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N^6-Methyladenine in mitochondrial DNA of higher plants

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After incubation of etiolated wheat seedlings in the presence of [8-^{14}C]adenine the radioactive N^6-methyladenine (m^6A) has been detected in the newly synthesized mitochondrial DNA (mtDNA) ($ML = 100 \cdot m^6A/(m^6A + A) = 0.4–0.6$). This DNA is a low molecular mass (7.7 S) fraction of the mtDNA population. The detection of N^6-methyladenine in the mtDNA of wheat seedlings indicates the presence of adenine DNA methylase in mitochondria of higher plants. The presence of m^6A in plant mtDNAs makes them distinct from animal mtDNAs in which, as we know, this additional base has not been found.

N^6-Methyladenine; DNA methylation; Mitochondrial DNA; (Higher plant)

1. INTRODUCTION

Although N^6-methyladenine was found in the higher plant DNA a long time ago [1-3], the functional role of this DNA modification as well as the location of the m^6A containing DNA in plant cells are unknown.

Here we have studied the ML of adenine residues in labeled mitochondrial (mt) and nuclear (n) DNA synthesized in etiolated wheat seedlings incubated in the presence of [8-^{14}C]adenine.

2. EXPERIMENTAL

Mironovskaya-808 wheat seeds were germinated and the growth of seedlings was synchronized, as described in [4]. Before the onset of mtDNA synthesis in the coleoptile or of the S-phase in the initial leaf cells [4], from 30 to 60 seedlings were cut off and incubated in a solution of [8-^{14}C]adenine (200 μCi/ml, Isotope, USSR) or [5-^{3}H]thymidine (10 μCi/ml, spec. act. 51 Ci/mmol, Isotope). nDNA and mtDNA for the determination of the degree of adenine methylation were isolated from the basal part of leaf (nDNA) and both coleoptile and distal part of the first leaf (mtDNA) by pronase and RNase treatment and CsCl density gradient fractionation of cellular lysates [4]. Undergraded mtDNA for the fractionation by the neutral sucrose density gradient centrifugation (5–20% sucrose in 1 M NaCl/0.001 M Na_4 EDTA) was isolated very carefully from coleoptiles of seedlings labeled with [5-^{3}H]thymidine and pulverised at liquid nitrogen temperature; the cellular lysate (0.5% SDS/0.01 M Na_4 EDTA) obtained was fractionated by a CsCl density gradient centrifugation [4,5]. DNA isolated were treated with 0.5 M NaOH at 37°C for 18 h to remove RNA; DNA was hydrolyzed to bases with 57% $HClO_4$ (100°C, 1 h). The bases were separated by two-dimensional TLC on cellulose, and their radioactivity was measured as in [4–6].

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Abbreviations: mtDNA, clDNA, nDNA, nsDNA, mitochondrial, chloroplast, nuclear and newly synthesized DNA, respectively; m^6A, N^6-methyladenine; m^5C, 5-methylcytosine; A, adenine; C, cytosine; ML, methylation level; TLC, thin-layer chromatography

3. RESULTS AND DISCUSSION

Replication of nDNA in a fully formed and ageing coleoptile or distal part of the first leaf of etiolated wheat seedlings is already completed and DNA synthesis proceeds, practically, only in mitochondria [4,7]. In the dividing cells of the basal part of first leaf of the same seedlings, radioactive precursors are incorporated practically into nDNA alone [4]. Wheat mtDNA differs from nuclear and chloroplast DNAs in base composition (buoyant density) [4,7,8] and thus can be separated from the bulk of the cellular DNA and effectively isolated

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Table 1

The adenine methylation level in mtDNA and nDNA from different organs of etiolated wheat seedlings

Nature and source of DNA	$100 \cdot m^6A/(m^6A + A)$ $(\bar{x} \pm \sigma)$
80-h-old seedlings	
mtDNA, coleoptile	0.46 ± 0.07
nDNA, leaf	0.09 ± 0.04
7-day-old seedlings	
mtDNA, coleoptile	0.55 ± 0.10
mtDNA, distal part of leaf	0.50 ± 0.06
nDNA, basal part of leaf	0.10 ± 0.10

$\bar{x}$, mean; σ, standard deviation. 80-h or 7-day-old seedlings were cut off and incubated for 20 h in the presence of [8-^{14}C]adenine (200 μCi/ml). The DNA synthesized in the first leaf and coleoptile cells were fractionated by CsCl density gradient centrifugation; mtDNA and nDNA fractions were isolated, hydrolyzed to bases and ML was determined

by CsCl density gradient centrifugation of the total labeled DNA preparations [4,7].

After incubation of 80 h or 7-day-old wheat seedlings with [8-^{14}C]adenine for 20 h and fractionation of nsDNA from the basal and distal parts of the first leaves and coleoptiles by a CsCl density gradient centrifugation, we isolated the nDNA and mtDNA and determined their MLs of adenine residues (table 1). m^6A was detected in mtDNA only. This base is either absent from the nDNA or its amount is below the sensitivity of the method used and so it could not be detected there with a good fidelity. According to our preliminary data clDNA as well as nDNA is either not methylated at adenine residues or the m^6A content in these DNAs is below the sensitivity of the assay method.

To assess the sensitivity of the methods used for assaying m^6A in labeled DNAs, we performed, concurrently with two-dimensional TLC, a chromatography of [8-^{14}C]adenine ($1-5 \times 10^5$ dpm) and assayed the radioactivity in the m^6A zone from the labeled adenine. We found by using standard chromatographic techniques [6], that no more than 0.05% of the [^{14}C]adenine radioactivity migrates into the m^6A zone. So, this value is the real limit of the assay method.

It is well known that the mtDNA of plants is heterogeneous in size and is represented by a population of molecules differing in length [9,10]. To assess the level of heterogeneity of the mtDNAs synthesized in the coleoptile, we labeled the

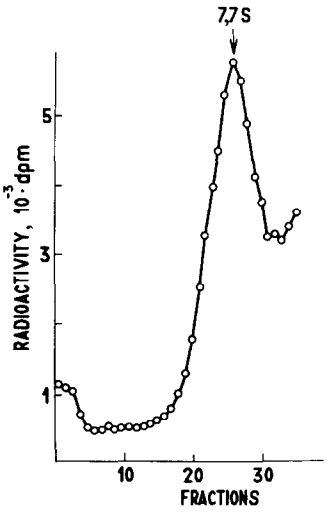

Fig.1. The radioactivity distribution pattern of [5-^{3}H]thymidine-labeled mtDNA from the coleoptiles of etiolated wheat seedlings which had been subjected to neutral sucrose density gradient centrifugation. Coleoptiles of the labeled seedlings (30) were homogenized at liquid nitrogen temperature and lyzed, and the labeled mtDNA was separated from nDNA by CsCl density gradient centrifugation, as described in [5]. Then the purified mtDNA was centrifuged in a neutral sucrose density gradient (5–20%) by an SW-50.1 rotor at 44 000 rev/min, at 20°C, for 4 h (Spinco L 8-70 preparative ultracentrifuge, Beckman, USA).

nsDNAs of wheat coleoptiles with [5-^{3}H]-thymidine, subjected these coleoptiles to mild lysis, purified their mtDNA by a CsCl density gradient centrifugation and fractionated by neutral sucrose gradient centrifugation. We see (fig.1) that practically the entire radioactivity from [5-^{3}H]-thymidine is localized in a comparatively narrow zone of the sucrose gradient with a sedimentation coefficient of 7.7 S. So, under the labeling conditions described here and elsewhere [4,7], precursors are incorporated mainly into the low molecular mass fraction of the wheat mtDNA population.

The detection of N^6-methyladenine in mitochondrial DNA of wheat seedlings indicates that these subcellular organelles must contain corresponding adenine DNA methylase(s) responsible for the modification of certain selective adenine residues in mtDNA. It is of major interest to identify and study the specificity of action, the structure and the properties of adenine DNA methylase of plant mitochondria. Further, it is important to find out

where this DNA methylase is encoded and where exactly it is synthesized in the cell.

Unfortunately, the functional role of the adenine residue methylation in higher plant DNA is unknown. In prokaryotes it is associated with the regulation of gene activity as well as of DNA replication and reparation [11,12]. At present we can only speculate that also in eukaryotes this enzymatic DNA modification may be engaged in a similar regulatory process of the genome functioning, reparation and replication. Anyway, detection of N^6-methyladenine in plants may support the idea on the possible new role of cytokinines (N^6-substituted adenine derivatives) in cell differentiation as modulators of adenine residue methylation in plant DNA [13].

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