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Structural analysis of length mutations in a hot-spot region of wheat chloroplast DNAs

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Summary. The hot-spot region related to length mutations in the chloroplast genome of the wheat group was precisely analyzed at the DNA sequence level. This region, located downstream from the *rbcL* gene, was highly enriched in A + T, and contained a number of direct and inverted repeats. Many deletions/insertions were observed in the region. In most deletions/insertions of multiple nucleotides, short repeated sequences were found at the mutation points. Furthermore, a pair of short repeated sequences was also observed at the border of the translocated gene. A sequence homologous with ORF512 of tobacco cpDNA was truncated in cpDNAs of the wheat group and found only in the mitochondrial DNA of *Ae. crassa*, suggesting the inter-organellar translocation of this sequence. Mechanisms that could generate structural alterations of the chloroplast genome in the wheat group are discussed.

Key words: Hypervariable region – Wheat chloroplast DNAs – Short repeats – Intramolecular recombination

Introduction

Most chloroplast genomes are small multicopy circular molecules (120–160 kbp), disrupted by large inverted repeats (20–26 kbp), and are highly conserved among vascular plants, mosses, and algae (Palmer 1985; Ozeki et al. 1987). Comparative studies of chloroplast DNAs based on restriction fragment analysis revealed that to some extent, chloroplast genomes in related plants, harbor structural rearrangements. These rearrangement occur as inversions (e.g., Howe 1985), deletion/insertions (Gordon et al. 1982; Palmer et al. 1985; Doebly et al. 1987), and translocations (Bowman et al. 1988; Ogihara et al. 1988). Structural mutations do not occur randomly through the genome among related plants (Palmer 1985). Furthermore, hot-spot regions related to length mutations have been reported (Tassopulu and Kung 1984; Ogihara and Tsunewaki 1988), indicating that some directed mechanism(s) operates to introduce the rearrangements in the chloroplast genome. One possibility is that AT-rich regions in the genome are associated with the recombination hot-spots which cause genome instability (Shih et al. 1984; Furano et al. 1986; Hyrien et al. 1987).

Recently, a number of inter-organellar translocations of genetic material have been reported (Scott and Timmis 1984; Schuster and Brennicke 1988). The region around the *rbcL* gene is of great interest for studies on genetic flux among organelles, because this region is commonly found in the mitochondrial genome of divergent plants (Nugent and Palmer 1988) and shows high variability of its translocated structure among related plants such as maize and rice (Lonsdale et al. 1983; Moon et al. 1988).

We previously reported (Ogihara et al. 1988) the deletion of a large segment of DNA in wheat chloroplast DNA, and the non-reciprocal translocation of the *rpl23* gene into the hot-spot region. Subsequently, we have carried out a structural analysis of the hot-spot region, in more detail. From a precise sequence analysis of the region, some additional characteristic features of the hot-spot were disclosed. In addition to these drastic rearrangements, a large number of deletions/insertions have also accumulated in the region. The region is highly AT-rich and contains many direct and inverted repeats that may contribute to the instability of this segment of the wheat chloroplast genome. Based on these analyses, the possible mechanisms of cpDNA rearrangements and the significance of short repeated sequences for structural alterations of the chloroplast genome are discussed. Furthermore, we have examined the possibility that the rearranged DNA segment had been translocated to other position(s) both inside the chloroplast genome as well as to other organelles.

Materials and methods

Two alloplasmic lines of *Triticum aestivum* cv. Chinese Spring, (*crassa*)-CS (designated as cpAcr and mtAcr) and (*squarrosa*)-CS (desig-

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nated as cpAsq and mtAsq), as well as a euplasmic line of Chinese Spring (designated as cpTa and mtTa), were used for the isolation of chloroplast (cp) and mitochondria (mt) fractions, according to standard procedures (Bonen and Gray 1980; Ogihara and Tsunewaki 1982). Nuclear fractions were prepared from 14-day-old etiolated seedlings of wheat (*T. aestivum* cv. Chinese Spring; designated as nTa) and two *Aegilops* species, i.e., *Aegilops crassa* (4×) (nAcr) and *Ae. squarrosa* var. typica (nAsq) (Watson and Thompson 1986). From these fractions, nuclear, chloroplast, and mitochondrial DNAs were extracted according to the procedure of Kolodner and Tewari (1975). These DNAs were digested with *Hin*dIII and *Bam*HI, fractionated on an 0.85% agarose gel, and transferred to a nylon membrane by the standard procedure (Maniatis et al. 1982). For construction of the specific probe for ORF512, the ORF512 coding region of tobacco cpDNA (probe 'orf512'), which is contained in the S5 fragment (Shinozaki et al. 1986), was prepared by *Dra*I digestion of S5 to create a 1 632-bp fragment (see Fig. 5). Specific probes for the detection of translocation were constructed by digestion of the cpDNA of *Ae. crassa* with restriction enzymes after a computer search of the sequence (Ogihara et al. 1991). *Xba*I digestion of the plasmid produced 349-bp (probe 'rpl23') and 801-bp (probe 'xb801') fragments, which correspond to the *rpl23'* region and the deleted segments in the cpDNAs of *T. aestivum* and *Ae. squarrosa*, respectively. Double digestion with *Nru*I and *Pst*I produced a 1 151-bp fragment (probe 'rbcL'), which corresponds to the internal coding sequence of the *rbcL* (ribulose, 1,5-bisphosphate carboxylase large subunit) gene (Terachi et al. 1987). The location of these probes on the physical map is shown in Fig. 5. The southern hybridization procedure employed has been reported previously (Katayama et al. 1991). Plasmid DNAs harboring the hypervariable region related to the length mutations of chloroplast DNAs in *T. aestivum* cv. Chinese Spring, *Ae. crassa* (4×), and *Ae. squarrosa* were used for sequence analysis (EMBL accession numbers X62117, X62118, and X62119). Subcloning of chloroplast DNA fragments into pUC119 and the sequence strategies employed were as described by Ogihara et al. (1991).

Results

Structural alterations of hypervariable region in wheat cpDNA

Of the three structural alterations that can occur in DNA, namely, deletions/insertions, inversions, and translocations, both deletions/insertions and translocations have been detected in the hypervariable region located downstream from *rbcL* by DNA sequencing (Ogihara et al. 1991). Six genes or ORFs were present in the hypervariable region including *rbcL* and ORF230 (Fig. 1). It is striking that a pseudogene of *rpl23* (designated as *rpl23'*) for chloroplast ribosomal protein L23 was non-reciprocally translocated into the region in place of the ORF512 of tobacco (Bowman et al. 1988; Ogihara et al. 1988). Although sequence homology with the ORF512 of tobacco was not detectable under the stringent conditions employed in previous work (Ogihara et al. 1988), we were successful in a further search using 'Harplot' analysis to find such homology. The homologous sequence of cpDNA in *Ae. crassa* (designated as ORF512') corresponded to only 10% of the length of the tobacco ORF512 (1 050–1 224 bases from the initiation codon) and had 58.1% similarity with it. Furthermore, the sequence had been deleted entirely in this region of cpDNA in *T. aestivum* and partly in that of *Ae. squarrosa*. Except for the *rbcL* and *psaI* (small peptide for photosystem I) genes, and ORFs including ORF512' and *rpl23'*, this region of gramineous cpDNA showed no homology with the corresponding region of tobacco cpDNA even under low-stringency conditions.

It is known that cpDNA is AT-rich. Thus the AT content of whole cpDNA is 62% in tobacco (Shinozaki et al. 1986) and 72% in liverwort (Ohyama et al. 1986). Spacer regions between genes are especially AT-rich, being about 80% in liverwort (Ozeki et al. 1987). Although overall the AT content of the sequenced region in the wheat complex is not so high (64% in *Ae. crassa*), genes and ORFs were bounded by AT-rich spacer regions as depicted in Fig. 1. The intergenic regions between *rbcL* and *rpl23'* and between ORF512' and *psaI* showed an AT content of more than 80%. It should be emphasized that the region around the sites where rearrangements took place, such as deletions/insertions with multiple nucleotides (see below), translocation and truncation, are highly AT-rich.

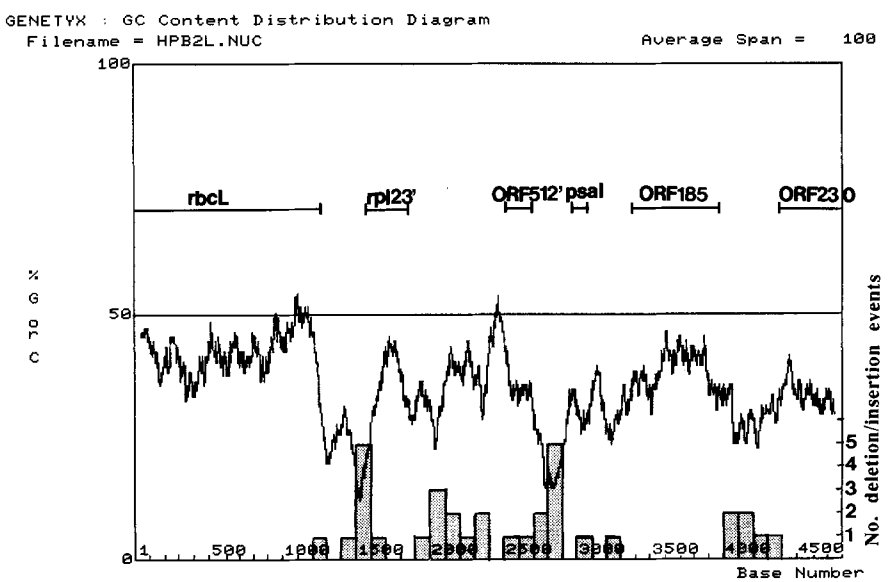

Fig. 1. Distribution of GC content and number of deletion/insertion events with multiple nucleotides in the hypervariable region of *Ae. crassa*. Each point is refers to the calculation of the percentage of GC content in every adjacent 50 nucleotides. *Bars* represent numbers of deletion/insertion events in every 100 nucleotides. The positions of *rbcL*, *rpl23'*, ORF512', *psaI*, ORF185 and ORF230 are indicated

Deletions/insertions in the hot-spot region of wheat cpDNA

Restriction fragment analysis of wheat cpDNAs had revealed that length mutations of DNA fragments were concentrated in the region adjacent to *rbcL*. DNA sequencing of the region in wheat cpDNAs enabled us to specify the position of two major length mutations, and we showed that these two mutations are deletions mediated by short direct repeats (Ogihara et al. 1988). In addition to these deletions, many other deletions/insertions were detectable in a comparison between wheat and *Aegilops*, and between the wheat group and rice. The number of nucleotides mutated and the number of deletion/insertion events are shown in Fig. 2. In total, 41 deletion/insertion events were found in the *Triticum-Aegilops* group, and 61 were observed in the wheat group-rice comparison. The majority of them (85.4% in the wheat group and 49.2% in the wheat-rice comparison) involved only a few nucleotides and were probably due to slippage during DNA replication (Levinson and Gutman 1987). Multi-nucleotide deletions/insertions (approximately 5–20 nucleotides) were also frequently observed, being 7.3% in the wheat-*Aegilops* comparison and 45.9% in the wheat group-rice comparison. Deletions/insertions showing more than 50 nucleotides were observed at a lower frequency (7.3% in the wheat group and 3.3% in the wheat rice comparison). It is noteworthy that in both groups of comparison repeated oligonucleotides, i.e., direct repeats and/or inverted repeats, were found around most deletions/insertions with multiple nucleotides. Of six deletional/insertional events containing more than five nucleotides of wheat-*Aegilops* cpDNA, five occurred at short direct repeats, and one, at an inverted repeat. In the comparison between the wheat group and rice of 34 deletional/insertional events harboring multiple (more than four), 16 cases contained short direct repeats and three contained inverted repeats. All those repeated oligonucleotides related to deletion/insertion as well as the numbers of nucleotides deleted/inserted are summarized in Table 1. Taking the divergence time of the differentiation between wheat and rice into account, we can conclude that most deletions/insertions of multiple nucleotides took place at, and/or around, repeated oligonucleotides as in the cases of plasmid DNA (DasGupta et al. 1987) and bacterial chromosomes (Albertini et al. 1982). A possible mechanism for a typical example of a repeat-mediated deletion is presented in Fig. 3. Since in this case, deletion events more easily explain the direction of structural changes than insertional and/or amplification events, structural rearrangements in this region have been traced from the rice sequence. From a comparison of the changed sequences in this region, the deletion between inverted repeats was deduced to have occurred first. Then, successive deletions, mediated by direct repeats, took place. Consequently, no more sequence homologies in this region were detectable by a simple comparison.

Most deletions/insertions were observed in the intergenic regions. Pseudogenes, such as *rpl23'* and ORF512', also harbor such alterations (Moon et al. 1988; Hiratsuka

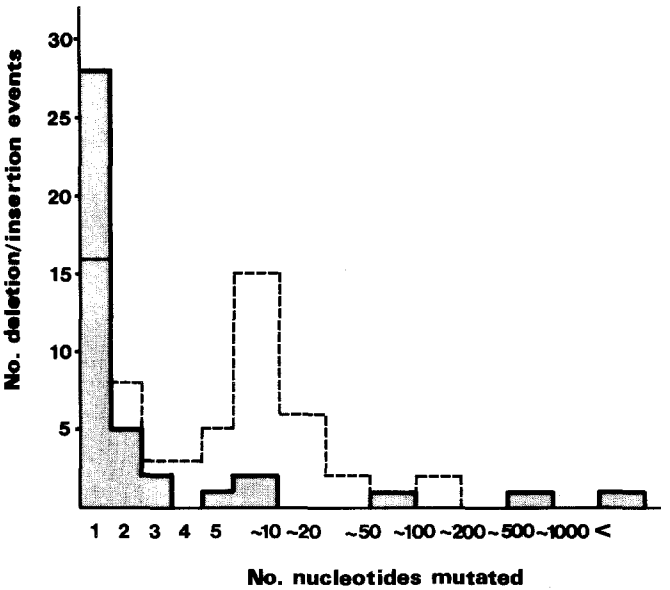

Fig. 2. Frequency of deletion/insertion events having various numbers of nucelotides found in the hypervariable region of cpDNAs. Deletion/insertion events were scored by a comparison of the DNA sequences published in Ogihara et al. 1991 within the wheat group (*Triticum* and *Aegilops*) (*bold line*) and between the wheat group and rice (*dotted line*). Deletion/insertion events involving more than ten nucleotides were grouped into a certain class depending on their frequencies

Table 1. Short repeated sequences found at the borders of deletions/insertions and the number of nucleotides deleted/inserted in the comparison among the wheat group and between the wheat groups and rice

Plants compared			
Among wheat group wheat group and rice		Between	
Repeat sequence	No. nucleotides deleted/ inserted	Repeat sequence	No. nucleotides deleted/ inserted
1 AGAAG	5	3 AAAT	21
2 AATAAAAT	8	4 ATTATCAT[a]	6
4 ATTATCAT[a]	6	5 ATAC	135
15 TTTTA	61	6 AGTTT	6
A CATTTTTTT	791	7 CTCCG	5
S ATTAT	1062	8 GAAATT[a]	75
		9 ATTC	4
		10 CTTTTTTTTTA GAATACTTT[a]	12
		11 TAAA	5
		12 TAAAAA	12
		13 AAAAT	17
		14 GAATAGAT	5
		15 TTTTA	11
		16 GAAGAA	6
		17 ACTA	7
		18 GAAATA	38
		19 GTAGA	5
		20 TTTGATT	7

[a] Inverted repeat
The number or letter preceding each repeat sequence is a designation from Ogihara et al. (1991)

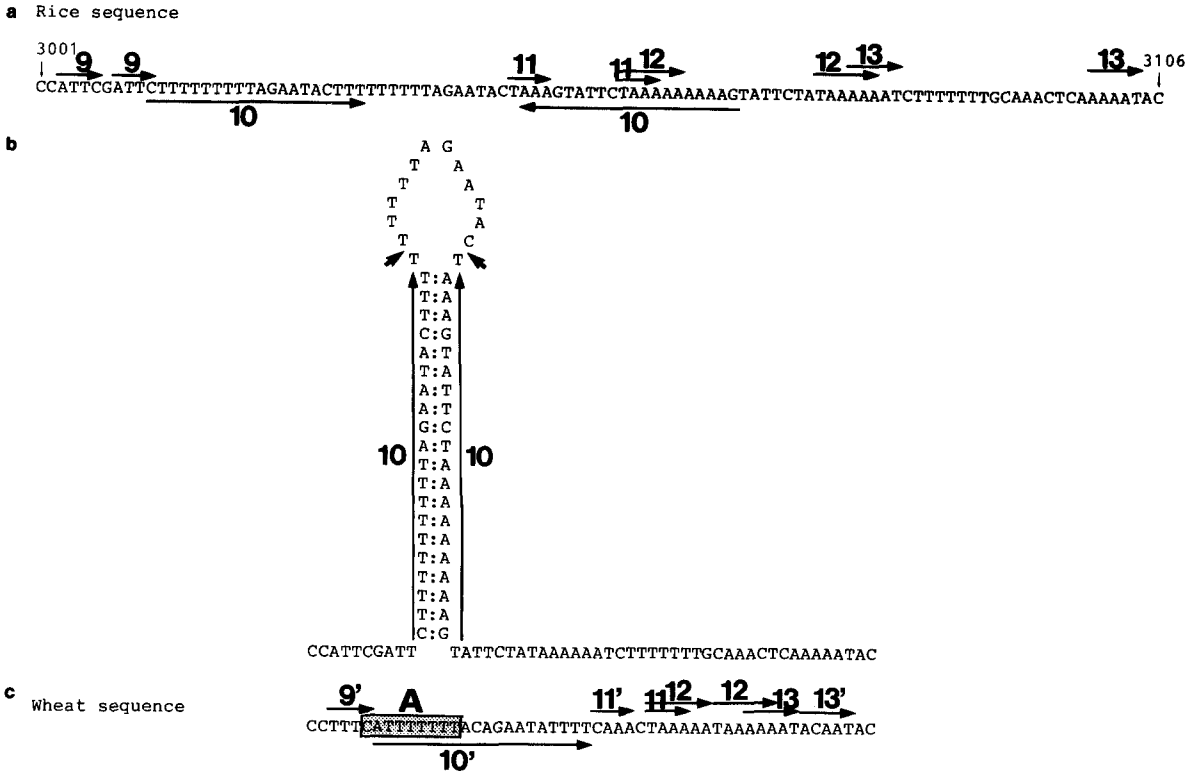

Fig. 3a–c. Successive deletion events in the hypervariable region of cpDNAs between rice (**a**) and the wheat group (**c**). Deletion events are assumed in this case (see text). *Numbered arrows* stand for short repeats thought to have participated in the deletions. First, deletions between the inverted repeats occurred at the positions of the *arrowheads* (**b**). Then, deletions mediated by direct repeats occurred successively through illegitimate recombination, and consequently nucleotide sequences were completely changed so as to show no sequence homology. *Primed numbered arrows* show incomplete homology with the counterparts of rice. Sequence '*A*' in the *hatched box* stands for the sequence that participated in the large deletion (792 bp) of the cpDNA of *T. aestivum* (Ogihara et al. 1988)

et al. 1989; Ogihara et al. 1991). However, the *rbcL* gene of *T. aestivum* carries a deletion when compared with the gene of the two *Aegilops* species, while the *rbcL* of the wheat group contains two deletional events, with eight nucleotides each at the 3′ terminus of the gene, when compared with the *rbcL* of rice (Terachi et al. 1987; Nishizawa and Hirai 1987). A and/or T clusters have a tendency to become targets for deletions/insertions (Aldrich et al. 1988). Additionally, the frequency of deletion/insertion events seems to correlate with the AT content of the region (Fig. 1).

Translocation of the rpl23′ gene into the hypervariable region

The pseudogene *rpl23′* has been non-reciprocally translocated to the AT-rich region. In order to trace the translocational event, the sequence of *rpl23′* was compared with the functional *rpl23* gene of wheat (Bowman et al. 1988). Critical alignment of *rpl23′* with the *rpl23* of *T. aestivum* indicated that homology starts at the fourth nucleotide downstream from the initiation codon, and continues to the eighth nucleotide downstream from the termination codon, being out of frame in *rpl23′*. At the border of the homologous region, there exist three sets of direct repeats consisting of four nucleotides, i.e., GAAT, designated as repeat 'T1' in Fig. 4. Moreover, we also found another direct repeat with eight nucleotides (repeat 'T2') inside the *rpl23* gene and just downstream from the direct repeat 'T1', as well as a repeat (T2′) having seven out of the eight nucleotides of repeat 'T2' and located upstream of the direct repeat 'T1'. Since no transposon-like structure was found around *rpl23′*, the *rpl23* gene was presumed to be translocated by illegitimate recombination mediated by these short repeat sequences, as proposed in Fig. 4. If the *rpl23′* sequence is omitted from the region, the recipient sequence before integration can be deduced, in which short repeats 'T1' and 'T2' are connected (Fig. 4a). If these short homologous sequences between *rpl23* and the recipient DNA are paired, then a recombination intermediate can be set up as shown in Fig. 4b. In the intermediate configuration, the second ATG from the initiation codon participates in the pairing, as does repeat 'T2'. If the DNA strands were nicked between ATG and GAAT, and non-reciprocal strand exchange took place in the paired 'GAAT' sequences boxed in Fig. 4b, the truncated *rpl23* gene would be connected to the 5′-recipient sequence. Again, if the free end of the recipient DNA sequence which encodes 'T1' and 'T2' was paired with the second 'T1' and T2′ in the *rpl23* gene, and strand transfer took place in the paired 'T1' (Fig. 4c), part of the *rpl23* gene would be integrated into the recipient DNA sequence. Consequently, the integrated sequence now

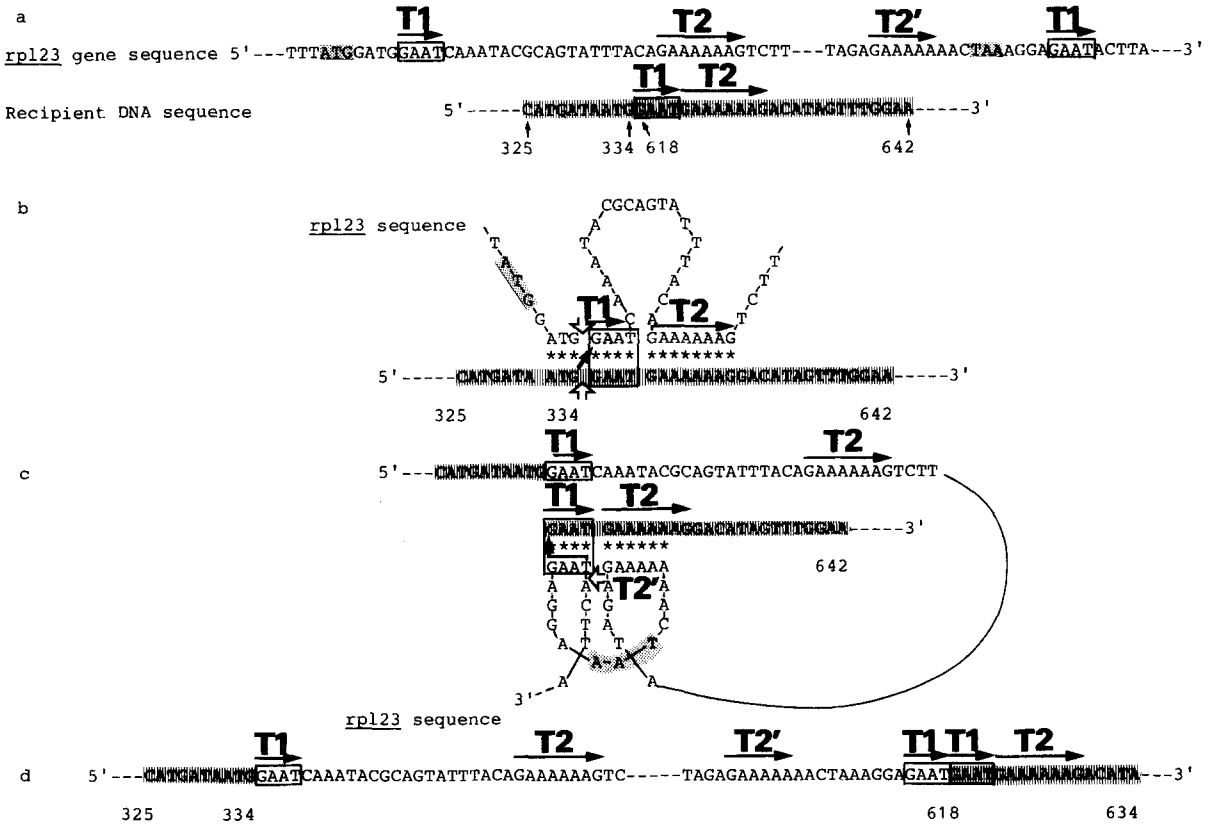

Fig. 4a–d. A model for the translocation of the *rpl23* gene mediated by illegitimate recombination through short repeat sequences. **a** The authentic *rpl23* sequence (*upper line*) located in the large inverted repeats, and the presumed recipient DNA sequence (*lower line*) before translocation of *rpl23*, because the translocation of *rpl23'* was found only in the cpDNA of grasses (Katayama and Ogihara, in preparation). *Dashes* indicate a stretch of DNA, for which the sequence is not shown. **b** A hypothetical intermediate of recombination. Direct repeat sequences (repeat T1 and T2), both of which are located around the initiation and termination signals of the *rpl23* gene and exist in the recipient sequence, are assumed to pair with each other to construct the intermediate. DNA strands are nicked at the position indicated by the *open arrows*, and unequal strand exchange takes place at the position indicated by the *short arrow*. **c** A subsequent recombination after **b**. The free end of the recipient DNA sequence is paired with the second T1 and T2 of the *rpl23* gene. The *rpl23* sequence is nicked at the position indicated by the *open arrow*, and DNA strands are ligated at T1 as shown by the *arrow*. **d** This novel sequence order is created after recombination. *Numbered large arrows* stand for repeat sequences, and strand transfer is assumed to occur at the boxed *sequence*. *Numbers below the sequence* are given from the termination codon of the *rbcL* gene

contains three sets of repeat 'T1', two sets of repeat 'T2', and one 'T2'' sequence, as shown in Fig. 4d.

Homology of the rearranged sequences in cpDNA of the wheat group with other organellar genomes

In order to search for the truncated ORF512 sequence in the wheat group, and the deleted segments of the cpDNA of *T. aestivum* and *Ae. squarrosa* that are present in *Ae. crassa*, we carried out Southern hybridization of nuclear, chloroplast and mitochondrial DNAs with different parts of the hypervariable region as probes (probes orf512, xb801, rbcL, and rpl23; see Fig. 5). As the nuclear and mitochondrial DNAs used for the experiment were contaminated with chloroplast DNA, [an approximately 10–20% contamination of cpDNA in each fraction was observed from the densitometer measurement (data not shown)], we regarded only those hybridized fragments with positions different from the cpDNAs as mitochondria- and/or nucleus-specific bands. As shown in Fig. 5, probe 'orf512' hybridized to the 9.0 kbp fragment of cpDNA of *T. aestivum* (cpTa), to the 9.8 kbp of cpDNA of *Ae. crassa* (cpAcr), and to the 8.8 kbp of cpDNA of *Ae. squarrosa* (cpAsq), all obtained by *Hin*dIII digestion, as well as to the 9.6 kbp fragment of cpTa, to the 10.5 kbp of cpAcr, and to the 9.2 kbp of cpAsq, all obtained by *Bam*HI digestion. In addition to the sites in the cpDNAs, homologous regions were strikingly detectable in three positions (12.5, 4.8, and 2.7 kbp) of the mitochondrial genome of *Ae. crassa*, but not in other species. This indicates the cpDNA sequence was inserted into three different regions of the mitochondrial genome, and/or three different fragments were produced as a result of intramolecular recombination in the mitochondrial genome and/or nucleotide changes.

All other probes (rbcL, rpl23 and xb801) revealed only hybridization signals with the chloroplast DNA fragments which had been mapped (data not shown).

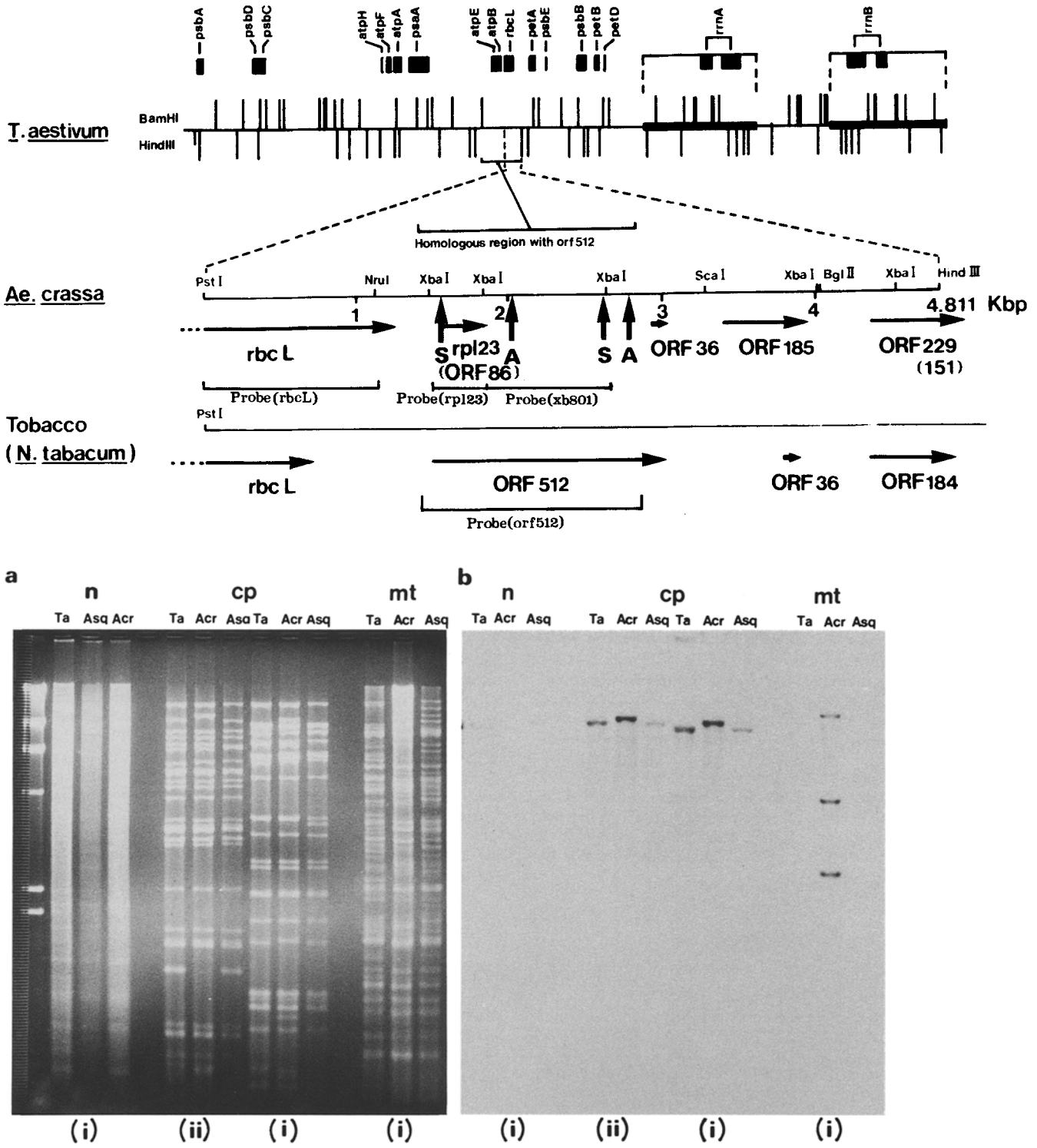

Fig. 5. A physical map (upper) of the hypervariable region for the length mutations among cpDNAs of *Triticum* and *Aegilops* species and the corresponding region of tobacco showing the positions of probes ('rbcL', 'rpl23', 'xb801' and 'orf512'), and hybridization profiles (lower) with tobacco ORF512 to the three organellar DNAs of three wheat species. (**a**) Profiles of the nuclear (*n*), chloroplast (*cp*), and mitochondrial (*mt*), DNAs digested with *Hin*dIII (marked as '*i*' on the bottom of the panels) and *Bam*HI (*ii*) after agarose gel electrophoresis. (**b**) Autoradiograph after hybridization with probe (orf512). The region homologous to tobacco ORF 512 is shown in the physical map

Discussion

The nature of structural alterations of the hypervariable regions in wheat cpDNAs

Deletions/insertions and a translocation were detected in the hypervariable region of wheat cpDNA. The majority of these length mutations were just a few nucleotides long due to slippage during DNA replication (Fig. 2). On the other hand, as depicted in Fig. 3, short repeated sequences, i.e., direct repeats or inverted repeats, were found around most length mutations comprising multiple nucleotides. From the present analysis, length mutations of chloroplast DNA mediated by short repeats can be classified into four groups: (1) Deletions of a DNA segment resulting from intramolecular recombination mediated by short direct repeats. In this process, only one of the direct repeats has been retained [the 'A' an 'S' sequences found in the cpDNAs of *T. aestivum* and *Ae. squarrosa*, respectively (Ogihara et al. 1988)]. (2) Loss/gain of one of several adjacent direct repeats due to slippage during replication (repeat '9' in Fig. 3). (3) Excision/addition between two direct repeats. In the present case, two direct repeats have been found at the border of the length mutations (direct repeats '11', '12', and '13' in Fig. 3). (4) Deletion/insertion between two inverted repeats (repeat '10' in Fig. 3). In relation to these four mechanisms, a recombination model by enzyme(s) promoting deletion at short sequence repeats (Singer and Westyle 1988) supports case (1). Case (2) can be explained by aberrant replication resulting from slippage-mispairing during DNA synthesis (Farabaugh et al. 1978; Das-Gupta et al. 1987; Brunier et al. 1988). Examples of an association of palindromic secondary structure and short direct repeats with length mutations of DNA segments (e.g., Glickman and Ripley 1984; Hasson et al. 1984; Nalbantoglu et al. 1986; Hyrien et al. 1987) are considered to support cases (3) and (4). These length mutations, i.e., deletion/insertion of DNA segments due to illegitimate recombination or slippage-mispairing between two distinct repeats, have been reported to occur in various genomes including plasmid DNA (e.g., Weston-Hafer and Berg 1989), the *E. coli* chromosome (Albertini et al. 1982), the mitochondrial genomes of filamentous fungi (Gross et al. 1984; Turker et al. 1987), yeast (Foury and Kolodynski 1983; Dieckmann and Gandy 1987), human (Schon et al. 1989) and plants (Rottmann et al. 1987; Joyce et al. 1988), and the nuclear DNA of mammals (Nalbantoglu et al. 1986; Canning and Dryja 1989). DNA segments changed by these length mutation varied in size from several to thousands of nucleotides. A number of these length mutations have been reported in cpDNAs (Zurawski and Clegg 1987; Aldrich et al. 1988; vom Stein and Hachtel 1988; Wolfson et al. 1991).

As in the cases of length mutations, the short repeat sequences are found at both ends of the translocated chloroplast *rpl23'* (see Fig. 4). In most transpositions of genes or DNA segments mediated by transposons, retrotransposons and illegitimate recombination (see references in Berg and Howe 1989), the target sites of the recipient genome were duplicated. But, in the translocation of the chloroplast *rpl23* gene, the target sequence ('T1' in Fig. 4) was triplicated, whereas two such sequences are found in the original *rpl23* sequence. This suggests that the *rpl23'* was translocated into the region via a novel illegitimate recombination mediated by the short repeat sequences, as shown in Fig. 4.

Furthermore, the large inversion detected in the comparison between the cpDNAs of gramineous plants and tobacco has also been ascribed to an intermolecular recombination mediated by short repeats (Hiratsuka et al. 1989). These lines of evidence indicate that short repeated sequences seem to play a substantial role in the reorganization of the chloroplast genome, though the enzyme systems that led to these rearrangements of the chloroplast genome remain to be identified and studied. One possible system is the illegitimate recombination mediated, and/or promoted, by topoisomerase (Ikeda 1986; Bae et al. 1988; Shuman 1989; Sperry et al. 1989), because both topoisomerases I and II have been found in chloroplasts (Nielsen and Tewari 1988; Pyke et al. 1989).

Translocations of cpDNA segments into other organelles

In the comparison of the hypervariable region of the wheat group with the corresponding region of tobacco cpDNA, several rearrangements were revealed: (1) almost all of the *rpl23* gene, which was originally located in the inverted repeat region of the chloroplast genome, had been non-reciprocally translocated to this region; (2) about 90% of ORF512 in tobacco had been truncated; (3) a sequence specific for the wheat species was found in the region. Sequences homologous with ORF512 of tobacco (ORF512') have been found in three chloroplast DNAs of the wheat group (Fig. 5). According to the sequence comparison of cpDNA among three wheat species, the full sequence of ORF512' was retained only in the cpDNA of *Ae. crassa* (Ogihara et al. 1991). Therefore, the homologous region of the three cpDNAs might involve another site close to the original, probably in the approximately 2.0 kbp region downstream from the *atpB,E* gene. But, strikingly strong homology with ORF512 was detected only in the mtDNA of *Ae. crassa*. This indicates recent transposition of ORF512' into the mitochondrial genome. It is, however, unclear whether these inserted segments encompass the full ORF512 sequence. The other portions of wheat cpDNA probed in the present study revealed no sequence homology with the other organellar genomes in terms of Southern hybridization. It should be emphasized that DNA sequence homology with the *rbcL* and *rpl23'* genes, both of which are detectable in the mitochondrial genome of rice and maize at positions close to one another (Lonsdale et al. 1983; Moon et al. 1988), could not be detected in the mitochondrial genome of the wheat group. This is suggestive of the recent transfer of a cpDNA segment to the mitochondrial genome, as in the case of *Brassica* (Nugent and Palmer 1988). Concerning the translocation of DNA segments into the nuclear genome, several cases have been reported (Scott and Timmis 1984; Pichersky and Tanksley 1988; Baldauf and Palmer 1990). However,

cpDNA segments located in the hot-spot region were not found in the wheat nuclear genome at the level of Southern hybridization.

As little is known about the mechanism(s) for transfer and subsequent maintenance of DNA segments among organellar genomes, further documentation of intra-organellar DNA transfers in related species such as *Triticum* and *Aegilops* can be expected to increase our understanding of the genetic flux that has occurred among organellar genomes during evolution.

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