The Mitochondrial Genome Organization of a Maize Fertile cmsT Revertant Line Is Generated Through Recombination Between Two Sets of Repeats

C. M.-R. Fauron,* M. Havlik* and R. I. S. Brettell

*Howard Hughes Medical Institute, University of Utah, Salt Lake City, Utah 84132, and *CSIRO, Division of Plant Industry, Canberra, Australia

Manuscript received July 26, 1989
Accepted for publication November 4, 1989

ABSTRACT

The mitochondrial genome (mtDNA) organization from a fertile revertant line (V3) derived from the maize cytoplasmic male sterile type T (cmsT) callus tissue culture has been determined. We report that the sequence complexity can be mapped to a circular "master chromosome" of 705 kb which includes a duplication of 165 kb of DNA when compared to its male sterile progenitor. Associated with this event is also a 0.423-kb deletion, which removed the cmsT-associated urfl3 gene. As found for the maize normal type (N) and cmsT mitochondrial genomes, the V3 master chromosome also exists as a multipartite structure generated by recombination through repeated sequences.

MATERIALS AND METHODS

Maize lines: Seeds from N and cmsT in a B37 nuclear background were supplied by Pioneer Hi-bred International. The genotype (Wf9T/W22 ♀ × A188Nrf) × W22rf was used as a source of T cytoplasm tissue culture from which the V3 plant was regenerated. More details on the line are given in C. M.-R. FAURON et al. (1990). V3 was propagated by repeated self-fertilization.

mtDNA library: Partially Sau3A-digested mtDNA from N, T, and V3 plants were cloned into the BamHI sites of cosmids vectors pHC79 (Hohn and Collins 1980) or pWE15 (Wahl et al. 1987). Complete BamHI digest of N, T, V3 were also cloned into the BamHI site of plasmid vector pUC8 or pUC18.

DNA purification and analysis: mtDNA was purified from 5- to 7-day dark grown maize coleoptiles as described elsewhere (FAURON et al. 1987). Cosmid or plasmid DNA was prepared as minipreps of 15 ml or maxipreps of 500 ml cultures (Maniatis 1982a). The genotype (Wf9T/W22 ♀ × A188Nrf) × W22rf was used as a source of T cytoplasm tissue culture from which the V3 plant was regenerated. More details on the line are given in C. M.-R. FAURON et al. (1990). V3 was propagated by repeated self-fertilization.

The entire sequence complexity of the mitochondrial genome of Zea mays from the normal fertile cytoplasm (N) and cytoplasmic male sterile cmsT, in a B37 and Wf9 nuclear background, is contained in a circular molecule, also called "master chromosome" of 570 kb and 540 kb, respectively (Lonsdale, Hodge and Fauron 1984; Fauron and Havlik 1988; Fauron et al. 1989a, C. M.-R. Fauron, unpublished results). The master circle containing the entire sequence complexity is part of the multipartite structure arising via recombination at repeated sequences. These subgenomic circular DNA molecules are evidenced by the existence of cosmid clones containing the repeats in the various environments. However, the diversity of repeated sequences dictates a unique population of subgenomic circular molecules for each genome (Fauron and Havlik 1989).

The T source of cytoplasmic male sterility (cmsT) was widely used in the United States as a convenient way to produce hybrid seeds until the late 1960s when it was found to be susceptible to the T toxin produced by the fungal pathogen Helminthosporium maydis race T (Ullstrup 1972; Tatum 1971). The susceptibility to this fungal pathogen is tightly linked to the male sterility factor of cmsT. In 1986, Dewey, Leving and Timothy identified a rearranged sequence unique to the cmsT mtDNA containing a gene encoding a 15-kd polypeptide associated with the male sterile phenotype (Dewey, Timothy and Leving 1987; Dewey et al. 1988). This sequence is located in cmsT on a 6.6-kd Xhol fragment that is absent in the revertant progenies obtained through tissue culture (Gengenbach et al. 1981; Abbott and Fauron 1986) with the exception of one mutant (Umbeck and Gengenbach 1983). This locus is located near a 4.6-kb repeat not found in the normal genome (Abbott and Fauron 1986; Fauron et al. 1987; Wise, Pring and Gengenbach 1987; Rottman et al. 1987).

The mitochondrial genome organization of the progeny of a cmsT tissue culture regenerated plant (Brettell, Goddart and Ingram 1979) that has reverted to fertility has been analyzed. We report here that intramolecular recombination involving two sets of repeated sequences and intermolecular recombination between some selected recombination products can account for the genome reorganization which includes a 0.423-kb deletion comprising the urfl3 gene, and a 165-kb duplication.
RESULTS

Comparison of V3 and cmsT mtDNAs by hybridization: The V3 mtDNA restriction fragment profile is very similar to that of its progenitor, the cmsT mtDNA. The cmsT mitochondrial map has already been established with three enzymes: BamHI, XhoI, and Smal (FAURON et al. 1989a). A comparison between the two genomes was done as follows. The 16 overlapping cmsT cosmid clones covering the entire cmsT mtDNA sequence complexity were sequentially used as probes against a blot containing cmsT and V3 mtDNAs digested with BamHI, XhoI and Smal and the hybridization patterns were compared. Fourteen cosmid clones hybridized to fragments of similar size in V3 and cmsT, indicating the presence of large stretches of homologous sequence between the two genomes. Only two cmsT cosmid clones (tu4A2 and tu4E6) identify some rearrangement in the V3 mtDNA. Clone tu4A2 identifies in V3 an extra BamHI fragment of 11 kb or Smal of 2.8 kb in V3 mtDNA. Clone tu4E6 identifies a shift in the migration of the cmsT band XhoI 6.6-kb to an XhoI 6.2-kb band in V3.

We already know from previous work (FAURON et al. 1987) that the disappearance of the XhoI 6.6-kb fragment characteristic of cmsT and the appearance of the XhoI 6.2-kb fragment in V3 are the result of a recombinational event within the genome. The 6.2-kb XhoI fragment was therefore used as a probe to identify the region in the cmsT genome involved in recombination. It hybridizes to two regions and the repeats were found to be located at coordinates 153 and 445 on the cmsT map (FAURON et al. 1989a). As shown in Figure 2, the 6.2-kb XhoI fragment is flanked on the right side by a 1.15-kb XhoI fragment as is the 6.6-kb XhoI fragment in cmsT. On the left side, the 6.2-kb XhoI fragment of V3 is flanked by a 2.0-kb XhoI fragment as found on the left side of the second copy of repeat R2 (Figure 2, b2). The mapping therefore agrees with homologous recombination through a repeat located within the 6.6-kb XhoI fragment and XhoI 3.2-kb fragment. Surprisingly, only the two forms b2 (parental) and c2 (recombinant) can be identified in the mtDNA. Even a Southern blot of a XhoI digest of V3 mtDNA probed with the 6.6-kb XhoI fragment, does not reveal any subliminal level of the other parental and recombinant products (data not shown). They have been deleted.

The mapping of V3 mitochondrial genome: The V3 genome is closely related to the cmsT parental genome. Only two regions have changed as a result of recombination between repeated sequences. In or-
The arrangement was described above with the probes the many isomeric forms resulting from recombination to understand the overall organization of the V3 mitochondrial genome. A set of V3 overlapping cosmid clones was mapped. They were identical to the cmsT genome (FAURON et al. 1989a). Cosmid clones representing the two parental forms containing each a copy of repeat R1 giving rise to the recombinant forms c1 and d1. Those four forms are present in the cmsT mtDNA. However, only the parental form b1 and recombinant c1 are present in the V3 mtDNA.

To understand the overall organization of the V3 mitochondrial genome, the physical map was constructed by identifying a set of overlapping cosmid clones as was done for the cmsT genome (FAURON et al. 1989a).

Fourteen specific mtDNA BamHI or XhoI fragments located approximately every 40 kb on the cmsT map were used as probes on a V3 cosmid clone library. For each probe at least a dozen positive clones were selected. Their DNA was extracted, digested with the three restriction enzymes BamHI, XhoI and SmaI, and separated on 0.8% agarose gel also containing as a control the cmsT clones covering the region of the genome being studied. A set of V3 overlapping cosmid clones was mapped. They were identical to the cmsT cosmid clones except for the two regions whose rearrangement was described above with the probes tu4A2 and tu4E6. In particular, the same repeated sequences were identified as in cmsT including the 1.5-kb repeat, the 6-kb repeat and the 4.6-kb repeat (FAURON et al. 1989a). Cosmid clones representing the many isomeric forms resulting from recombination between the three copies of the 1.5 kb repeat were identified as well as the four forms resulting from recombination between the two copies of the 6-kb repeats. However, one striking feature was that only two forms out of the possible four obtained through recombination within the 4.6-kb repeat were identified. Of the four possible forms shown in Figure 3 the parental form (a1) and the recombinant form (d1) were not found.

**The model:** The hybridization studies performed in order to compare the cmsT and V3 mitochondrial genomes have shown two differences, both related to recombinational events:

1. Recombination through the repeat called R1 (4.6-kb repeat) is associated with the loss of one parental form (Figure 3, a1) and one recombinant form (Figure 3, d1), both containing the *urf13* gene.

2. Recombination through the repeat R2 gives rise to the 6.2-kb XhoI fragment found in equimolar amount as the parental form containing the 3.2-kb XhoI fragment. The two other forms, the parental (Figure 2, a2) and the other recombinant (Figure 2, d2), both containing the *urf13* gene have been deleted.

The model shown in Figure 4, which can account for all the data, indicates the result of an intermolecular recombination event operating between subgenomic recombinant products selectively maintained. As part of the multipartite structure, recombination through the 4.6-kb repeats (R1) in cmsT gives rise to two circular molecules of 83 kb (A1) and 457 kb (A2). Another intramolecular recombination through the R2 repeats given rise to two molecules of 292 kb (B1) and 248 kb (B2). Then an intermolecular recombination event between the circular forms A2 and B2 either through the R1 repeat, the R2 repeat, or the other homologous sequence produces a circular molecule of 705 kb. This new master chromosome has two striking features: a deletion of 0.5 kb representing the sequences found between the repeats R1 and R2 including the *urf13* gene, and a duplication of 165 kb of the sequence localized between the R'1 and R'2 repeats.

Though no mechanism is known to explain the loss of the recombination intermediate A1 and B1 or their combined form (Figure 4, A1B1), all of the data from the V3 mapping fit into this model: the loss of the 6.6-kb XhoI fragment, the appearance of the 6.2-kb XhoI fragment, the integrity of the 3.2-kb XhoI fragment as it was in cmsT and the integrity of the two copies of the R1 repeat or the R2 repeat. This mechanism is probably identical to the one proposed by SMALL, SULFOLK and LEAVER 1989 to operate with repeats located near the AtpA gene.

**CONCLUSION**

**The V3 master chromosome:** A diagram of the V3 vs. the cmsT master chromosomes is shown on Figure 5. The 165-kb duplication in V3 contains the following genes: *atp6, atp4, atp9* and *cob*. The sequences deleted from the cmsT genome encompass only the *urf13* gene.

The cmsT phenotype is a very stable trait; no spontaneous reversions have been observed in the field.
although the T cytoplasm represented nine-tenths of the United States maize production by the end of the 1960s. However, regenerated plants from cellus tissue culture revealed a fair number of discrete cytoplasmic mutations responsible for the reversion to a fertile phenotype (Brettel et al. 1980; Gengenbach, Green and Donovan 1977; Gengenbach et al. 1980).

For the regenerant V3, we have seen that the reversion to fertility has occurred through a very precise rearrangement of the mitochondrial genome via recombination between two selected sets of repeats R1 (4.6 kb) and R2 (0.127 kb). The recombination events are also associated with an unknown mechanism that selectively eliminates or amplifies specific recombinant intermediates to reconstruct a novel master chromosome exhibiting loss of some discrete sequences (the urf13 gene) and duplication of some other sequences.

The major difference between cmsT and V3 is the activation of repeat R2 as a recombination site re-
quired to generate the V3 mitochondrial genome. In the cmsT plant R2 does not seem to participate in some recombination event at any detectable level, at least as seen through hybridization experiments. On the contrary, the repeat R1 in cmsT plants has been found to be involved in recombination as we have mapped some of these recombinant clones from our cosmid libraries.

The novel mitochondrial chromosome for the fertile revertant V3 still contains the two sets of repeats R1 and R2 and therefore has the potential to continue to evolve into a new structure. However, the urf13 gene that has been strongly correlated to the cms phenotype is lost, thus preventing a possible reversion of the V3 progeny to sterility. In other words, the V3 fertile phenotype should be very stable unless some undetectable level of recombinant intermediates containing the urf13 gene is still present in the mitochondria or a new locus becomes responsible for the cms phenotype. The urf13 locus itself, which required a large number of recombination events for its creation, is very unlikely to come to existence spontaneously.

Two mechanisms are involved in the mitochondrial genomic reorganization in the revertant V3: activation of localized recombination and selection of recombinant products. The enhanced recombination frequency within the repeat R2 might be the result of the tissue culture process itself. The selective elimination of recombinant intermediates might be the result of a faulty replication system, lacking some replicative function either to initiate or terminate a complete cycle of replication. Alternatively, the recombinant intermediates that became fixed in V3 might have already been present at a very low subliminal level and have been amplified as in the case of the AtpA gene (Leaver et al. 1988). In any case, the recombinant intermediates carrying the urf13 locus have been lost.

One possible functional utility for the presence of short repeats around the maize mitochondrial chromosome might be in the induction of recombination allowing a subdivision of the genome and elimination of subgenomic molecules carrying a detrimental phenotype to the plant.

We are grateful to Raymond Gesteland for his support and advice and Toni Adkins for the typing of the manuscript.

LITERATURE CITED


Communicating editor: M. R. HANSON