtDNA comprised 20% of the total DNA in G. *graminis* grown to stationary phase and since the fungus is haploid and hyphal compartments are predominantly uninucleate (Asher & Shipton, 1981), it can be calculated that there are about 100 copies of mtDNA per hyphal compartment. This compares with 100 copies of mtDNA found in stationary phase cells of *S. cerevisiae* (Caron *et al.*, 1979).

Many isolates of *G. graminis* are infected with double-stranded (ds) RNA virus particles (Buck *et al.*, 1981). Knowledge of the sizes of the n and mt genomes reported here, together with determination of the cellular dsRNA/DNA ratio, has recently enabled us to determine the average number of dsRNA molecules (and hence virus particles) per hyphal compartment (300

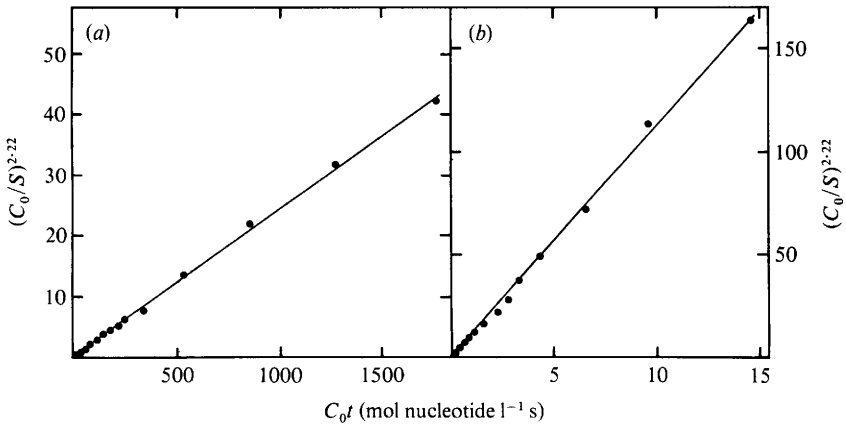

Fig. 1. Reciprocal C_0t plots of (*a*) nDNA and (*b*) mtDNA from *G. graminis*. See Methods for terminology.

for *G. graminis* isolate 3b1a; McFadden *et al.*, 1983). This value compares with 200 to 1000 molecules of L dsRNA per cell found in most isolates of *S. cerevisiae* (Brewer & Fangman, 1980).

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