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A nuclear sequence associated with self-incompatibility in *Nicotiana alata* has homology with mitochondrial DNA

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Summary. A 1.0-kb nuclear fragment located 5′ to a coding sequence associated with self-incompatibility in *N. alata* shows homology with mitochondrial chromosomal DNA on Southern blots. This sequence is also present in the mitochondrial DNA of two species of tomato, *L. esculentum* and *L. pennellii*, but shows no homology to mtDNA of *Zea mays*. The homologous mitochondrial fragment from *N. alata* was cloned and sequenced. A short region of 56 bp matches the nuclear sequence in 53/56 bp. Other matched but misaligned segments flank the 3′ end. The nuclear sequence is marked at the 5′ end by two 8 bp direct repeats. The function of the nuclear sequence is not known although, it is located 397 bp upstream from the site of transcription of the self-incompatibility gene. The mitochondrial sequence contains only limited open reading frames and the nuclear sequence has none. There is evidence that additional segments of the mitochondrial clone hybridize to other nuclear sequences. The exchange of sequences between the mitochondrial and nuclear genomes of plants is discussed.

Key words: *Nicotiana alata* – Self-incompatibility – Plant mitochondrial DNA – Interorganeller sequence transfer – *Lycopersicon*

Introduction

Plant mitochondrial genomes are generally much larger than those of animals, although each code for a similar, small number of proteins (Leaver and Gray 1982). The coding requirements of plant mitochondria account for only a fraction of their mtDNA and this *C* value paradox still defies explanation.

The amount of this excess mtDNA varies widely across the plant kingdom (for review, see Palmer 1985). For example, within the family Cucurbitaceae, the size of the mitochondrial genomes vary seven-fold (Ward et al. 1981). However, it appears that only a small proportion (5%–10%) of the mtDNA of these cucurbits is repeated, suggesting that amplification of sequences cannot account for the increased size of these genomes. Some mitochondrial sequences have homology with chloroplast DNA and these sequences have probably been transposed from the chloroplast genome (Stern and Lonsdale 1982; Stern and Palmer 1984). However, there is no correlation between increased genome size and the amount of chloroplast-related sequences (Stern et al. 1983; Palmer 1985). It is therefore likely that some of the excess DNA of plant mitochondria is derived from the nucleus. Recently, mitochondrial sequences of *Oenothera* have been found that have homology to transcribed nuclear DNA (Schuster and Brennicke 1987). These authors suggest that interorganelle transfer of DNA may require an RNA intermediate since, to date, only transcribed sequences have been involved in such transfers.

In this paper, we present sequence analysis of mtDNA from *N. alata* that has homology to a nuclear fragment that is 5′ to the coding region of a gene associated with self-incompatibility. The homologous sequences are short with little, if any, coding capacity but do contain features that are reminiscent of mobile genetic elements.

Materials and methods

The following plant materials were used: *Nicotiana alata* homozygous for the S_2 allele of the incompatibility locus; *Lycopersicon esculentum* (tomato) cv. Grosse-Lisse; *L. pennellii*

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(LA716) (a wild relative of tomato obtained from C. M. Rick, University of California, Davis); *Zea mays* (corn) cv. Flat Red.

Techniques for total DNA isolation have been previously described (Bernatzky and Tanksley 1986a). Total DNA was isolated from leaves and primarily contains nuclear DNA with varying amounts of chloroplast and mitochondrial DNA contamination. Mitochondrial DNA was isolated by the DNase I procedure (Kolodner and Terwari 1972).

Restriction enzyme digestion with *Eco*RI, *Hind*III or *Hinc*II was according to manufacturer specifications (International Biotechnologies). Southern blots were produced from restriction fragments that were separated on 0.9% agarose gels, treated for 12 min in 0.25 N HCl and transferred to Zetaprobe nylon membrane (Biorad) in 0.4 N NaOH. Probes were made by random priming (Maniatis et al. 1982) of inserts. Filters were hybridized (Bernatzky and Tanksley 1986b) at 68 °C overnight and were washed to a final stringency of 1 × SSC, 0.1% SDS at 68 °C.

Clones of mtDNA were made from purified mtDNA of *N. alata* that was digested with *Hind*III, ligated into pGEM vector (Promega) and transformed into JM109. The homologous mitochondrial fragment was identified by screening colony lifts with a 1.0 kb *Hinc*II fragment that is upstream from the *S*-associated cloned genomic sequence. Sequencing was accomplished with Gem Seq K/RT sequencing system (Promega).

Results

A 3.1-kb *Eco*RI fragment that contains a coding sequence associated with self-incompatibility (Anderson et al. 1986) has been cloned from *N. alata* bearing the S_2 allele (Mau et al., in preparation). Subclones of this fragment were generated with *Hinc*II. A 1.0-kb fragment that is 334 bp upstream from the coding sequence was used as a probe on Southern blots of total DNA from *N. alata* and *L. esculentum* digested with *Hind*III. This probe produced a highly repeated pattern on *N. alata* but only one major band of about 750 bp on *L. esculentum* (Fig. 1 A). Subsequent hybridizations with DNA from *L. esculentum* and a wild relative, *L. pennelli*, that were digested with 12 different enzymes revealed no polymorphism for this sequence (data not shown). This high degree of conservation suggested that this homologous sequence may, in fact, be cytoplasmic. MtDNA was then isolated from *N. alata* and *L. esculentum* and Southern blots containing 200 ng of each mtDNA and 5 µg total DNA were probed with the 1.0-kb nuclear fragment. The hybridization signals clearly indicate that the homologous segment is in the mtDNA of both species (Fig. 1 A). An undigested sample of *L. esculentum* mtDNA demonstrates that the sequence is integrated into the high molecular weight chromosomal DNA and not in an extrachromosomal element.

The 750-bp mtDNA sequence of *N. alata* was cloned and, when used as a probe, it hybridized to a single fragment in both the total and mtDNA of *N. alata* and *L. esculentum* (Fig. 1 B). This is evidence that the sequence responsible for the repeated pattern of *N. alata* in Fig. 1 A and the sequence that is homologous to mtDNA

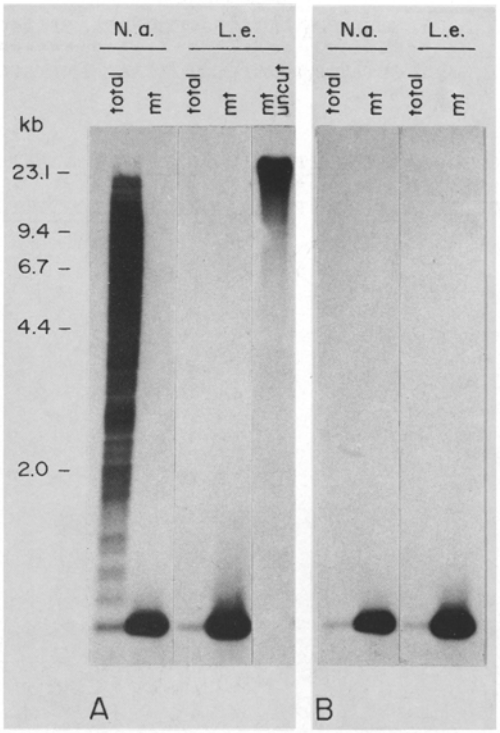

Fig. 1 A and B. Southern hybridization of *Nicotiana alata* (*N. a.*) and *Lycopersicon esculentum* (*L. e.*) total and mtDNA digested with *Hind*III. **A** Hybridization probe was the 1.0-kb genomic fragment that is upstream of the *S*-associated locus. The total DNA samples are 5 µg and the mtDNA samples are approximately 200 ng. Lane 5 is an undigested sample of *Lycopersicon esculentum* mtDNA. Molecular weight references in kilobase pairs are shown at the far left. **B** The hybridization probe was the 750-bp mitochondrial clone from *N. alata*. The DNA samples are the same as in **A**

are separate elements of the 1.0-kb genomic clone. In order to evaluate the significance of this mt sequence across the plant kingdom, the mt clone was hybridized to mtDNA from maize. Under moderate stringencies of hybridization and wash, and with long exposures to film, there was no detectable signal (data not shown).

It was determined that the homologous sequence occurs on a 315-bp *Hind*III/*Hinc*II mtDNA fragment. Sequencing and alignment of the mitochondrial and nuclear fragments reveal a 56-bp segment of very high homology (53/56 bp) (Fig. 2). There are two additional short, perfectly matched sequences of 8 and 15 bp 3′ from the main region of homology, However, these short segments are not aligned properly, suggesting that some rearrangement has occured since the interoganelle transfer. This is a common pattern of organization for plant mtDNA – nucleotide sequences tend to evolve slowly while structural rearrangements are more common (Palmer 1985; Sederoff et al. 1981).

The lack of sequence divergence between these two fragments could be explained in two ways. One is that the

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Nuc  ACAAAAAGTACCTATAAAAAGTATGTCCCAACAATTTAGCCTGAAATGAAAAAAAG
     *     * * ************  ********************** *********
Mt   AGCTTGAATCCCTATAAAAAGTCCGTCCCAACAATTTAGCCTGAAAAGAAAAAAAG

Nuc  TGGGGTAGAAACTAAGTTTCTTTTAGATCCTTTTGAAATCCTCATACAACTGATGG
     ********** *    **   **   *    *     *   * * *       *
Mt   TGGGGTAGAAGTTTCTATTGAATTGAGTAAGATCCTTTTGAATAGAAGATGCCATG
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Fig. 2. Nucleotide sequence of the region of homology between the nuclear (Nuc) and mitochondrial (Mt) fragments from *N. alata*. The *asterisks* indicate a match of sequence. The *bars* below the sequences show additional segments of homology that do not align properly. The *arrows* indicate the direct repeats of the nuclear sequence

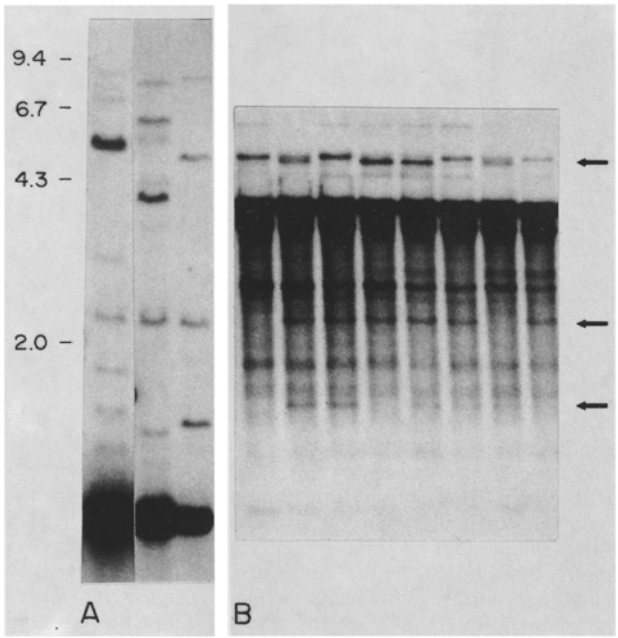

Fig. 3 A and B. Additional fragment homologies to the mitochondrial clone of *N. alata*. **A** *N. alata* (*N. a.*), *L. esculentum* (*L. e.*) and *L. pennellii* (*L. p.*) total DNA (5 μg) digested with *Hind*III and probed with the 750-bp mitochondrial clone of *N. alata*. Variation in the signal of the strongly hybridizing 750-bp fragment is due to different amounts of mtDNA contamination between samples. Molecular weight references in kilobase pairs are shown at the far left. **B** A sample of F_2 progeny from a cross between *L. esculentum* and *L. pennellii*. The total DNA (5 μg) was digested with *Eco*RI. The probe was the same as in **A**. *Arrows* indicate segregating fragments

sequence has been recently transferred between these genomes and base substitutions have not accumulated. Alternatively, the lack of divergence could indicate some functional constraint on the nuclear sequence. The homologous sequence is found within 397 bp of the start of transcription of a gene involved in self-incompatibility. Whether this sequence influences the function of the gene is not presently known. The nuclear sequence has no significant open reading frames and the mt sequence has only one that is opposite to the strand in Fig. 2. Evidence that this sequence is important in the expression of the downstream coding sequence would come from the finding of this sequence associated with other *S*-alleles of *N. alata*. An even stronger case for function would be the presence of this sequence near a homologous locus in a related genus such as *Lycopersicon*. However, we have not yet determined if the self-incompatibility systems of the two genera are based on the same biochemical principles. Allelic diversity of the *S*-associated coding sequences of *N. alata* is very high (Bernatzky et al. 1988; Anderson et al., in preparation), thus making the identification of related sequences in *Lycopersicon* very difficult.

The nuclear sequence contains a short 8-bp direct repeat that immediately flanks the 5′ region of homology (one of the repeats is within the homologous sequence). It is of interest to note that the first 7 bp of this repeat (AAAAGTA) perfectly match the terminal portion of the inverted repeat of the *S*-2 plasmid of maize that is found in the mitochondria of the *S* male-sterile cytoplasm (Levings and Sederoff 1983). The homology with the maize mt plasmid goes no further. Also, there is very little homology of either the 56-bp sequence of the entire 315-bp mt sequence with any of the plant, organelle, viral or structural sequences contained in the GenBank database (accessed through the University of Wisconsin Genetics Group software).

When Southern blots of total DNA from *N. alata*, *L. esulentum* and *L. pennellii* are probed with the 750-bp mitochondrial clone, other fragments become visible after long exposures to film (Fig. 3 A). It is likely that at least some of these fragments are nuclear, since equivalent signals are not seen with purified mtDNA (Figs. 1 and 3 A). Evidence that these fragments are nuclear comes from the analysis of F_2 progeny from a cross between *L. esculentum* × *L. pennellii*. Samples of total DNA from six progeny were digested with *Eco*RI and probed with the 750-bp mt clone (Fig. 3 B). Since all of these progeny have the same cytoplasm, the difference in patterns between individuals is probably due to segregation of nuclear fragments. In addition, when subclones of the 750-bp mtDNA sequences are used on Southern blots individually (data not shown), differences in the restriction patterns for these nuclear fragments are observed, raising the possibility that there is more than one element on the mitochondrial clone with homology to nuclear DNA.

Discussion

The mechanism by which this sequence has been transfered between organelles is not obvious. The presence of direct repeats in the nuclear sequence are consistent with features of transposable element excision (Nevers et al. 1986). There is indirect evidence that transposable elements are capable of producing duplications of DNA (Courage-Tebbe et al. 1983). One of the direct repeats immediately flanks the major homologous region and further suggests that these repeat sequences are associated with the transfer process.

There are only a few reports of transfer of sequences between nuclear and chromosomal mtDNA of plants (Farrelly and Butow 1983; Schuster and Brennicke 1987). The direction of transfer has been inferred by identifying the organelle in which the sequence usually functions. For example, a sequence has been found in the nucleus of yeast that is a mosaic of segments from known mitochondrial genes, suggesting that the direction of transfer was to the nucleus (Farrelly and Butow 1983). Two discoveries establish the ability of mtDNA to take up foreign sequences. One is the finding of widespread homologies between chloroplast and mtDNA (Stern and Lonsdale 1982; Stern and Palmer 1984). The sequences involved are known to be true chloroplast genes and so the direction of transfer is considered to be from the chloroplast to the mitochondria. The other report is the finding of a sequence in *Oenothera* mtDNA that has homology to a mosaic of coding sequences and structural RNAs of the nucleus (Schuster and Brennicke 1987). Within this segment there is also a region of reasonable homology to reverse transcriptase. These authors consider that interorganelle transfer of DNA may occur through RNA with subsequent local reverse transcription and genomic integration.

We cannot infer the direction of transfer for the sequence in *N. alata*. The lack of homology with maize mtDNA suggests that it is not a conserved coding sequence of the mitochondria. The absence of a significant open reading frame makes it difficult to assign it to one genome or the other. It may be that some interorganelle transfers do not require RNA but that other structural features are important for duplication and mobility. The evidence presented here indicates that some of the excess mtDNA of plants may be derived from non-coding nuclear DNA. How widespread the occurrence is of such mitochondria-nuclear homologies remains to be seen.

The proximity of the mtDNA related sequence to the *S*-locus suggests that it may influence the regulation of this gene. The type of self-incompatibility of *N. alata* is termed gametophytic. In this system, any haploid pollen that carries an allele that matches an allele in the diploid sporophytic tissue upon which it germinates is inhibited and prevented from fertilizing the ovule. Although a cytoplasmic component is not usually associated with self-incompatibility, there are certain unexplained observations such as the generation of new allelic specificities that appear first in the stylar (maternal) tissue (Nettancourt et al. 1971), that could be explained by such a cytoplasmic component. The role that this sequence may play in the function and expression of self-incompatibility will be investigated further.

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