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

By J. J. P. McFADDEN AND K. W. BUCK*

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

(Received 13 June 1983)

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 $0.022 \text{ M}^{-1} \text{ s}^{-1}$ for nDNA and $10.9 \text{ M}^{-1} \text{ s}^{-1}$ 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 *Hind*III and *Eco*RI restriction endonuclease digests of phage λ DNA and ϕ X174 DNA as standards as described by McDonnell *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 , $2 \text{ mM-Na}_2\text{EDTA}$, pH 7.6 buffer (for denaturation) and 10 mM-Tris/HCl , 0.15 M-NaCl , $2 \text{ mM-Na}_2\text{EDTA}$, 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 $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ 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

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 ^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 $0.022 \text{ M}^{-1} \text{ s}^{-1}$ for *G. graminis* nDNA, $10.9 \text{ M}^{-1} \text{ s}^{-1}$ for *G. graminis* mtDNA and $0.14 \text{ M}^{-1} \text{ s}^{-1}$ 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).

We thank Dr C. J. Rawlinson for providing *G. graminis* isolates and for useful discussions, Dr P. W. J. Rigby for advice and the Science and Engineering Research Council for the award of a studentship (to J. J. P. M.)

REFERENCES

- ASHER, M. J. C. & SHIPTON, P. J. (1981). *Biology and Control of Take-all*. London & New York: Academic Press.
- BERNARD, U., BADE, E. & KÜNTZEL, H. (1975). Specific fragmentation of mitochondrial DNA from *Neurospora crassa* by restriction endonuclease *EcoRI*. *Biochemical and Biophysical Research Communications* **64**, 783–789.
- BREWER, B. J. & FANGMAN, W. L. (1980). Preferential inclusion of extrachromosomal genetic elements in yeast meiotic spores. *Proceedings of the National Academy of Sciences of the United States of America* **77**, 5380–5384.
- BRITTEN, R. J., GRAHAM, D. E. & NEUFELD, B. R. (1974). Analysis of repeating DNA sequences by reassociation. *Methods in Enzymology* **29**, 363–418.
- BUCK, K. W., ALMOND, M. R., MCFADDEN, J. J. P., ROMANOS, M. A. & RAWLINSON, C. J. (1981). Properties of thirteen viruses and virus variants obtained from eight isolates of the wheat take-all fungus *Gaeumannomyces graminis* var. *tritici*. *Journal of General Virology* **53**, 235–245.
- CARON, F., JACQ, C. & ROUVIERE-YANIV, J. (1979). Characterisation of a histone-like protein extracted from yeast mitochondria. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 4265–4269.
- CUMMINGS, D. J., BELCOUR, L. & GRANDCHAMP, C. (1979). Mitochondrial DNA from *Podospira anserina*. I. Isolation and characterisation. *Molecular and General Genetics* **171**, 229–238.
- GRISI, E., BROWN, T. A., WARING, R. B., SCAZZOCHIO, C. & DAVIES, R. W. (1982). Nucleotide sequence of a region of the mitochondrial genome of *Aspergillus nidulans* including the gene for ATPase subunit 6. *Nucleic Acids Research* **10**, 3531–3539.
- HASTIE, N. D., BRENNAN, V. & BRUENN, J. A. (1978). No homology between double-stranded RNA and nuclear DNA of yeast. *Journal of Virology* **28**, 1002–1005.
- KRUMLAUF, R. & MARZLUF, G. A. (1980). Genome organisation and characterisation of the repetitive and inverted repeat DNA sequences in *Neurospora crassa*. *Journal of Biological Chemistry* **255**, 1138–1145.
- MCDONELL, M. W., SIMON, M. N. & STUDIER, F. W. (1977). Analysis of restriction fragments of T7 DNA and determination of molecular weights by electrophoresis in neutral and alkaline gels. *Journal of Molecular Biology* **110**, 119–146.
- MCFADDEN, J. J. P., BUCK, K. W. & RAWLINSON, C. J. (1983). Infrequent transmission of double-stranded RNA particles but absence of DNA proviruses in single ascospore cultures of *Gaeumannomyces graminis*. *Journal of General Virology* **64**, 927–937.
- RIGBY, P. W. J., DIECKMANN, M., RHODES, C. & BERG, P. (1977). Labelling of deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. *Journal of Molecular Biology* **113**, 237–251.
- STAHL, U., LEMKE, P. A., TUDZYNSKI, P., KÜCK, U. & ESSER, K. (1978). Evidence for plasmid-like DNA in a filamentous fungus, the ascomycete *Podospira anserina*. *Molecular and General Genetics* **162**, 341–343.
- SZEKELY, M. & SANGER, F. (1969). Use of polynucleotide kinase in fingerprinting non-radioactive nucleic acids. *Journal of Molecular Biology* **43**, 607–617.
- TIMBERLAKE, W. E. (1978). Low repetitive DNA content in *Aspergillus nidulans*. *Science* **202**, 973–975.
- ZIMMERMAN, J. L. & GOLDBERG, R. B. (1977). DNA sequence organisation in the genome of *Nicotiana tabacum*. *Chromosoma* **59**, 227–252.