Available online at www.sciencedirect.com
ScienceDirect
Mitochondrion 8 (2008) 15–25
Mitochondrion
www.elsevier.com/locate/mito

Linear plasmids in plant mitochondria: Peaceful coexistences or malicious invasions?

Hirokazu Handa *

Plant Genome Research Unit, National Institute of Agrobiological Sciences, 2-1-2 Kan-non-dai, Tsukuba 305-8602, Japan
Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Ten-noh-dai, Tsukuba 305-8572, Japan

Received 15 August 2007; received in revised form 24 August 2007; accepted 2 October 2007
Available online 11 October 2007

Abstract

Plant mitochondria contain small extrachromosomal DNAs in addition to a large and complex main mitochondrial genome. These molecules can be regarded as extrachromosomal replicons or plasmids, of which there are two forms, circular and linear. Linear mitochondrial plasmids are present in many fungi and in some plants, but they seem to be absent from most animal cells. They usually have a common structural feature, called an invertron, that is characterized by the presence of terminal inverted repeats and proteins covalently attached to their 5′ termini. Linear mitochondrial plasmids possess one to six ORFs that can encode unknown proteins but often code for the DNA and RNA polymerases. Although the functions of most linear plasmids in plant mitochondria are unknown, some plasmids may be associated with mitochondrial genome rearrangements and may have phenotypic effects due to their integration into mitochondrial genome. The *Brassica* 11.6-kb plasmid, one of the linear mitochondrial plasmids in plants, shows a non-maternal inheritance, in contrast to mitochondrial genomes. The origin of these plasmids is still a mystery, but indirect evidence indicates the possibility of horizontal transfer from fungal mitochondria. In this review, the main features of these unique DNAs present in plant mitochondria are described.

Keywords: Linear DNA; Mitochondria; Plants; Plasmids

1. Introduction

Mitochondria, the power plants of eukaryotic cells, are sites of respiration. Most eukaryotic cells produce energy necessary for their survival by a series of oxidative metabolic processes in mitochondria. According to the endosymbiont hypothesis, mitochondria are descended from the symbiosis of α-proteobacteria into ancestral eukaryotic cells. The genome size and gene content of present-day mitochondrial genomes are much smaller than those of the ancestral eubacterial genome, suggesting that massive gene transfer events from the mitochondrion to the nucleus occurred during the evolution of this cellular organelle. However, the mitochondrial genome still retains a subset of genes that encode many of the protein subunits involved in the energy-metabolizing enzyme complexes, ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and several ribosomal proteins. Although the mitochondrial function is conserved in all eukaryotic cells, including animal, fungal, and plant cells, higher plant mitochondrial genomes have a number of unique features compared to their counterparts in animals or fungi. Higher plant mitochondrial genomes are not only larger in size but their structure is also complicated by structural rearrangements due to homologous intra- or inter-molecular recombination events. The mitochondrial genomes of higher plants are the largest mitochondrial genomes so far reported (see review by Kubo and Newton, 2007 in this issue).

In addition to a large and complex main mitochondrial genome, the mitochondria of many higher plant species contain a variety of smaller DNA molecules. These mole-

* Address: Plant Genome Research Unit, National Institute of Agrobiological Sciences, 2-1-2 Kan-non-dai, Tsukuba 305-8602, Japan. Tel.: +81 29 838 8374; fax: +81 29 838 7417.
E-mail address: hirokazu@affrc.go.jp

1567-7249/$ - see front matter
doi:10.1016/j.mito.2007.10.002

cules can be regarded as extrachromosomal replicons or plasmids that can be autonomously replicated in mitochondria because they are usually present at a high stoichiometry relative to the main genome. Extrachromosomal mitochondrial DNA molecules are found in many fungi and in some higher plants, but they seem to be absent from most animal cells. These smaller DNA molecules can be classified into two types, circular DNAs and linear DNAs. Circular DNAs have been found in a diverse group of plant species, ranging in size from approximately 1 to 2 kb (reviewed in Lonsdale and Grienenberger, 1992). Although these circular DNAs usually do not have an open reading frame of significant length, the presence of transcripts, short regions of homology among circular DNAs, and sequences homologous to circular DNAs in the nuclear genome, have been reported (broad bean, Wahleithner and Wolstenholme, 1987; Flamand et al., 1992; date palm, Benslimane et al., 1994; maize, Abbott et al., 1985; Smith and Pring, 1987; rice, Shikanai et al., 1989; sugar beet, Thomas, 1992; sunflower, de la Canal et al., 1991). The linear DNA molecules in plant mitochondria are larger than the circular DNAs, ranging from 2 to 13 kb in size. Sequence analyses conducted so far have revealed that most of them have terminal inverted repeats and contain several long, expressed open reading frames. Their structures are usually very similar to those of mitochondrial plasmids found in several fungal species.

In this review, the structure and gene expression of plant mitochondrial linear plasmids are described in comparison to those of fungal linear mitochondrial plasmids and their possible function in plant mitochondria is discussed.

2. Discovery of linear DNA molecules in plant mitochondria

The presence of linear DNA molecules in mitochondria was initially postulated in *Saccharomyces cerevisiae* (Sinclair et al., 1967). The first linear plasmids actually described in mitochondria were the maize S1 and S2 plasmids, identified as additional bands to the main mitochondrial genome by agarose gel electrophoresis (Pring et al., 1977). A mitochondrial linear plasmid, pAI, was then detected in the fungal species *Ascobolus immerses* (Francou, 1981). The number of linear plasmids identified in fungal and plant mitochondria has since been increasing continuously. More than 50 linear plasmids have been found in mitochondria of more than 20 fungal species (reviewed in Meinhardt et al., 1990, 1997). In contrast, the presence of linear mitochondrial plasmids in higher plants is very rare. To date, 14 linear plasmids have been reported in only eight plant species, *Beta vulgaris*, *Brassica napus*, *Brassica rapa*, *Daucus carota*, *Sorghum bicolor*, *Zea diploperennis*, *Zea luxurians*, and *Zea mays* (see Table 1). As mentioned in Section 1, there is no report on the presence of linear plasmids in animal mitochondria and linear plasmids seem to be absent from most animal cells. This unbalanced distribution of linear plasmids among eukaryotic cells is very interesting from the point of view of the origin, transmission and function of linear plasmids in mitochondria.

3. Structure and coding capacities of plant mitochondrial linear plasmids

Linear mitochondrial plasmids in plants usually contain terminal inverted repeats and also proteins covalently attached to their 5′ termini. They share this DNA structure not only with fungal mitochondrial plasmids but also with some DNA viruses, including adenovirus and *Bacillus subtilis* bacteriophage Φ29, and with various transposable elements such as Ac and Ds of maize and Tam from *Antirrhinum majus* (reviewed by Sakaguchi, 1990). The name "invertrons" was given to this group of linear DNA molecules.

So far, five linear mitochondrial plasmids have been completely sequenced. These linear plasmids are the maize S1 (Paillard et al., 1985), S2 (Levings and Sederoff, 1983), and 2.3-kb plasmids (Leon et al., 1989), the sugar beet 10.4-kb plasmid (Accession No. Y10854), and the *Brassica* 11.6-kb plasmid (Handa et al., 2002). These plasmids all contain terminal inverted repeats (TIRs) at their 5′ ends, although the repeat sizes vary from 170 bp (maize 2.3-kb plasmid) to 407 bp (sugar beet 10.4-kb plasmid) (see Table 1). However, there is no sequence homology among the TIRs of these five plasmids except for those of S1 and S2 (which are completely identical to each other). Attachments of proteins to the 5′ termini have also been found in the sugar beet 10.4-kb plasmid (Saumitou-Laprade et al., 1989), the *Brassica* 11.6-kb plasmid (Turpen et al., 1987), the carrot 9.2-kb plasmid (Robison and Wolyn, 2005), the *Sorghum* N1 and N2 plasmids (Chase and Pring, 1986), and the maize S1 and S2 plasmids (Kemble and Thompson, 1982), and also the 2.3-kb plasmid (Bedinger et al., 1986). These findings suggest that most of the linear mitochondrial plasmids in plants belong to a group of invertrons (Sakaguchi, 1990). Invertron structure, relatively long inverted terminal repeats with proteins covalently bound to their 5′ ends, is also a common feature of linear mitochondrial plasmids in fungi, indicating that there are some relationships between plant and fungal linear plasmids.

Although the sizes of linear mitochondrial plasmids in plants sequenced so far vary from 2312 bp for the maize 2.3-kb plasmid to 11 640 bp for the *Brassica* 11.6-kb plasmid (Table 1), all plasmid DNAs contain one or more open reading frames (ORFs) (Fig. 1). The smallest linear plasmid in plant mitochondria sequenced so far, the maize 2.3-kb plasmid, has one open reading frame that potentially encodes a 294-amino-acid protein (Leon et al., 1989). The 2.3-kb plasmid also contains two tRNA genes, *trnP* and *trnW*, the only such case so far found in linear plant mitochondrial plasmids. Maize S1 and S2 have three ORFs and two ORFs, respectively, one of which, URF2 encoding a 339-amino-acid protein, is common to both plasmids. S1 and S2 have almost identical sequences (about