Volume 298, number 2,3, 109–112 FEBS 10717 February 1992

Superproduction of heavy minicircular mitochondrial DNA in aging wheat coleoptiles

M.D. Kirnos, N.I. Alexandrushkina, G.Ya. Zagorskaya, I.I. Kireev and B.F. Vanyushin

A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, 119899, USSR

Received 29 November 1991

On incubation of 7-day-old wheat seedlings in the presence of [^{3}H]thymidine, the radioactivity incorporated into coleoptile DNA is found to be localized mainly (>95%) in the fraction of heavy mitochondrial DNA (H-mt DNA; ρ = 1.716 gm/cm^3). Upon long (48–72 h) incubation of cut-off seedlings in water, the amount of this DNA shows a dramatic increase and corresponds to about 10% of the total coleoptile DNA. H-mtDNA is represented by open circular molecules with a contour length varying from 0.12 to 0.6 μm. The functional role of this DNA is still unknown.

Heavy wheat mitochondrial DNA; Synthesis; Size

1. INTRODUCTION

In contrast to the widely accepted concept of a more or less unimodal character of higher plant mitochondrial DNAs in GC-content [1,2], there is evidence that these DNAs may be heterogeneous in buoyant density [3–8]. For example, recently we detected a heavy mtDNA fraction in total wheat mtDNA as well as in mtDNA of other higher plants [3,4]. In differentiated leaf and coleoptile cells of wheat seedlings, the radioactive DNA precursors are incorporated more intensively in this heavy (ρ = 1.716 gm/cm^3) fraction of mitochondrial DNA when compared with other mtDNAs or nuclear DNAs [3,4].

Analysis of nucleotide sequences of light plasmide-like mtDNA of maize, sunflower and winter cress also clearly shows that plant mtDNA molecules are polymodal in the GC-content in one and the same plant as well as in various plant species [5–8].

Unfortunately, the autonomous replication ability, the replication pattern, the regulation of synthesis and the biological role of individual mtDNA populations in plants at different stages of ontogenesis have actually not been studied as yet. In this respect the heavy mtDNA detected in wheat [3,4,9,10] is a very good model for studying the different aspects of plant mtDNA replication because (1) this is the only DNA that most actively replicates in the differentiated cells of plant organs already formed, and (2) it can be easily discriminated amongst various cellular DNAs by its density and can be isolated by menas of CsCl density gradient centrifugations. The newly formed H-mtDNA is metabolically stable [3]. In wheat seedlings, the synthesis of H-mtDNA is periodic, well coordinated with nuclear DNA replication in meristem cells of the leaf basal part and regulated by exogenous phytohormones [9,10].

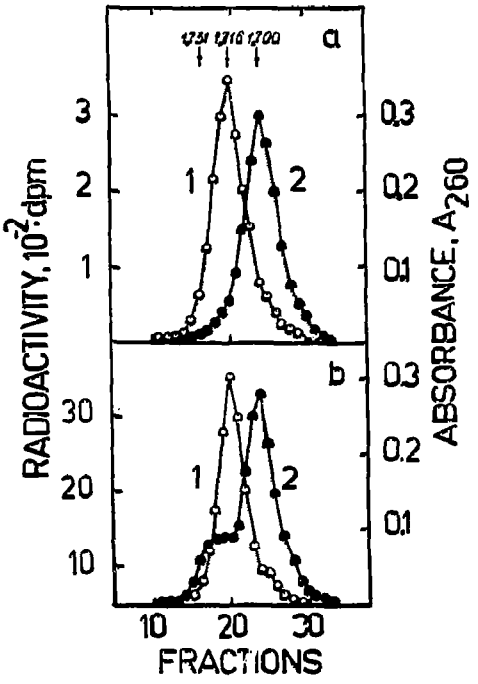

Fig. 1. Distribution pattern of the newly formed [^{3}H-methyl]thymidine-labeled (1) and the total (2) wheat coleoptile DNA in a CsCl density gradient. Coleoptile DNA from 7-day-old cut-off seedlings incubated for 2 h (a) and for 48 h (b) with radioactive thymidine.

Abbreviations: mtDNA, mitochondrial DNA; H-mtDNA, heavy mitochondrial DNA; nDNA, nuclear DNA

Correspondence address: M.D. Kirnos, A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, 119899, USSR.

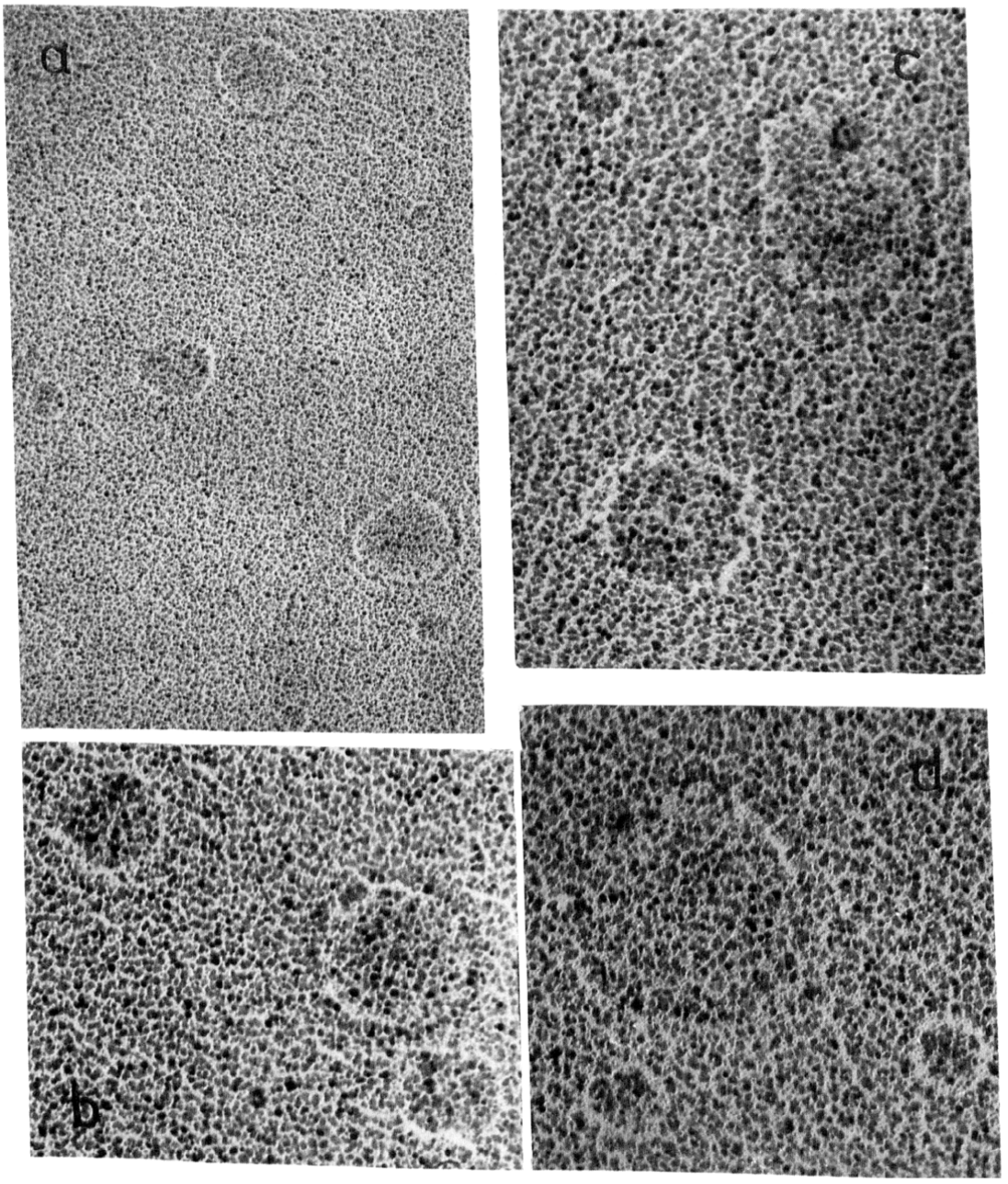

Fig. 2. Electron microscopy photographs of wheat heavy (ρ = 1.716 gm/cm^3) mitochondrial DNA. (a) × 80,000; (b, c, d) × 160,000.

In this paper we present data on the isolation and some structural characteristics of wheat heavy mitochondrial DNA.

2. MATERIALS AND METHODS

Seed germination and growth synchronization of winter Mironovskaya 808 wheat seedlings were carried out as described earlier [9]. 7-day-old seedlings were harvested by cutting off and incubated for 24–72 hours in water in the presence of [^{3}H-methyl]thymidine (10–100 μCi/ml, specific radioactivity 52 Ci/mM, Isotop, USSR).

Coleoptiles were isolated and ground in a mortar at temperature of the liquid nitrogen; the fine plant powder thus obtained was extracted at 0–4°C with a solution containing 1% Triton X-100, 0.10 M NaCl and 0.05 M Tris-HCl, pH 7.5 (conditions of minimal chromatin solubility). Insoluble chromatin and cell debris were separated by centrifugation (3,000–10,000 × g, 0–4°C, 10 min); the supernatant obtained was supplemented with 0.1 vols. of 10% sodium dodecyl sulfate and 1.0 M EDTA solutions (each), as well as with dry CsCl (up to its final concentration equal to 56%, w/v). Denatured proteins, floated in this CsCl solution, were removed by centrifugation (15,000 × g, 20°C, 30 min), and the lysate was centrifuged (48 hours, 20°C, 44,000 rpm) in a U-65 rotor of a K-32 ultracentrifuge (USSR). 0.2 ml fractions of the gradient were collected from the tube bottom, and their radioactivity and DNA content were determined. H-mtDNA was located by radioactivity measurement. The isolated H-mtDNA was dialyzed against a 0.001 M NaCl, 0.001 M EDTA solution and centrifuged once more in a CsCl density gradient.

The purified H-mtDNA fraction was studied by means of electron microscopy (Hitachi HU-11 Model Electron Microscope, Japan) according to the Kleinschmidt and Zahn procedure [11].

H-mtDNA electrophoresis was carried out according to the known procedure [12] for 2–3 h in 1.2% horizontal agarose gels at 2–3 V/cm in a 0.089 M Tris-borate buffer solution pH 8.3 containing 0.5 μg/ml ethidium bromide.

The total DNA from coleoptiles fixed with ethanol was isolated by pronase E (Serva) and RNase A (Sigma) treatments with subsequent chloroform deproteinization; then it was centrifuged in a CsCl density gradient [3,4,9,10].

3. RESULTS AND DISCUSSION

We found earlier that H-mtDNA replication proceeds periodically during wheat seedling growth [9]. In coleoptile the maxima of H-mtDNA synthesis are well coordinated with the replication cycles of nuclear DNA in leaf basal meristem cells [9]. When the replication of nDNA in cut-off seedlings is blocked by prolonged incubation in water or by cycloheximide treatment [3], the H-mtDNA synthesis is very significant and is modulated by exogenous phytohormones [10].

In this particular work we have found that in coleoptiles of cut-off deficient wheat seedlings with blocked nDNA synthesis the H-mtDNA synthesis, proceeding at least for a few days, results in a strong increase in the H-mtDNA amount in coleoptile cells. This can be easily seen as an essential inflection on the UV-light adsorption profiles of the total coleoptile DNA in the CsCl gradient zone with the buoyant density value (ρ) equal to 1.716 gm/cm^3 (Fig. 1). Thus, in this particular case, the amount of H-mtDNA formed in coleoptiles under our experimental conditions is sufficiently high for detection not only by radioactivity but also by UV-light adsorbance measurements. In the total DNA or the coleoptile DNA isolated from normally grown seedlings, the H-mtDNA is practically undetectable by UV-light adsorbance measurement [3,9].

The share of the H-mtDNA fraction is cut-off deficient seedlings makes up about 10% of the total coleoptile DNA (Fig. 1). In this case the amount of H-mtDNA is about 10-fold higher as compared with the total mitochondrial DNA content in normal wheat seedlings. This is a unique fact showing that the prolonged starvation of seedlings (incubation in water) is accompanied by significant superproduction of the specific plant mtDNA fraction.

The selective accumulation of H-mtDNA in coleoptiles of deficient wheat seedlings has allowed us to isolate molecules of this plant mtDNA with relative ease (see section 2) and study them by means of electron microscopy and electrophoresis.

Fig. 2 shows that the heavy wheat mtDNA is represented by open circular molecules of various contour lengths. The minimal contour length of this mtDNA is equal to 0.12 μm, which corresponds to about 360 base pairs in these DNA molecules. As far as we know, 360 bp circular molecules are the smallest ones found in the plant mtDNA thus far. Wheat H-mtDNA with the maximal contour length 0.6 μm contains about 1800 base pairs.

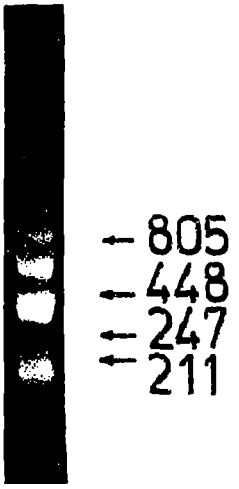

Fig. 3. Electrophoregram of the open circular heavy wheat mtDNA fraction in a 1.2% agarose gel. Products of λ phage DNA hydrolysis with *Pst*I restriction nuclease are used as markers. The size of DNA fragments (base pairs) is indicated.

Small circular mtDNAs are common in plants. Thus, different maize cytoplasms contain low-molecular-weight mtDNA species of 1.42 and 1.57 kb (C cytoplasm), 2.35 kb (N, C, S cytoplasms) and 1.94 kb (N, T, C, S cytoplasms) [13]. 2.1 kb [14] and 0.97 kb mitochondrial plasmid-like DNA, designated as B4 [15], were found in rice. 1–2 kb small circular mtDNA molecules were detected in *Vicia faba* plants [16]. More than 90% of the total circular mtDNA molecules from the broad bean have a contour length within the range of 0.4–0.54 μm [2]. Minicircular DNA with a contour length of 0.2–0.6 μm was found in a supercoiled state in sugar beet mitochondria [17]. Plasmid-like DNA of 0.3–1.2 kb have been detected in mitochondria from various *Epilobium* species [18].

Trying to fractionate the purified H-mtDNA molecules according to their size, we kept in mind that all the molecules in the investigated wheat H-mtDNA fraction are similar or even identical in conformation (Fig. 2). This population of isolated wheat H-mtDNA was separated by electrophoresis in 1.2% agarose gels into at least five individual fractions which seem to be different in size (Fig. 3).

Thus, using CsCl density gradient centrifugation in conjunction with agarose gel electrophoresis, it is possible to effectively isolate the heavy plant mitochondrial DNA and fractionate it into molecules more or less homogenous in the GC-content, size and shape; this may be quite useful for further research into their primary structure and function. In particular, in this way a new class of plant heavy minicircular mitochondrial

DNAs free of nuclear, chloroplast and other mtDNAs is obtained.

REFERENCES

[1] Lejene, B., Quetier, F., Jubier, M.F., Falconet, D., Rode, A. and Hartmann, C. (1988) Bull. Soc. Bot. Fr. Actual Bot. 135, 49–55.
[2] Balley-Serres, J., Leroy, P., Jones, S.S., Wahleithner, J.A. and Wolstenholme, D.R. (1987) Curr. Genet. 12, 49–53.
[3] Kirnos, M.D., Bakeeva, L.E., Volkova, S.A., Ganicheva, N.I. and Vanyushin, B.F. (1983) Biochimiya (Russ) 48, 1505–1512.
[4] Vanyushin, B.F., Alexandrushkina, N.I. and Kirnos, M.D. (1988) FEBS Lett. 233, 397–399.
[5] Paillard, M., Sederoff, R.R. and Levings, C.S. (1985) EMBO J. 4, 1125–1128.
[6] Levings, C.S. and Sederoff, R.R. (1983) Proc. Natl. Acad. Sci. USA 80, 4055–4059.
[7] Crouzillat, D., Gentzbitte, L., Canal, L., de la Vaury, C., Perrault, A., Nicolas, P. and Ledoigt, G. (1989) Curr. Genet. 15, 283–289.
[8] Turpen, T., Garger, S.J., Marks, M.D. and Grill, L.H. (1987) Mol. Gen. Genet. 209, 227–233.
[9] Kirnos, M.D., Volkova, S.A., Ganicheva, N.I., Kudryashova, I.B. and Vanyushin, B.F. (1983) Biochimiya (Russ) 48, 1587–1596.
[10] Alexandrushkina, N.I., Kudryashova, I.B., Kirnos, M.D. and Vanyushin, B.F. (1990) Biochimiya (Russ) 55, 2038–2044.
[11] Kleinschmidt, A. and Zahn, R.K. (1959) Z. Naturforsch. 14b, 770.
[12] Maniatis, T.F., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York.
[13] Kemble, R.J. and Bedbrook, J.R. (1980) Nature 284, 565–566.
[14] Shikanai, T., Yang, Z.Q. and Yamada, Y. (1987) Plant Cell Physiol. 28, 1243–1251.
[15] Shikanai, T. and Yamada, Y. (1988) Curr. Genet. 13, 441–443.
[16] Negruk, V.I., Cherny, D.I., Nikiforova, I.D., Aleksandrov, A.A. and Butenko, R.G. (1982) FEBS Lett. 142, 115–117.
[17] Dudareva, N.A., Kiseleva, E.V., Boyarintseva, A.E., Maystrenko, A.G., Khristolyubova, N.B. and Salganik, R.I. (1988) Theor. Appl. Genet. 76, 753–759.
[18] Schmitz, U.K. (1988) Curr. Genet. 13, 411–415.