II. Recombination: Effects on Structure and Function of the Mitochondrial Genome

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1. Introduction

Three decades ago, it was shown for the first time that mitochondria contain DNA (Luck and Reich 1964, cited in Lonsdale and Grienenberger 1992). In the meantime, the mitochondrial DNA (mtDNA) of eukaryotes has been studied in detail and some of the mitochondrial genomes have already been sequenced. In many cases, mtDNAs are small (approx. 16 kb) and circular. Furthermore, it can now be generalized for all vertebrates and invertebrates that the genetic information of mitochondria is compactly organized and predominantly on one strand of the mtDNA (for ref. cf. review of Lonsdale and Grienenberger 1992).

In contrast, the size of the mitochondrial genome of plant species differs in a wide range. For fungi, sizes of predominantly circular genomes reported to date vary between 18.9 and 176 kb. Mitochondrial genomes of higher plants can be even larger; the largest one described so far is that of muskmelon, with a size of 2400 kb (cf. Lonsdale and Grienenberger 1992). Most of the plant species studied up to now possess multi-circular mitochondrial genomes, where the circular organization is determined by repeated sequences (Palmer 1992). This multipartite structure of the mitochondrial DNA is a consequence of homologous recombinations between large sequence duplications and inversions. Recombination events within and between circular structures can possibly lead to multimeric circles and to highly complex forms of the mitochondrial genomes (cf. Lonsdale and Grienenberger 1992).

The evolution of plant mitochondrial genomes has obviously been driven by rearrangements and by the loss of sequences as a result of recombination events. The present chapter summarizes recent findings in this field.

2. Organization of the Mitochondrial Genome

a) Recombination Repeats and Rare Recombination Events

The high frequency of recombination via repeated sequences is partly responsible for the complexity of plant mitochondrial DNA. Repeated sequences which are present in two
copies relative to other sequences in the genome, but occur in four genomic environments because they are involved in frequent recombination events, are called recombination repeats (Stern and Palmer 1984).

Depending on the orientation of the repeats, either direct or indirect, intramolecular recombination in the master chromosome leads to subgenomic circles or to isomeric forms (Fauron et al. 1991). Recombination repeats have been found in all mitochondrial genomes mapped to date, with the exception of *Brassica hirta* (Palmer and Herbon 1987) and *Marchantia polymorpha* (Oda et al. 1992). However, the lack of recombination repeats in these two genomes shows that recombination repeats are not an essential feature of plants mitochondrial DNAs. The size of most known recombination repeats range from 1 to 12 kb (Palmer and Herborn 1986; Stern and Palmer 1986; Siculella and Palmer 1988); the smallest known to date is 266 bp (Schuster and Brennicke 1986) but larger recombination repeats have also been reported (Fauron et al. 1990; Folkerts and Hanson 1991). A summary of the known recombination repeats, the master circle sizes, and the subgenomic circles that can be predicted is provided by Hanson and Folkerts (1992).

In addition to (and distinct from) the frequently occurring recombination across defined recombination repeats, rare recombinations between very short repeats also occur in plant mitochondrial DNA. The result are DNA molecules present in substoichiometric amounts relative to the main mitochondrial genome which are termed "sublimons" (Small et al. 1987). To explain structural changes observed in plant mitochondrial genomes, models involving frequent recombination events as well as rare recombinations have been developed.

Three copies of a recombination repeat, two in direct and a third in indirect orientation, are present on the 443 kb master chromosome in *Petunia* line 3704 (Folkerts and Hanson 1989). In *Petunia* CMS line 3688, one of these three 6.6-kb repeats is truncated (Folkerts and Hanson 1991). Sequence analysis revealed that the normal and the truncated repeats are nearly identical over 1.42 kb (Conklin and Hanson 1993). The deletion in the truncated repeat has probably arisen from a rare recombination event between two copies of an 18 bp repeat which are localized in direct orientation at the position of the deletion breakpoints in the truncated repeat. The lack of sequence homology shared by different recombination repeats suggests that recombination may occur by a homologous mechanism rather than being strictly site-specific, although there seems to be also some site specificity, as the mere presence of repeats is not sufficient for apparent high-frequent recombination (Hanson and Folkerts 1992).

When a number of recombination repeats in direct and indirect orientation are present in a master chromosome, the situation becomes very complex, as for example, in maize (Fauron et al. 1991).

A recombination model involving two steps can explain the different rearrangements in the mitochondrial genome observed in fertile revertants in maize CMS-T after tissue culture (Fauron et al. 1992). At first, intramolecular recombination across two different sets of direct repeats gives rise to subgenomic circles. A second intermolecular recombination between defined subgenomes creates a newly rearranged master chromosome containing deletions and duplications. In the revertant V3, intramolecular recombination between two sets of direct repeats R2 (0.127 kb) and R1 (4.6 kb) is followed by intramolecular recombination either through the R1 repeat, the R2 repeat, or other homologous sequences resulting in a new master chromosome of 735 kb (Fauron et al. 1990).