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Minireview

The dynamic nature of plant mitochondrial genome organization*

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The characteristic features of higher plant mitochondrial genomes: size, structure, recombination activity and evolutionary dynamics, are reviewed with the emphasis on the mitochondrial DNA (mtDNA) of *Phaseolus vulgaris*. Among all examined eukaryotic organisms, higher plants were found to contain the largest and most complex mitochondrial genomes. The plant mtDNA structure *in vivo* and mechanisms of evolution are controversial. We present the currently accepted models and how these models correspond to mitochondrial genomes of several common bean lines.

Recent studies of the plant mitochondrial genome organization have revealed intriguing features differentiating the plant genome from its mammalian and fungal counterparts [1–4]. In this review we will discuss some aspects of the plant mtDNA organization, with the emphasis on the *Phaseolus vulgaris* genome which has been the subject of our research for the last few years.

GENOME SIZE

The largest mitochondrial genomes have been identified in higher plants. Even the smallest plant mitochondrial genome of 208 kb in *Brassica hirta* is several times larger than any examined animal (16–20 kb) and most fungal (80 kb in yeast) mtDNAs [1, 2]. Among the plant species studied so far the

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Abbreviations: kb, 1000 base pairs; mtDNA, mitochondrial DNA; CMS, cytoplasmic male sterility; *pvs*, *Phaseolus vulgaris* sterility sequence; PFGE, pulsed-field gel electrophoresis; *atp9*, gene of subunit 9 of the F_0ATPase complex; *atpA*, gene of subunit 1 of F_1ATPase; *cob*, gene of cytochrome *b*, *nad1*, gene of subunit 1 of the NADH dehydrogenase complex; *orf*, open reading frame; *urf*, open (unidentified) reading frame; *coxII*, gene of subunit II of the cytochrome *c* oxidase complex; *Fr*, fertility restoring gene.

mitochondrial genome of muskmelon (2500 kb) is the largest and may be compared with some bacterial genomes. However, most plant mtDNAs are 300–600 kb in size. This amazing disproportion between the mitochondrial genomes of higher plants and other eukaryota is the result of the presence of additional genes, internal duplications and integrated nuclear and chloroplast sequences [4]. Strong size variation of mtDNA exists between very closely related plant species as well. For example within the *Cucurbitaceae* family the size of mtDNAs varies 7 to 8-fold [2]. On the other hand, size differences are less pronounced for *Brassica/Raphanus* (208–242 kb) [5] and *Phaseolus* (324–456 kb) [6]. In the case of the genus *Phaseolus* the mitochondrial genome sizes were estimated by the summation of fragment sizes from restriction profiles [6]. The most accurate method is, however, the physical mapping. Up to now, physical maps have been available for only two lines of *P. vulgaris* [7]. Based on the linear representations of physical mapping data, the mitochondrial genomes of two *P. vulgaris* lines are 417 kb and 420 kb in size. These estimates agree with the earlier values derived from restriction profiles. However, if the multiple chromosome models for the mitochondrial genomes in common bean (see chapter "Reappraisal of the master circle" and Fig. 2) are considered in the calculations, the size of the genomes would increase substantially (651 kb and 861 kb) [7]. This discrepancy can be explained by the fact that the determination of genome size using restriction patterns does not allow accurate determination of the fragment copy number. According to the multiple chromosome model, regions present at one copy number per genome would appear in digestion profiles as submolar bands.

MULTIPARTITE STRUCTURE

In most thoroughly studied systems (mammalian and fungal) mitochondria contain multiple copies of a circular DNA molecule [2, 3]. Therefore it was initially presumed that the plant mitochondrial genomes would be similar. However, restriction enzyme analyses and electron microscope studies suggested that the structure of plant mitochondrial genome was more complex and that it existed as a collection of molecules differing in size and at varying stoichiometry [8]. The first model proposed for plant mtDNA organization was based on mapping the *Brassica campestris* and *Zea mays* mitochondrial genomes [2, 8]. According to this model, the entire sequence of plant mtDNA was arranged in a circular molecule, the so-called master chromosome containing various numbers of large repeated sequences. These large repeats could be recombinationally active. Recombination between such repeats in direct orientation generates a number of smaller circular molecules (subgenomic molecules) [2]. The size of the subgenomic molecules depends on the distance separating two copies of the repeat. Recombination between inverted repeats leads to isomeric forms. The isomeric forms have the same size but differ from each other by the orientation of a sequence located between the large repeats [2]. Furthermore, the homologous recombination is reversible and the whole system is maintained in a state of dynamic equilibrium [9]. Thus the complexity of plant mitochondrial genome would depend on the number, location and orientation of recombinogenic repeats, which vary from species to species.

RECOMBINATION REPEATS

A recombination repeat is defined as a sequence which is present in at least two copies in the genome and it is flanked by different genomic environments [2]. The sites from which two or more flanking sequences start are called branching points and they define the ends of the repeat. A number of genomic environments surrounding the repeated sequences is identified during the construction of a physical map. Two major families of recombinationally active repeats, R1 and R2, were identified in the mitochondrial genome of common bean line WPR-3 [7, 10]. Three copies of each repeat are present on the physical map of the mitochondrial genome of this line. Two copies are in direct orientation

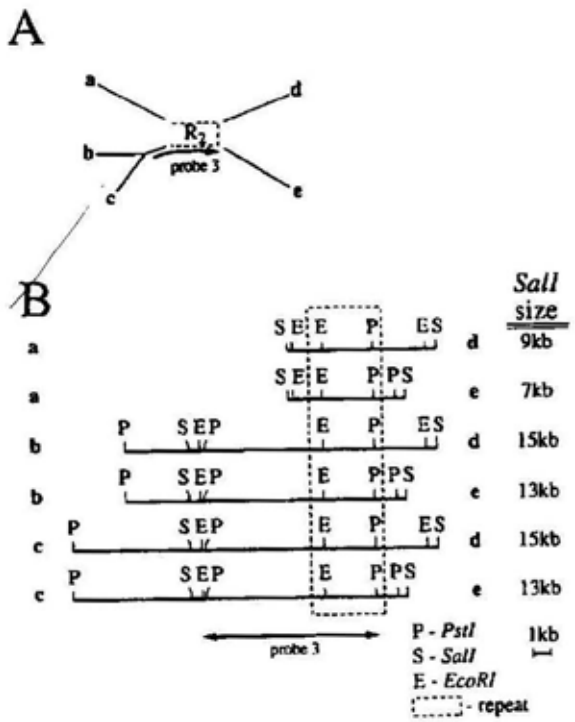

Figure 1. Genomic environments of the large repeat R2 found in the mitochondrial genome of common bean line WPR-3.

A. Diagram of the branch points identified around the repeat R2. Different flanking sequences are designated a, b, c, d, e. B. Six genomic environments were found by hybridization of the probe 3 containing R2 to *Sal*I digested mtDNA from WPR-3. The location of the probe is indicated by the arrow. The sizes of hybridizing *Sal*I fragments are shown at the right. (Adapted from Ref. [7]).

and the third in inverted orientation. Either of the repeats had three flanking sequences on one end of the repeat and two on the other end. As a consequence, either repeat was present in six genomic environments. As shown in Fig. 1, two flanking sequences share a segment of homology forming an additional branching point to one side of the R2 repeat. We are currently investigating the nature of this branching point. Our results suggest that this point results from the recombination through a small repeat (see below) localized near the larger, more active repeat R2. The comparison of sequences of large recombination repeats from different plant mitochondrial genomes indicates that all copies of this same repeat are identical but there is little or no primary sequence homology between repeats from various species [9, 11]. Moreover, the DNA sequences repeated in one species could exist as single-copy DNA in others. Consequently, no motif is common to the sequences repeated in the mitochondrial genomes of different plants. The factors promoting homologous recombination are still not known [11]. Besides the apparent high-frequency recombination involving the large repeats, rare recombination events between very short regions of similarity have also been detected in plant mitochondrial genomes [8]. In contrast to the large repeats, the recombination mediated by small repeats is not readily reversible because of their small size, thus leading to irreversible changes in the genome. The small repeats recombine infrequently in response to stress, e.g. passage through a tissue culture [2]. It has been postulated that the products of their recombination are either maintained at a low level in the genome (sublimons) or can be amplified to normal stoichiometry [12, 13]. It has been suggested [2, 12, 13] that such recombinations are involved in generation of mutants with altered mitochondrial function and that they are a major force in the evolution of plant mitochondrial genomes. It is widely accepted that recombination events across large repeats create the multipartite structure of plant mitochondrial genomes, while short repeated sequences account for the evolutionary dynamics of higher plant mtDNA.

REAPPRAISAL OF THE MASTER CIRCLE

To-date, there is no direct evidence from either electron microscopy or gel electrophoresis to support the existence *in vivo* of the master chromosome. Thus, it seems that, at least in some species, the mitochondrial

genome exists as multiple circular chromosomes that replicate independently [7, 14]. The configuration of these molecules could not be predicted based on recombination between large repeats and therefore they can not be considered as subgenomic molecules. In contrast to this, rare recombination events across small repeats are probably responsible for generation of the chromosomes present at subgenomic levels. However, intramolecular and intermolecular recombination events between large repeats localized on these autonomous molecules may also occur. The proposed models for the structure of the mitochondrial genomes of the cytoplasmic male sterile common bean line (CMS-Sprite) and its fertile revertant line (WPR-3) are consistent with the above hypothesis [7, 10]. Based on cosmid mapping data, the mitochondrial genome of the CMS line consists of three autonomous chromosomes differing only in short, unique regions (Fig. 2). The unique region present in one of these chromosomes is the male sterility associated sequence designated *pvs* (*Phaseolus vulgaris* sterility sequence) [14]. The mitochondrial genome of the fertile revertant line appeared to contain only two of the three chromosomes, having apparently lost the *pvs*-containing molecule [7]. The mapping data cannot exclude the presence of a molecule corresponding to the master circle in CMS and revertant mitochondria. However, the existence of such a circle would require a very large (over 200 kb) duplication in the case of WPR-3 and even triplication in the case of CMS-Sprite within the master chromosome. It is very interesting that the mitochondrial genomes of two fertile progenitors of CMS-Sprite were mapped as a single master chromosome without any large duplications or triplications (H. Jańska and M. Wołoszyńska, unpublished).

STRUCTURE *IN VIVO*

So far conventional restriction mapping has produced circular maps for all plant mito-

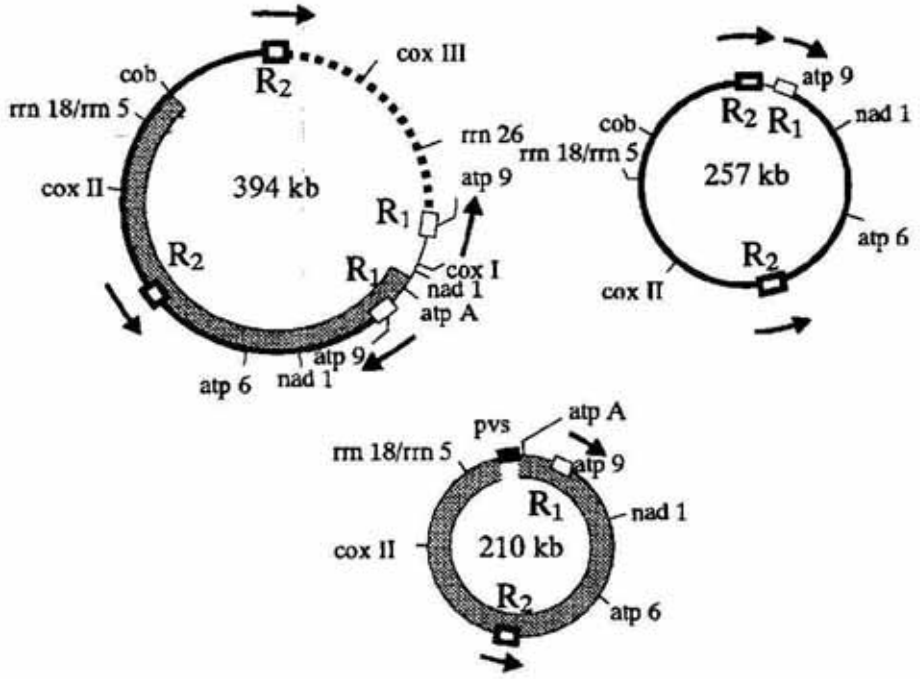

Figure 2. Three-circular model of the CMS-Sprite mitochondrial genome.

Regions shared by more than one chromosome are designated by different line styles: the thick black line (394 kb and 257 kb chromosomes) and the thick gray line (394 kb and 210 kb chromosomes). The dotted line on the 394 kb chromosome represents the sequence between the R1 and R2 repeats, which is exchanged between chromosomes. The thin lines of the 394 kb and 257 kb chromosomes indicate regions unique for these chromosomes. The sterility determinant 3-kb *pvs* sequence (black box) is the unique region of the 210 kb chromosome. The large repeated sequences are visualized by white (R1) and striped (R2) boxes, arrows indicate their relative orientation. (Adapted from Ref. [10].

chondrial genomes irrespective of the model (one or more autonomous chromosomes) [15]. However, the circularity of plant mtDNA has not yet been verified by direct visualization. Electron microscopic analysis of mtDNA isolated from plant tissue usually reveals a broad range of molecules differing in size, which are mainly linear with a low percentage of small circles. The largest circular structure identified by electron microscopy is the *Marchantia polymorpha* mtDNA of 184.4 kb [2, 4]. Pulsed-field gel electrophoresis (PFGE) of higher plant mtDNA also yields patterns inconsistent with the predicted circular nature of mtDNA molecules [3, 16]. Figure 3 shows the PFGE profile for the undigested mtDNA from *P. vulgaris* seedlings. In order to visualize only mtDNA, and not the contaminating plastid or nuclear DNA, the gel was hybridized with probes of mitochondrial origin. Three forms of mtDNA were detected: part of the mtDNA did not enter the gel at all, a part migrated in the compression zone of the gel and another portion migrated as a smear of linear molecules of about 50 kb to 200 kb. There were no distinct bands that could represent circular molecules. Similar results were reported for several higher plant species which have mitochondrial genomes different from that of *P. vulgaris* with respect to size and proposed organization [16]. The striking discrepancy between the circular model for the mitochondrial genome based on physical mapping and the results of electron microscopic and electrophoretic studies led to alternative hypotheses. The first is, that most of plant mtDNA exists as complex branched structures or simple linear molecules derived from the complex forms, but not as circular molecules [3]. The second hypothesis proposes that the genome exists primarily in a circular conformation and that some unique features of mitochondrial genome organization account for the extreme fragility of the mtDNA [15]. On the other hand, the existence of a number of circular molecules in maize mitochondria was proved using PFGE in combination with infrequently cutting enzymes [15]. The molecules were colinear with the master chromosome of the cosmid-derived map except at the point of circularization.

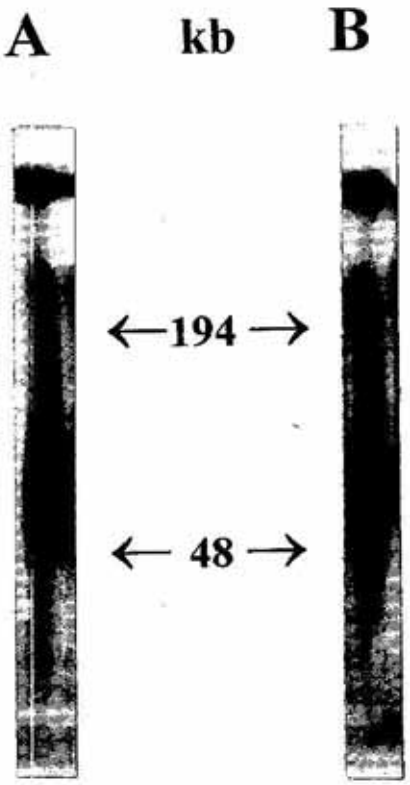

Figure 3. Autoradiogram of undigested mtDNA from *Phaseolus vulgaris* obtained after a CHEF-gel electrophoresis and hybridization with the *atp9* gene from maize (A) and the *pvs* sequence from CMS-Sprite (B).

Mitochondria purified according to the method described in [7] were embedded in LMT agarose and lysed by 0.5 mg/ml proteinase K and 1% SDS (final concentrations) followed by incubation at 55°C overnight. Then plugs were washed twice in subsequent solutions: TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 7.5) at room temperature, TE buffer with 0.04 mg/ml phenylmethylsulfonyl fluoride (PMSF) at 50°C and finally TE buffer at room temperature. The plugs were stored in 0.5 M EDTA, pH 8.0, at 4°C. Agarose embedded mtDNA was electrophoresed on a CHEF-gel at 175 V with 30 s pulse time in 1% agarose for 22 h. Size marker was a mixture of λ DNA concatamers, the positions of the 48 kb and 194 kb bands are indicated by arrows in the middle of the picture (H. Jańska, unpublished).

The master chromosome of 570 kb, which was predicted from restriction mapping data, was not detected. Recently *Arabidopsis thaliana* mtDNA fragments larger than 300 kb were integrated into yeast artificial chromosomes [4]. This gives no evidence for a particular mtDNA configuration *in vivo* but shows that such large DNA molecules are present in plant mitochondria. Thus the configuration of mtDNA molecules *in vivo* is still

not clear. However, the most accepted model assumes the circular form of mtDNA and cosmid derived maps are still used as a tool for studies of the mitochondrial genome [2].

PLASMID-LIKE MOLECULES

Several plant species have been found to carry species-specific, and in some cases cytoplasm-specific, small linear or circular DNA or RNA molecules [17–19]. Small supercoiled DNA molecules have been observed in mitochondrial preparations from common bean by Dale *et al.* [18]. However in our laboratory, we did not observe those molecules in mitochondrial DNA preparations from the following lines of *P. vulgaris*: CMS-Sprite, WPR-3, GO8063, NEP-2 and POP ([7], and H. Jańska and M. Wołoszyńska, unpublished). This could reflect differences in mitochondrial DNA isolation procedures or differences in plant genotypes. Two large double stranded RNA molecules were identified in common bean [19]. To-date the role of these molecules is not known.

DNA CONTENT OF MITOCHONDRIA AND THEIR INHERITANCE

The number of mitochondria varies considerably between different cell types [9]. However, the ratio between nuclear and mtDNA remains approximately constant. This discrepancy can be explained if we assume that an individual mitochondrion does not contain a complete genome as it was shown for tabacco [20]. Moreover, mitochondria can fuse, thus allowing the genomes to mix and recombine readily [9, 17]. To summarize, the mitochondrial genome organization cannot be visualized as a rigid structure but rather must be seen as a dynamic system, generated through continuous fusions and divisions of organelles. In the past, the mitochondrial genome of higher plants was thought to be transmitted only maternally [21]. Recent studies revealed exceptions to this rule, since a paternal and biparental contribution of mitochondria to the progeny has been reported [21–23]. Kuck *et al.* [22] found "maternal" and "paternal" mtDNA fragments in hybrids resulting from crosses between different *Triticale* lines. The use of the PCR technology allowed to show that the "paternal" fragments were maintained at a substoichiometric level in the mtDNA of maternal parent [22]. Thus, a selective amplification of these molecules in the absence of paternal and biparental inheritance could explain these observations.

GENE CONTENT AND STRUCTURE

In plants, like in other eukaryotic organisms, mitochondrial genomes code only for a small number of proteins while other mitochondrial peptides are encoded by nuclear genes [1]. Although the total number of mitochondrial genes in flowering plants is unknown, it may be similar to the number identified in *M. polymorpha* — namely 94 [2, 4]. So far, in the higher plants, the largest set of 47 mitochondrial genes has been mapped on the master chromosome of maize [2]. The genes identified in mitochondria of different plants may be divided into five groups: genes for the respiratory enzymes, transfer RNA genes, ribosomal RNA genes, ribosomal protein genes and genes of unknown function [2]. Some of these genes were localized on the common bean physical map using heterologous probes [7, 10]. The majority of genes were present in only one position. The *atp9* gene, which is part of the R1 repeat, exists in several different environments. This results from the recombinogenic activity of R1. The potential influence of various flanking sequences on the gene expression remains to be elucidated. It is not known, whether the inverted orientation of two R1 copies can result in the antisense regulation of this gene expression. In the sterile line the *atpA* gene is present in two environments, while in the fertile revertant line it appears only in one [7, 10, 24]. Similarly, mtDNA in the sterile line, but not in the revertant, contains the 111-bp DNA fragment coding the C-terminal part of the *cob* gene in addition to its full length copy [7, 10, 24]. Both of these duplications are associated with cytoplasmic male sterility, since in all examined CMS lines of

P. vulgaris and *P. coccineus* the *pvs* sequence was flanked by a full copy of the *atpA* gene and the fragment of the *cob* gene [25].

A number of plant mitochondrial genes contain intron sequences [1, 2, 4]. Some introns are divided into two parts separated by DNA stretches up to 300 kb in length. Each part of the divided intron is attached to the exon. The exon flanked by portions of introns forms a separate transcription. The divided introns associate to form a functional intron, which is subsequently excised. This process is called *trans*-splicing. In some genes the *trans*-splicing is exceptionally complex because of exon duplications. This is the case with maize, where the exon a of the *nad1* gene is duplicated [2]. The exact structure of the *nad1* gene in common bean was not determined, although hybridization showed that the internal region (exon b) of the *nad1* gene mapped to two different places of mitochondrial genome [7]. However, the two bands observed on the autoradiograms were of different intensity. This suggests, that in the common bean genome at least a portion of *nad1* exon b is duplicated. This duplication could be a part of pseudogene or chimeric gene [2, 4]. The pseudogenes are derived from functional genes by base substitutions, additions and deletions. They are usually not transcribed, hence their name. The chimeric genes are generated when stretches of DNA sequences (unique or homologous to various regions of the genome) are fused to form the same reading frame. The chimeric genes are often associated with cytoplasmic male sterility [26], and were also observed in the mitochondrial genome of the common bean CMS line [14, 24]. The *pvs* contains at least two open reading frames: *orf239* and *orf 98*. Hybridization analysis of these *orfs* indicates their chimeric nature. The 5′ end of *orf98* is unique, while its 3′ flanking and 3′ coding sequences are derived from an internal region of the chloroplast alanine-tRNA intron. The structure of *orf239* is even more complicated. Three short regions of homology to the maize mitochondrial 5-kb repeat and a segment almost identical to the fragment of the gene coding for the chloroplast photosystem II D2 protein are interspaced by unique DNA sequences.

GENE TRANSFER

The sequences of chloroplast origin were found integrated in all examined plant mitochondrial genomes [1, 2, 4]. The chloroplast-derived DNA regions vary from species to species in size and identity and are randomly distributed over various locations within the plant mitochondrial genomes. The mitochondrial genes for tRNAs, sharing 90–100% homology with their chloroplast counterparts, are called "chloroplast-like" and are considered part of chloroplast DNA regions inserted into the mitochondrial regions during evolution [27]. Most of them are potentially functional. In *P. vulgaris* six of these genes were identified [27]. In contrast, those considered to be native mitochondrial genes exhibit a lower homology to the chloroplast genes ranging from 65% to 80%.

Evidence has been found for the presence of sequences of not only chloroplast but also nuclear origin integrated into mtDNA [4]. Besides the gene transfer to mitochondria, sequence movements from the mitochondrial genome into the nucleus have also been identified [2, 4, 28]. It is suggested that the above observations reflect the process of evolution. Mitochondria are believed to originate from a bacteria-like progenitor, whose genes were lost or transferred to the nuclear genome during evolution. The transfer from mitochondrion to the nucleus of the *coxII* gene has been most extensively studied in legumes [28]. Most of those plants have a copy of this gene both in the nucleus and in the mitochondrion, but only one form is expressionally active. In common bean *coxII* is expressed only in the nucleus.

MITOCHONDRIAL MUTATIONS

Since mitochondria are involved in the basic metabolism of the cell, most mutations in the mitochondrial genes are expected to be deleterious. In many cases such mutations are lethal. The lack of viable mutants has made it difficult to study plant mitochondrial functions and nuclear-mitochondrial interactions. However, a few plant mitochondrial mutations are known: cytoplasmic male ste-

rility (CMS) found in a large number of species [26], nonchromosomal stripe (NCS) in maize [29], the "teosinte cytoplasmic-associated miniature" (tcm) in maize [30] and a leaf variegation phenotype associated with mitochondrial genome rearrangements due to a nuclear mutation in *Arabidopsis* [31].

CYTOPLASMIC MALE STERILITY

CMS is a maternally inherited trait characterized by the inability of the plant to produce viable pollen [26]. In agriculture, CMS mutants have been very useful in production of hybrid lines with increased productivity by eliminating the need for hand emasculation. CMS is caused by a lesion in mtDNA but this trait is observed phenotypically only in a certain nuclear background. This is due to nuclear fertility restorer genes which suppress CMS mutations. Cytoplasmic male sterility is thus thought to result from incompatibility between the nucleus and mitochondria. At least five sources of CMS have been found in the genus *Phaseolus* [32]. In the first case, a sterility-inducing mitochondrial genome of *P. vulgaris* was recovered from the fertile line GO8063. This line contains the CMS-specific sequence — *pvs* but it is fertile due to the presence of a fertility restoring nuclear genotype. The CMS-Sprite was derived from a cross between, GO8063 and a stable maintainer line Sprite. The four other CMS lines were obtained by crosses of different *P. coccineus* genotypes as female with *P. vulgaris*. All CMS lines from *P. vulgaris* and *P. coccineus* known to date contain the same CMS-specific sequence [25]. A high frequency of plants carrying this sequence was also observed in the wild *Phaseolus* population. It was thus confirmed that these plants contain fertility restorer genes [25].

CHANGES REGULATED BY NUCLEUS

Two nuclear genes, *Fr* and *Fr2*, have been found to restore pollen fertility to the cytoplasmic male sterile common bean by apparently distinct mechanisms [33, 34]. *Fr2* influences the expression of the *pvs* sequence [33], whereas *Fr* restores pollen fertility by altering the mitochondrial genome organization [34]. The mitochondrial genome alteration can also occur spontaneously although at low frequency. Both events, spontaneous reversion and *Fr* mediated restoration, involve the loss of the *pvs* containing chromosome [7, 10]. Genetic analysis revealed that two copies of the *Fr* gene per nuclear genome are needed for permanent restoration of fertility [34]. It was also demonstrated that the *Fr*-directed disappearance of the *pvs* sequence occurred during flower development [34]. Moreover, it was suggested that *Fr* action depends on the expression of *pvs*. This conclusion was based on the observation that in the presence of the restorer gene *Fr2* the gene *Fr* no longer directed the elimination of the *pvs* containing chromosome. The *Fr*-mediated alteration of mitochondrial genome ilustrates how the nuclear genotype influences the copy number of mtDNA molecules. Other examples of this phenomenon are also known [12, 35]. The nuclear background can also increase the frequency of mitochondrial recombinations [29]. It has been suggested that both these mechanisms are not only involved in the generation of mutants but may also play an important role in the evolution of the plant mitochondrial genome [31].

EVOLUTION

In contrast to animal mtDNA and the highly conserved gene arrangement in chloroplast, plant mitochondrial genomes have been shown to evolve rapidly in structure but very slowly in sequence [5]. The rate of point mutations in plant mtDNA is 40–100-fold lower than in animal mtDNA. A few inversions, duplications or deletions were observed between closely related plant species in genus *Brassica*, while the genomes of more distant species differ virtually randomly with respect to sequence arrangement. Two main mechanisms account for the presence of the rearranged forms of the plant mitochondrial genome: novel recombination events through small repeats as well as selective amplification of pre-existing substoichiometric sequence arrangements [2]. These mechanisms

are not mutually exclusive because the sublimon molecules are indeed the recombination products passed from generation to generation at the substoichiometric level. Basing on these mechanisms Small *et al.* [12] and Fauron *et al.* [2] proposed models of mtDNA evolution. Both models involve a double recombination process with substoichiometric intermediates followed by selective amplification of one of the recombination products (Fig. 4). According to the Small's model only small repeats participate in the double recombination, while Fauron assumes that recombinations occur at both small and large repeats. In both models copies of the same repeats are in direct orientation and the event promoting the generation of a new genome is the activation of small repeats. Deletions and duplications often detected in plant mtDNA and the consequent rapid evolutionary changes in the mitochondrial genome structure can be explained by these models. Any DNA sequence located between copies of two different direct repeats can be deleted or duplicated. Small *et al.* [12] described how the double recombination could have created the 12 kb DNA fragment duplication found in modern maize and Fauron [2] explained by double recombination the loss of a deleterious *urf13* gene from *cms*T callus tissue that returned to fertility [2]. This suggests that the mitochondrial genome could have been changed by either a gain or/and a loss of DNA sequence from the common ancestor. It has been postulated, that products of double recombination are sometimes maintained in the mitochondrial genome for many generations at low level before amplification [2, 12, 13]. Stress caused by a change in environmental conditions or plant crosses might induce the amplification of such molecules to normal stoichiometry. Sublimon amplification might have also been affected by the developmental stages of the plants [36]. CMS-related sequences in the form of sublimons have been found in fertile lines of sunflower and petunia [36]. We propose that the appearance of the CMS-Sprite genome is ac-

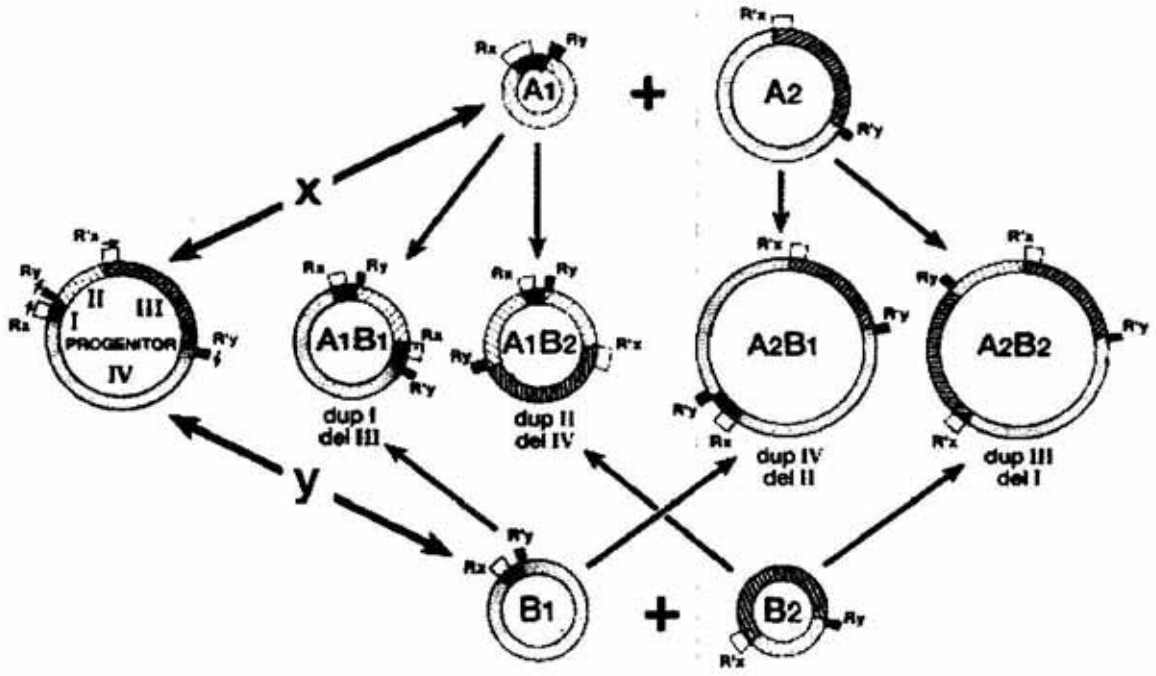

Figure 4. The model of higher plant mitochondrial genomes evolution proposed by Fauron *et al.* [2].

The progenitor chromosome contains two sets of direct repeats: highly recombinogenic large repeat x (Rx, R'x) and active small repeat y (Ry, R'y). Regions between repeats are designated I, II, III, IV. Recombination through the repeat x generate A1 and A2 DNA molecules and recombination involving the repeat y produces B1 and B2. Intramolecular recombination in a pairwise manner between any two of these DNA molecules will create new chromosomes: A1B1, A1B2, A2B1, A2B2. Relative to progenitor genome new chromosomes contain a specific duplication (dup) and specific deletion (del). For example, the chromosome A2B2 has region III duplicated and region I deleted. (Reproduced from Ref. [2]).

companied by amplification of the *pvs*-containing sublimon and *vice versa* — reversion to fertility is associated with a dramatic decrease of this chromosome (H. Jańska and M. Wołoszyńska, unpublished). Furthermore, the *pvs* sequence was probably generated by an ancient recombination event(s) during evolution. Hervieu *et al.* [25, 32] suggest that this process took place at least 7000 years ago within the genome of the common ancestor for *P. vulgaris, P. coccineus* and *P. polyanthus*. The mechanisms by which a novel arrangement, initially at a very low level in the genome, can become dominant, are not well understood. They may involve preferential replication of substoichiometric molecules and/or selective segregation of these molecules during cell division [7, 12]. Phylogenetic relationships between closely related common bean lines [37] and between three *Phaseolus* species [6] have been investigated based on mtDNA molecular anlysis. Considerable variation was present among the three *Phaseolus* species in contrast to the high homogeneity of the mitochondrial genome within *P. vulgaris*. However, five restriction fragment length polymorphisms were found dividing the common bean lines into two groups corresponding to the Mesoamerican and Andean gene pools of *P. vulgaris*. These two major gene pools were previously distinguished by seed size, growth habits, environmental adaptation, disease resistance, isoenzyme variants and phaseolin types [37]. It was deduced that at least two out of the five identified restriction fragments length polymorphisms resulted from point mutations. The nucleotide substitutions were also shown to be responsible for intraspecies variability in barley and tomatoes [37]. This is inconsistent with the findings of Palmer [5], who investigated mtDNA variation within the *Brassica* species. He detected no point mutations but only structural changes. Similarly, most of the restriction fragments length polymorphisms in the genus *Beta* are due to sequence rearrangements rather than to point mutations [38]. It can not be determined from the present study which kind of mutation is more important to the intraspecies evolution of higher plant mitochondrial genomes. However, it seems to be well documented that most of interspecies variability results from mtDNA structural rearrangements [5]. Our knowledge concerning the evolution of the higher plant mitochondrial genomes is based only on studies of a limited number of plant species. Obviously, more data is needed to conclude what mechanisms are predominantly involved in mtDNA evolution.

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