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The retrotransposon *RTip1* is integrated into a novel type of minisatellite, *MiniSip1*, in the genome of the common morning glory and carries another new type of minisatellite, *MiniSip2*

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Abstract Transposable elements have often been discovered as new insertion sequences in known genes, and minisatellites are often employed as molecular markers in diagnostic and mapping studies. We compared the genes for flower pigmentation in a line of the common morning glory bearing fully colored flowers with those in two *anthocyanin*^{flaked} mutable lines producing variegated flowers and found RFLPs at the region of the *ANS* gene for anthocyanin biosynthesis. The DNA rearrangements detected by the RFLPs are due to integration of a novel type of minisatellite, *MiniSip1*, having a long LTR retrotransposon, *RTip1*, inserted in the mutable lines. The structural analysis of the rearranged region revealed that the 12.4-kb *RTip1* element is flanked by 5-bp target duplications within the *MiniSip1* sequence and contains two LTR sequences of about 590 bp, a primer binding site for tRNA^{Lys}, a typical polypurine tract and another new type of minisatellite, *MiniSip2*. Since no long open reading frame corresponding to the *gag* and *pol* genes was found, *RTip1* appears to be a defective *Ty3*/*gypsy*-like element. Interestingly, the 269-bp-long *MiniSip1* element comprises two alternating motifs of 41 bp and 19 bp, whereas the 962 bp long *MiniSip2* element consists of two partially alternating motifs of 86 bp and 90 bp which are partially homologous to each other. Possible evolutionary processes that may have generated the rearranged structure at the *ANS* gene region are also discussed.

Key words DNA rearrangement · Minisatellite · Morning glory · Repetitive DNA · Retrotransposon

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Introduction

Repetitive DNA is an integral component of eukaryotic genomes and can be classified into two groups; interspersed repeats and tandem repeats (Weising et al. 1995). Retrotransposons or retroelements that transpose via RNA intermediates reside as interspersed repeats in the genomes, and a class of these elements carry long terminal repeats (LTRs) at both termini (Berg and Howe 1989; Smyth 1993; Wessler et al. 1995; Kunze et al. 1997). The LTRs are flanked by short inverted repeats which terminate with the sequences 5′-TG and CA-3′. Upon integration, the LTR retrotransposons generate target duplications, usually 5 bp in length. A primer binding site (PBS) resides a few nucleotides inside of the 3′ end of the 5′-LTR sequence, and tRNA binds to the PBS and serves as a primer for the first(−)-strand cDNA synthesis. In most plant LTR retrotransposons, the initiator tRNA^{Met} is utilized as a primer. The second(+)-strand cDNA synthesis is primed at a polypurine tract (PPT) located immediately upstream of the 3′-LTR.

The active, or autonomous, LTR retrotransposons contain open reading frames (ORFs) encoding for proteins necessary for the replication and transposition of the elements. They include *gag*, which carryies an RNA binding motif, and a polyprotein gene, *pol*, containing domains for proteinase (PR), integrase (IN), reverse transcriptase (RT) and RNaseH (RH) activities. The

LTR retrotransposons are further divided into two groups; the *Ty1/copia*-like and *Ty3/gypsy*-like elements, according to their order of the domains in the *pol* genes and the amino acid sequences of the RT proteins.

Tandem repeats can generally be classified into three groups; satellites, minisatellites and microsatellites (Tautz 1993; Weising et al. 1995). Satellite DNA consists of very long stretches of sequences with a very high number of repetitions (more than 1000 copies) of a basic motif unit generally about 100–300 bp long, and it usually occurs at a few limited genomic loci. Minisatellites consist of a lower number of repetitions of a shorter motif, usually 10–60 bp long, and they occur at many loci in the genome. Microsatellites show a low degree of repetitions of very short motifs, around 1–10 bp long, and they are dispersed over various genomic loci. Minisatellites have been shown to have a high degree of polymorphism due to variations in the number of the repeats as well as to the sequence alterations within the repeats, and they are also termed VNTR (variable number of tandem repeats). The polymorphic nature of the minisatellites makes them useful as molecular markers in the diagnostic identification of strains and in mapping studies in various organisms (Dallas 1988; Tourmente et al. 1994; Weising et al. 1995).

During studies to identify the mutable allele *anthocyanin*^{flaked} (*a*^{flaked}) conferring flower variegation in the common morning glory (*Ipomoea purpurea* or *Pharbitis purpurea*) (Iida et al. 1995; Hisatomi et al. 1997), we have found distinct RFLPs (restriction fragment length polymorphisms) at the region of the *ANS* gene for anthocyanin biosynthesis (Forkmann 1993; Martin and Gerates 1993). The DNA rearrangements detected by the RFLPs are due to the integration of a novel type of minisatellite, *MiniSip1*, that has a 12,422-bp-long LTR retrotransposon, *RTip1*, inserted. In this report, we present a structural analysis of *MiniSip1* carrying *RTip1* and show that *RTip1* contains another new type of minisatellite, *MiniSip2*. Interestingly, the 269-bp-long *MiniSip1* element was found to comprise two alternating motifs of 41 bp and 19 bp, whereas the 962-bp-long *MiniSip*2 element consists of two alternating motifs of 86 bp and 90 bp which are partially homologous to each other.

Materials and methods

Plant materials

The lines of the common morning glory KK/FP-36 and KK/VR-37 bearing wild-type dark-purple flowers and white flowers with red variegation, respectively, have been described previously (Iida et al. 1995; Hisatomi et al. 1997). In this study, we also employed the line KK/VR-40, which produces white flowers with red variegation, and it was obtained from K. Kasahara's private collection. Both lines KK/VR-37 and KK/VR-40 carry the mutable *flaked* allele (Barker 1917; Imai and Tabuchi 1935; Hisatomi et al. 1997). Plants were grown in pollinator-free greenhouses.

Nucleic acids procedures

Plant DNA was extracted from their leaves by the CTAB (hexadecyltrimethlammonium bromide) method (Murray and Thompson 1980) and subjected to Southern hybridization analysis (Sambrook et al. 1989; Inagaki et al. 1994; Hisatomi et al. 1997). The 1.3-kb snapdragon '*Candi*' (*ANS*) cDNA (Martin et al. 1991) was used as a probe.

General methods for genomic cloning were according to Sambrook et al. (1989) and were modified as described earlier (Inagaki et al. 1994; Hisatomi et al. 1997). Using the '*Candi*' (*ANS*) cDNA as a probe, we cloned the genomic 12-kb *Bgl*II fragments containing the *ANS* gene from the variegated lines KK/VR-37 and KK/VR-40 into the *Bam*HI site of λGEM11 (Promega) to yield λV-Ans-1c and λV-Ans-3c, respectively. Since no apparent difference was detected in the cloned fragments from KK/VR-37 and KK/VR-40 by restriction analysis, we further characterized only the fragment on λV-Ans-1c from KK/VR-37. The 10-kb *Bam*HI fragment carrying the *ANS* gene from the wild-type line KK/FP-36 was also cloned into λGEM11. Actually, two independent λ clones carrying the identical segment were obtained, and one of them, λF-Ans-5c, was used for further studies. Using the probes A and B derived from the cloned segment on λF-Ans-5c (see Fig. 2A), we cloned two different *Eco*RI fragments, each of about 10 kb, separately into λDASH II (Stratagene). Five independent λ clones carrying an identical fragment were obtained using probe A (1.5-kb *Sac*I-*Pst*I), and these were denoted λV-Ans-6a. Similarly, three independent λ clones containing the same fragment were obtained using probe B (1.5-kb *Nhe*I-*Bgl*II), and these were denoted λV-Ans3′-7b. These cloned segments were subcloned into the pBluescript II SK$^-$ (Stratagene) for further molecular analysis.

To clone the internal sequence of the LTR retrotransposon, *RTip1*, we empolyed the procedure for the long and accurate polymerase chain reaction (LA-PCR) amplification (Barnes 1994; Chen et al. 1994) using the LA-PCR kit (TaKaRa-Syuzo). The locations of the primers for the LA-PCR amplification are shown in Fig. 2. The primers used are: Pr(5′)-1, 5′-GCGGCCGCAAATTAGCTAGCTTATTCTTGATTCAATTGCATCC-3′; Pr(5′)-2, 5′-TGTATTGAAAGGATGTCCAAATGTGGCCGTTTGTC-3′; Pr(3′)-3, 5′-CGCGGTATCTTTTCACCGCCTGCTGGTACTCAACC-3′; Pr(3′)-4, 5′-GCGGCCGCCAGATTGAGGAGTCAGAAATCAGGCATCATTGTCC-3′. Either primers Pr(5′)-1 and Pr(3′)-3 or Pr(5′)-2 and Pr(3′)-4 were used in the LA-PCR amplification, and the cycles were as follows: initial denaturation at 94°C for 1 min, 14 cycles consisting of denaturation at 98°C for 20 s and annealing and extension at 68°C for 20 min, and then 16 cycles consisting of denaturation at 98°C for 20 s and annealing and extension at 68°C for 20 min using the autoextension feature to add 15 s per cycle.

The DNA segments used for the probes D and E (see Figs. 2 and 5) were prepared by PCR amplification: 30 cycles of denaturation at 94°C for 1 min, annealing at 68°C for 1 min and extension at 72°C for 3 min. The primers used for the probe D are 5′-GTGCATGGGTTACAGCCAATCGTCCC-3′ and 5′-CTTCTAAGGCTTTGAACCTCACTTTTTCC-3′, and those for the probe E, 5′-CTAAAAGACCCTAGGCTACTGGGGAGGGG-3′ and 5′-GACCCGCCGGAGGGTTTTGTGGGTATCCAC-3′. Southern hybridization was performed under the standard hybridization conditions: 6 × SSC, 0.5% SDS, 50% formamide at 42°C for about 12 h (Sambrook et al. 1989). After hybridization, the nylon membranes were successively washed with 2 × SSC and 0.5% SDS at 20°C for 5 min, 2 × SSC and 0.1% SDS at 20°C for 15 min, 0.1 × SSC and 0.5% SDS at 37°C for 45 min, and 0.1 × SSC and 0.5% SDS at 68°C for 45 min.

For probe F, the 62-bp oligonucleotides 5′-AATTCGTGTGTATATGTCTTGTGTTATCTACATAAATCATATTTGAGAATAAGTAAATATAG-3′ and 5′-AATTCTATATTTACTTATTCTCAAATATGATTTATGTAGATAACACAAGACATATACACACG-3′ were synthesized and phosphorylated at their 5′ ends by T4 polynucleotide kinase (TaKaRa-Syuzo). After annealing, the synthesized double-stranded oligonucleotides were ligated and cloned

into the *Eco*RI site of the plasmid vector Bluescript II SK$^-$ (Stratagene). One of the recombinant plasmids carrying four copies of the tandemly repeated oligonucleotides was chosen, and the DNA fragment for probe F was cleaved out of the vector by restriction enzymes *Bam*HI and *Xho*I. Southern hybridization was performed under hybridization conditions of low stringency: 6 × SSC, 0.5% SDS, 40% formamide at 42°C for about 12 h. After hybridization, the nylon membranes were successively washed with 3 × SSC and 0.5% SDS at 20°C for 5 min, 3 × SSC and 0.1% SDS at 20°C for 15 min, 3 × SSC and 0.5% SDS at 37°C for 45 min and 3 × SSC and 0.5% SDS at 68°C for 45 min.

DNA sequencing was carried out by the chain termination method (Sambrook et al. 1989). The nuleotide sequence data reported here will appear in the EMBL, GenBank and DDJB Nucleotide Sequence Database under the accession number AB000394-AB000397.

Results and discussion

Cloning of the *ANS* gene region

The a^{flaked} variegated line of the common morning glory, KK/VR-37, bears white flowers with normal-colored flakes and sectors, and it carries multiple DNA rearrangements including insertions of mobile element-like sequences, *MELSIP1* and *MELSIP2*, in the region carrying the gene encoding dihydroflavonol 4-reductase (DFR) (Hisatomi et al. 1997). An interspecific complementation test was carried out between the a^{flaked} line of the common morning glory and the mutable $a\text{-}3^{flecked}$ line of the Japanese morning glory, which carries the transposable element *Tpn1* inserted within the *DFR* gene. The results indicated that the *DFR* gene in the a^{flaked} mutable line is active and complements the *DFR* gene in the mutable $a\text{-}3^{flecked}$ line since the interspecifec F_1 hybrids bear lightly colored flowers (Hisatomi et al. 1997). We also found RFLPs at the region containing the *ANS* gene for flower pigmentation in the mutable a^{flaked} lines of the common morning glory, KK/VR-37 and KK/VR-40 (Fig. 1). The patterns of the Southern hybridization suggested that both mutable lines carry at least a 7-kb insertion.

To characterize the insertion further, we compared the 12-kb *Bgl*II fragment from the mutable line KK/VR-37 on λV-Ans-1c with the 10-kb *Bam*HI fragment from the wild-type KK/FP-36 on λF-Ans-5c. As Fig. 2B shows, λV-Ans-1c contains only the part of the insertion sequence proximal to the *ANS* gene. Since the insertion was able to be localized near the *Pst*I site on λF-Ans-5c, we screened a single restriction fragment hybridizable with probes A and B and found an *Eco*RI band of about 10 kb that appeared to be hybridized with both probes (data not shown). Cloning of *Eco*RI fragments hybridizable with these probes revealed that the 10-kb *Eco*RI band consists of two different fragments of the same size and that one of them, on λV-Ans-6a, contains the probe the A sequence, and the other, on λV-Ans3′-7b, is hybridizable with probe B.

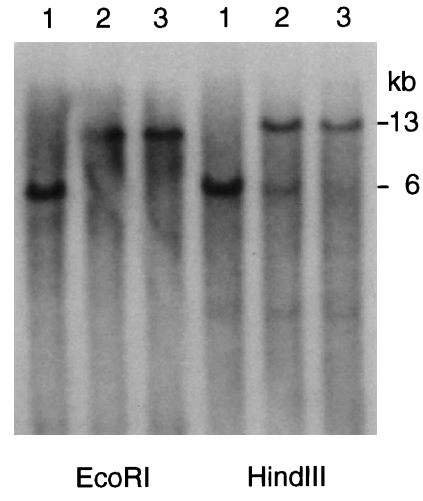

Fig. 1 Southern hybridization analysis of the *ANS* region of the common morning glory. Genomic DNAs were cleaved with the restriction enzymes indicated and subjected to Southern hybridization analysis using the snapdragon '*Candi*' (*ANS*) cDNA (Martin et al. 1991): *slot 1* KK/FP-36, *slot 2* KK/VR-37, *slot 3* KK/VR-40. Estimated lengths of hybridizing fragments are indicated in kilobases

Since we were unable to clone the entire insertion in an appropriate λ vector, we next attempted to obtain DNA segments containing the junction sequences between the cloned fragments on λV-Ans-6a and λV-Ans3′-7b by employing LA-PCR amplification. Since probes A and B were found to be unique sequences in the genome of the morning glory, two pairs of PCR primers were designed; one pair consisted of Pr(5′)-1 at the probe A and Pr(3′)-3 at the insertion on λV-Ans3′-7b and another of Pr(5′)-2 at the insertion on λV-Ans-6a and Pr(3′)-4 at probe B (Fig. 2B). The product of the LA-PCR amplification using primers Pr(5′)-1 and Pr(3′)-3 was 6.5 kb long, whereas that using primers Pr(5′)-2 and Pr(3′)-4 was 7 kb in size. Sequencing of these amplified fragments showed that the two cloned fragments on λV-Ans-6a and λV-Ans3′-7b are juxtaposed to each other on the genome of the mutable lines (Fig. 2B). Compared with the wild-type line KK/FP-36, the mutable line KK/VR-37 (and probably also KK/VR-40) carries about a 12.6-kb insertion at the *ANS* gene region.

Characterization of the insertion at the *ANS* gene region in the variegated line

To characterize the insertion near the *ANS* gene region in the line KK/VR-37, we sequenced about 13.5 kb of a segment that included the entire insertion sequence in KK/VR-37 and compared it with the corresponding sequence of about 1.5 kb in the wild-type line KK/FP-36. As Fig. 3 shows, the insertion resides about 0.5 kb away from the *ANS* structural gene in KK/VR-37 and

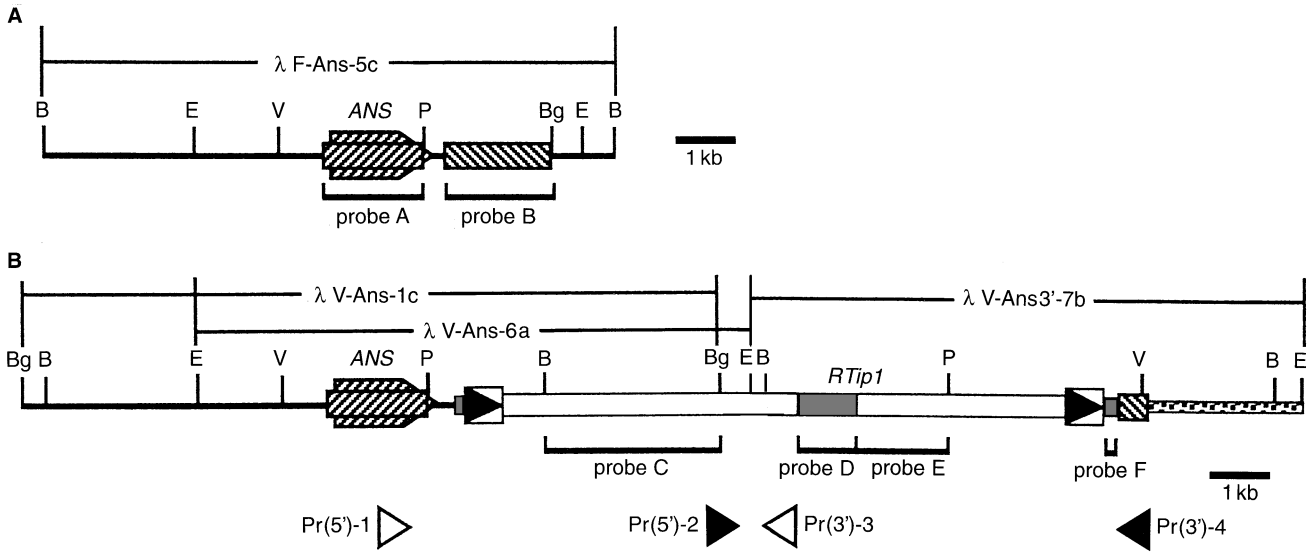

Fig. 2A, B Physical maps of the *ANS* gene region. **A** The wild-type line KK/FP-36, **B** the mutable line KK/VR-37. The fragments cloned on the λ vectors used are indicated *above* the maps. The segments used for probes A and B from KK/FP-36 and their corresponding sequences on KK/VR-37 are indicated by the *hatched boxes*. The *filled arrowheads in the boxes* at both ends of *RTip1* indicate the LTR sequences, and the *small shadowed rectangles flanking RTip1* indicate *MiniSip1*. The location of *MiniSip2* is indicated by the *shadowed rectangle within the internal RTip1 box*. The *dotted box at the right end* of the map in KK/VR-37 represents the nonhomologous sequence appearing to the *right* of the probe B sequence. Preparation of the DNA segments used for the probes C, D, E and F to detect an *RTip1* internal sequence, *MiniSip2,* another *RTip1* internal sequence and *MiniSip1*, respectively, in the morning glory genomes (see **Fig. 5**) is described in Materials and methods. The *horizontal arrowheads below* the maps indicate the sites of primers for PCR amplification. Restriction sites are: B *Bam*HI, Bg *Bgl*II, E *Eco*RI, P *Pst*I, V *Eco*RV

consists of at least two repetitive elements, a minisatellite-like element, *MiniSip1*, of 269 bp and a 12,422-bp-long LTR retrotransposon, *RTip1*. Since *RTip1* is flanked by 5-bp target duplications within the *MiniSip1* sequence, it is clear that *RTip1* must have been integrated into *MiniSip1*. Moreover, *RTip1* contains a 962-bp-long element, *MiniSip2*, in the middle of the *RTip1* internal sequence flanked by LTRs, and *MiniSip2* carries tandemly repeated motifs longer than the motifs found in the usual minisatellites (see below).

Although the mode of integration of *MiniSip1* is not known, we noticed that a copy of the 19-bp motif-B sequence is present at the insertion site in the wild-type line KK/FP-36 and that minor sequence alterations occur in the flanking region to the left of the insertion in KK/VR-37 (Fig. 4A). To the right of the insertion site in KK/VR-37, only a segment of about 0.5 kb was found to be homologous to the corresponding sequence in KK/FP-36 (Fig. 2). Since a completely new sequence appeared at the right of the 500-bp homologous segment (data not shown), it is likely that another DNA rearrangement occured either in KK/FP-36 or KK/VR-37. It should be mentioned here that the 12.7-kb insertion in the close vicinity of the *ANS* gene is unlikely to have anything to do with the mutable a^{flaked} allele because germinal revertants containing the reverted *A* alleles homozygously still carry these insertions at the *ANS* region and no apparent additional DNA rearrangement appears to have occurred in the *ANS* region during the reversion (data not shown).

Sequence characteristics of the LTR retrotransposon *RTip1*

The LTR retrotransposon *RTip1* is 334 bp longer than the barley LTR retrotransposon *BARE-1*, one of the

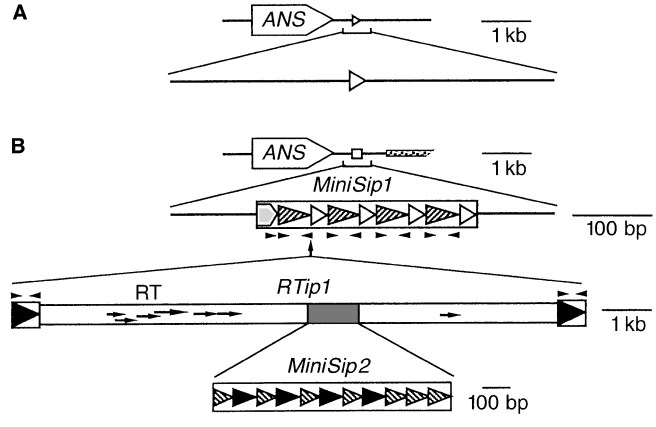

Fig. 3A,B Elements causing the DNA rearrangements at the *ANS* gene region. **A** The wild-type line KK/FP-36 contains one copy of the motif B sequence, represented by the *open horizontal arrowhead*, near the 3′ end of the *ANS* gene. **B** The line KK/VR-37 carries *MiniSip1*, indicated by the *rectangle*, near the 3′ end of the *ANS* gene. The *arrowheads within* the *MiniSip1* and *MiniSip2 boxes* indicate the motif sequences shown in **Fig. 4**. *RTip1* is drawn as in **Fig. 2**, and the *small horizontal arrows within the internal RTip1 box* indicate the open reading frames (ORFs) longer than 400 bp. The *tiny filled arrowheads below MiniSip1* and *above* LTRs of *RTip1* indicate small inverted repeats shown in **Fig. 4**

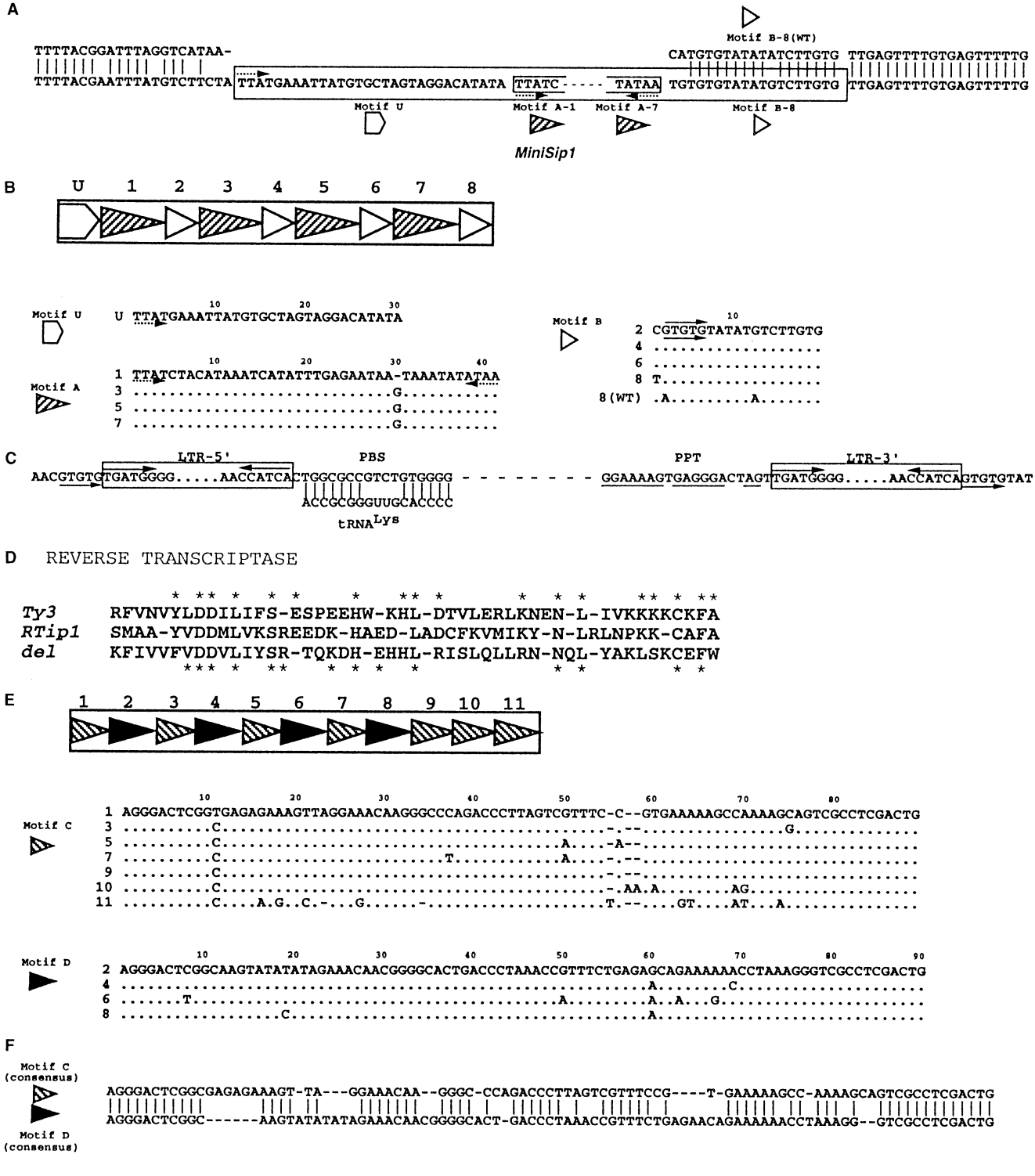

Fig. 4A–F Relevant sequences found in the regions carrying the DNA rearrangements. **A** Comparison of the junction sequences at the *MiniSip1* insertion site between KK/FP-36 and KK/VR-37 **B** motif sequences in *MiniSip1*, **C** relevant sequences in the retrotransposon *RTip1,* **D** comparison of amino acid sequence of the RT domains in retrotransposon, **E** motif sequences in *MiniSip2*, **F** comparison of the consensus sequences of the two motifs C and D in *MiniSip2*. The symbols for the motif sequences are drawn in the same way as **Fig. 3**. Deleted nucleotides or amino acids are represented by hyphens (−), and nucleotide sequences identical to the top lines in the motif sequences A, B, C and D are marked with *periods* (·). The *vertical short bars* between two sequences in **A** and **F** indicate identical nucleotides, whereas those in **C** signify complementary sequences at the primer binding site (PBS). The purine nucleotides at the polypurine tract (PPT) are *underlined*. The ends of the motif A sequence within the *MiniSip1 box* in **A** are marked by the *square brackets*. The small terminal inverted repeats of the motif A sequences and LTRs of *RTip1* are indicated by the *horizontal arrows below* and *above* the sequences, respectively. The 5-bp target duplication sequence GTGTG generated upon *RTip1* integration is indicated by the two *horizontal arrows* sandwiching the sequence in **B** and **C**. The *asterisks above* and *below* the sequences in **D** indicate identical amino acids between *Ty3* and *RTip1* and between *RTip1* and *del*, respectively

longest LTR-retrotransposons of plants reported previously (Manninen and Schulman 1993). *RTip1* is flanked by a 588-bp LTR at the 5′ terminus and a 591-bp LTR at the 3′ end, and both LTR sequences in themselves are flanked by the 6-bp terminal inverted repeats, 5′-TGATGG-3′. The sequence divergence of these two LTRs is 3.5%, suggesting that the integration of *RTip1* into *MiniSip1* is highly unlikely to be a recent event (Smyth 1993).

RTip1 carries a characteristic primer binding site (PBS) for a primer tRNA to synthesize the first(−)-strand cDNA next to the 5′ LTR (Fig. 4C). Although the initiator $tRNA^{Met}$ is usually used as the primer in the plant LTR retrotransposons, $tRNA^{Lys}$ seems to be utilized in *RTip1* because its PBS is much more homologous to the sequence at the 3′ end of $tRNA^{Lys}$ than to that of $tRNA^{Met}$ (Sprinzl et al. 1987). The only other known LTR retrotransposon in plants carrying PBS for $tRNA^{Lys}$ is the maize LTR retrotransposon, *Zeon-1* (Hu et al. 1995). *RTip1* also carries a typical polypurine tract (PPT) immediately upstream of the 3′-LTR for the second(+)-strand cDNA synthesis.

Although the internal part between LTRs of *RTip1* is 11 kb long, the longest ORF is 747 bp long and part of the RT domain was found in the 486-bp ORF (Fig. 3). On the basis of the homology in the RT domains and another region shown in Fig. 4D, *RTip1* appears to be one of the *Ty3/gypsy*-like elements. No ORF corresponding to the *gag* and *pol* genes was found, indicating that *RTip1* is an inactive element. The occurrence of sequence divergence in its LTRs supports this view. Moreover, *RTip1* and its relatives are present in multiple copies in the genomes of the morning glory (Fig. 5), and the copy numbers of the LTR sequences are higher than those of the sequences containing the RT domain region (data not shown).

Sequence characteristics of the minisatellite-like elements *MiniSip1* and *MiniSip2*

Two minisatellite-like elements, *MiniSip1* and *MiniSip2*, were found in the 12.6-kb insertion near the *ANS* gene in KK/VR-37. *MiniSip1* is a 269-bp-long AT-rich element, and it comprises two alternating motifs of 41 bp and 19 bp (Fig. 4B). Interestingly, these motif sequences in each repeat unit are identical except for one repeat unit, and the 41-bp motif is flanked by 4-bp terminal inverted repeats. Thus, one may call this element a "composite minisatellite" because it is composed of two different motifs.

DNA gel blot analysis using the synthesized probe for *MiniSip1* indicated that the genome of KK/VR-37 contains a few copies of *MiniSip1*, most likely only one copy, at the *ANS* gene (Fig. 5D). One strong band together with a few weaker bands also appeared in the wild-type line KK/FP-36. It should be noted that some ambiguity exists concerning the copy number of the element because the highly AT-rich probe gave faint bands under low-stringent hybridization conditions. Nonetheless, *MiniSip1* may be an exceptional element in this respect because usually minisatellites occur at many loci in the genome.

A second element, *MiniSip2*, found in the middle of *RTip1*, is also not a typical minisatellite. The 962-bp-long element, *MiniSip2*, also comprises two alternating motifs of 86-bp and 90-bp together with two additional copies of the 86-bp motif as tandem repeats, and these motif sequences in each repeat unit are almost identical

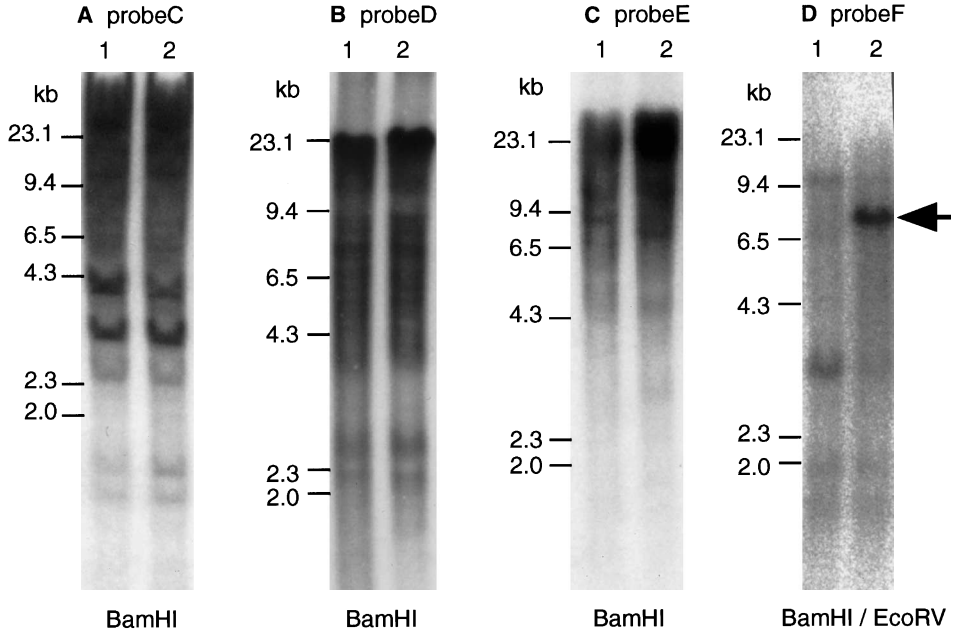

Fig. 5A–D Presence of the *MiniSip1* and *RTip1* sequences in the genomes of the common morning glory. **A** *RTip1* internal sequence containing the region encoding RT, **B** *MiniSip2*, **C** *RTip1* internal sequence adjacent to *MiniSip2*, **D** *MiniSip1*. Genomic DNAs were cleaved with the restriction enzymes indicated and subjected to Southern hybridization analysis using the probes indicated in **Fig. 2**: *slot 1* KK/FP-36, *slot 2* KK/VR-37. The *arrow* in **D** points to the band corresponding to the fragment near the *ANS* gene in **Fig. 2**. Estimated lengths of hybridizing fragments are indicated in kilobases

(Fig. 4E). Although *MiniSip2* also appears to be another "composite minisatellite", a close examination of these longer motif sequences revealed that their consensus sequences are homologous to each other (Fig. 4F). Since the repeating motifs in minisatellites and satellites are around 10–60 bp and about 100–300 bp, respectively (Weising et al 1995), the lengths of the motifs in *MiniSip2* fit satellites better than minisatellites. However, *MiniSip2* is too short to be a satellite, and its relatives are present in multiple copies in the genome of the common morning glory (Fig. 5B). The copy numbers of *MiniSip2* appear to be much higher than those of the *RTip1* internal sequence adjacent to *MiniSip2* (Fig. 5), suggesting that *MiniSip2* sequences not associated with *RTip1* must also be present in the morning glory genomes. Therefore, *MiniSip2* can be regarded as a new type of minisatellite. In both *MiniSip1* and *MiniSip2*, the fact that little variation was observed in the motif sequences of each repeat unit may also be structural characteristics of these elements.

Although it is not known whether minisatellites are able to transpose in the genome, they have been implicated to be hot spots for homologous recombination (Jarman and Wells 1989; Wahls et al. 1990; Weising et al. 1995). Since both *MiniSip1* and *RTip1* are present near the *ANS* gene in KK/VR-37 and are absent in KK/FP-36, integration of *MiniSip1* into the *ANS* region must have taken place in a progenitor of KK/VR-37. Alternatively, it is equally plausible that excision of the element from the *ANS* gene occurred in an ancestral line of KK/FP-36. Although a copy of the 19-bp motif-B sequence is present at the insertion site in the wild type line KK/FP-36, a simple homologous recombination event can not account for the generation of the rearranged structure.

The LTR retrotransposon *RTip1* must have been integrated later into *MiniSip1* because a 5-bp target duplication is present within the *MiniSip1* sequence in KK/VR-37. Incorporation of another minisatellite, *MiniSip2*, into *RTip1* probably occurred after the integration of *RTip1* into *MiniSip1*, although we could not rule out the possibility that *MiniSip2* incorporation might have taken place prior to *RTip1* integration into *MiniSip1*. A transposon-like element, WIS*1*, from wheat is reported to contain a typical minisatellite array (Martienssen and Baulcombe 1989). It should also be pointed out that the structural features of tandemly repeated sequences found in the subterminal repeated regions of some transposable elements belonging to the *En*/*Spm* family bear a curious resemblance to those of the minisatellites described here (Hoshino et al. 1995; Saedler and Gierl 1996). For example, the two different tandem repeats of 104 bp and 122 bp were found in the subterminal repeated regions of the transposable element *Tpn1*, and they are present in multiple copies in the genome of the Japanese morning glory (Inagaki et al. 1994; Hoshino et al. 1995). *MiniSip2* must have been able to duplicate and transpose as a part of *RTip1*, since the retrotransposons transpose via RNA intermediates. Nonetheless, it is interesting to see whether the new minisatellites described here can serve as molecular markers in diagnostic and mapping studies.

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