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

By J. J. P. MCFA DDEN and K. W. BUCK*

Department of Pure and Applied Biology, Imperial College of Science and Technology, London SW7 2BB, U.K.

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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 $29 \times 10^6$ base pairs and $60 \times 10^3$ 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 3bl a 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 HindIII and EcoRI restriction endonuclease digests of phage $\lambda$ DNA and pX174 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$_2$EDTA, pH 7.6 buffer (for denaturation) and 10 mM-Tris/HCl, 0.15 M-NaCl, 2 mM-Na$_2$EDTA, 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 $[^{32}$P]ATP and polynucleotide kinase (Szekely & Sanger, 1969), and *Escherichia coli* DNA, which was used as an internal standard, was labelled with $^3$H, 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 $S/C_0 = \frac{1}{(1 + kC_0t)^{2/3}}$, 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 $1 + kC_0t = (C_0/S)^{2/3}$, so that $k$ can be determined from plots of $(C_0/S)^{2/3}$ 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 3515-3517.
25 × 10³ base pairs (bp). After isopycnic centrifugation in cesium 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 cesium 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 ³²P-labelled DNA and ³H-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 C₀t plots are shown in Fig. 1. Taking the genome size for E. coli to be 4·6 × 10⁶ bp (Zimmerman & Goldberg, 1977), the sequence complexity of G. graminis nDNA was calculated to be 29 × 10⁶ bp. This value is similar to the nuclear genome sizes of two other ascomycete fungi, Aspergillus nidulans (26 × 10⁶ bp, Timberlake, 1978) and Neurospora crassa (27 × 10⁶ 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 × 10³ bp. This is within the range of mitochondrial genome sizes found for other ascomycetes (cf. A. nidulans, 32 × 10³ bp, Grisi et al., 1982; N. crassa, 62 × 10³ bp, Bernard et al., 1975; Saccharomyces cerevisiae, 70 × 10³ bp, Caron et al., 1979; P. anserina, 95 × 10³ 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

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**Fig. 1.** Reciprocal C₀t plots of (a) nDNA and (b) mtDNA from G. graminis. See Methods for terminology.
for *G. graminis* isolate 3bla; 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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**REFERENCES**


