Theor Appl Genet (2002) 105:167–172
DOI 10.1007/s00122-002-0950-0

Fukashi Shibata · Masahiro Hizume

Evolution of 5S rDNA units and their chromosomal localization in *Allium cepa* and *Allium schoenoprasum* revealed by microdissection and FISH

Received: 14 August 2001 / Accepted: 10 December 2001 / Published online: 21 June 2002

Abstract *Allium cepa* and *Allium schoenoprasum* each possess 5S rDNA units of two different sizes. The evolution of the two repeat units and their chromosomal localization were investigated. *A. cepa* has 5S rDNA loci in the proximal and distal regions of the short arm of chromosome 7. When the proximal and distal segments of the short arm of chromosome 7 were microdissected separately, and used as templates for PCR, the short and long 5S rDNA fragments were amplified predominantly from the proximal and distal segments, respectively. The nucleotide sequence of the long 5S rDNA unit resulted from partial duplication of a non-transcribed spacer (NTS) and the insertion of a unique sequence. FISH using a probe consisting of the unique sequence demonstrated that the long unit was distally localized. In *A. cepa*, the long 5S rDNA unit is only present distally and the short unit is predominantly located proximally on the short arm of chromosome 7. In *A. schoenoprasum*, the NTSs of the two different-sized 5S rDNAs had quite different sequences. The two 5S rDNA loci were localized very close together in the interstitial region of chromosome 6. FISH, using long and short 5S rDNA unit probes with a competitor of a 120-bp sequence of the 5S rRNA gene, indicated that the long 5S rDNA unit was localized proximally and the short unit distally. Although the NTSs of the 5S rDNA of *A. cepa* and *A. schoenoprasum* had quite different nucleotide sequences, the long 5S rDNA units of *A. cepa* and *A. schoenoprasum* share a common 75-bp sequence. This sequence might act in the formation of the long 5S rDNA unit in *Allium* species.

Communicated by J.S. Heslop-Harrison

F. Shibata (✉)
Biological Institute, Faculty of Science, Ehime University, Matsuyama 790-8577, Japan
e-mail: shibatafukashi@hotmail.com
Fax: +81-89-933-2029

M. Hizume
Biological Institute, Faculty of Education, Ehime University, Matsuyama 790-8577, Japan

Keywords *Allium cepa* · *Allium schoenoprasum* · Chromosome · Fluorescence in situ hybridization · Microdissection · 5S rDNA

Introduction

The 5S rDNA unit is composed of a highly conserved 120-bp sequence encoding 5S rRNA and a non-transcribed spacer (NTS), and is organized as tandem repeats at specific locations in the genome. The NTS shows length and nucleotide-sequence variation among species: most species contain a single kind of 5S rDNA unit, while two or more kinds of 5S rDNA units of different size are present in some species (Ellis et al. 1988; Gottlob-McHugh et al. 1990; Singh et al. 1994; Singh and Singh 2001). Naturally, in a species possessing one kind of 5S rDNA unit, the 5S rDNA loci encode a single kind of 5S rDNA unit. In species with multiple kinds of 5S rDNA units of different sizes, the loci do not always encode the same kind of 5S rDNA unit. *Pisum sativum* possesses two kinds of 5S rDNA units of different size that are repeated together (Ellis et al. 1988); little is known of the relationship between multiple-sized 5S rDNA units and the 5S rDNA loci. Studying the evolution of multiple-sized 5S rDNA units and their chromosomal localization provides an important clue to help understanding the evolution of genes and tandemly arranged repetitive sequences.

Allium cepa and *Allium schoenoprasum* are classified in the same subgenus *Rhizirideum* (Hanelt 1990) and each have two 5S rDNA units of different size (Shigyo et al. 1996). In the subgenus *Rhizirideum*, other closely related species only have one 5S rDNA unit (Do and Seo 2000). In *A. cepa*, the 5S rDNA gene has been mapped to two loci on the short arm of chromosome 7 (Hizume 1994; Lee and Seo 1997; Lee et al. 1999). In *A. schoenoprasum*, mapping of the 5S rDNA has not been reported. This study analyzed the relationship between the two different-sized 5S rDNAs and their chromosomal loci by PCR of microdissected chromosome segments

carrying 5S rDNA loci, fluorescence in situ hybridization (FISH) and by sequencing.

Materials and methods

Plants

A. cepa L., *A. schoenoprasum* L., *A. schoenoprasum* var. *foliosum* Regel., *Allium fistulosum* L., *Allium thunbergii* G. Don, *Allium chinense* G. Don, *Allium virgunculae* F. Maekawa et Kiramura, *Allium grayi* Regel, *Allium sativum* L. and *Allium tuberosum* Rottl. were collected and planted at Ehime University. For a PCR template, genomic DNA was extracted from leaves by the CTAB method (Murray and Thompson 1980).

Chromosome preparation

Root tips were collected and treated with 2 mM 8-hydroxyquinoline at 20 °C for 3 h. After fixation in acetic alcohol (acetic acid: ethanol = 1:3), the root tips were treated with 2% Cellulase RS (Yakult) and 0.5% Pectolyase Y-23 (Seishin Pharma.) in 2 × SSC (pH 4.5) containing 10 mM of EDTA at 37 °C for 30 min and squashed in 45% acetic acid. The coverslips were removed by the dry ice method and the slides were air-dried.

Microdissection

Each air-dried chromosome specimen was immediately used for microdissection. In the chromosome complements prepared by enzymatic maceration, chromosome 7 was unambiguously identified by its size and shape. The distal and proximal chromosome segments were dissected from the short arm of chromosome 7 using an inverted microscope equipped with a micromanipulator (ON-T1, Olympus) and transferred by a glass needle into 10 μl of distilled water. Each segment from a single chromosome was collected separately into a microtube and six duplicate samples were used for PCR.

PCR

The 5S rDNA was amplified by PCR using a universal primer set specific for 5S rDNA. The sequences of primers 5SL1 and 5SL2 were 5′-CGGTGCATTAATGCTGGTAT-3′ and 5′-CCATCAGAACTCCGCAGTTA-3′, respectively (Hizume 1993). PCR was performed using conventional thermal conditions. Each microdissected chromosome segment was used as a PCR template directly, without further treatment. When no product of the first PCR was visible on an agarose gel, 0.5 μl of the reaction mixture was used as a template for a second round of PCR under the same conditions.

DNA sequencing

The PCR-amplified 5S rDNA was cloned using a TOPO TA cloning kit (Invitrogen), and the clones were sequenced using M13 forward and reverse primers by the dye terminator method employed with an automated sequencer (310 and 373 DNA Sequencing System, Applied Biosystems). For *A. cepa*, four clones (pAc5S-15, -24, -33 and -45) contained the long 5S rDNA (accession numbers: AB056586, AB056587, AB056588 and AB056594), and seven clones (pAc5S-09, -10, -16, -18, -20, -27 and -29) contained the short 5S rDNA (accession numbers: AB056584, AB056585, AB056589, AB056590, AB056591, AB056592 and AB056593). For *A. schoenoprasum* var. *foliosum*, three clones (pAs5Sa2, a3, a5) contained the short 5S rDNA unit (accession numbers: AB066471, AB066472 and AB066473), and three clones (pAs5Sa7, a34, a35) contained the long 5S rDNA unit (accession numbers: AB066474, AB066475 and AB066476). For *A. schoenoprasum*, three clones (pAs5Sc2, c6, c7) contained the short 5S rDNA unit (accession numbers: AB066477, AB066478 and AB066479), three clones (pAs5Sc1, c3, c4) contained the long 5S rDNA unit (accession numbers: AB066480, AB066481 and AB066482), and clone pAs5Sc900-8 (accession number: AB066483) contained a 900-bp 5S rDNA fragment.

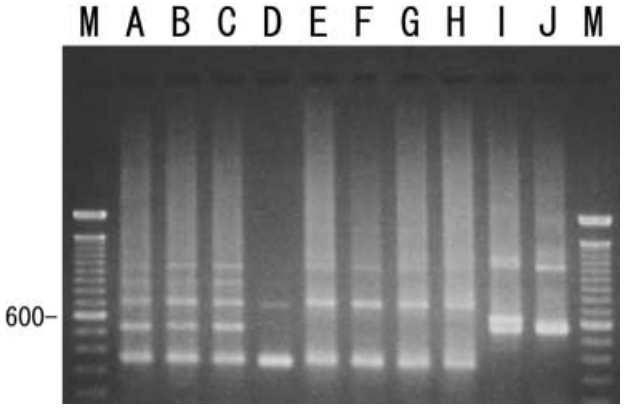

Fig. 1 Agarose-gel electrophoresis of PCR-amplified 5S rDNA from ten *Allium* taxa: *A. cepa* (**A**), *A. schoenoprasum* var. *foliosum* (**B**), *A. schoenoprasum* (**C**), *A. fistulosum* (**D**), *A. thunbergii* (**E**), *A. chinense* (**F**), *A. virgunculae* (**G**), *A. grayi* (**H**), *A. sativum* (**I**), and *A. tuberosum* (**J**). (*M*) contains a 100-bp ladder marker

FISH

The probes consisted of short and long 5S rDNA clones of *A. cepa* and *A. schoenoprasum*, and a sequence specific for the long 5S rDNA unit of *A. cepa* was amplified by PCR (primers EP6: 5′-TAGGACGATACGGTTC-3′, EP7: 5′-CTATTTGCTCACTCTA-3′). As a probe and blocking DNA, the coding region of 5S rDNA (120-bp) was amplified from genomic DNA of *Rumex acetosa* (primers 5SS1: 5′-GGGTGCGATCATACCAGC-3′, and 5SS2: 5′-GGGTGCAACACAAGGACTTCC-3′). Biotin and digoxigenin (DIG) labeling of probe DNA was accomplished by nick translation using the BioNick labeling system (BRL) and random prime amplification using the DIG-High Prime (Roche Diagnostics), respectively. The procedures used for FISH followed Shibata et al. (2000).

Results and discussion

Size variation of 5S rDNA units revealed by PCR in ten *Allium* taxa

In ten taxa of the genus *Allium*, 5S rDNA units were amplified by PCR using universal primers, as shown in Fig. 1. Of the ten taxa examined, *A. sativum* is classified in the subgenus *Allium*, and the other species are classified in the subgenus *Rhizirideum*. A 350-bp 5S rDNA repeat unit was amplified in *A. cepa*, *A. schoenoprasum* var. *foliosum*, *A. schoenoprasum*, *A. fistulosum*, *A. thunbergii*, *A. chinense*, *A. virgunculae* and *A. grayi*. In addition to the short unit, *A. cepa* and *A. schoenoprasum* possess a unit about 520-bp long. *A. sativum* has 570-bp and 620-bp repeat units and *A. tuberosum* has a 550-bp re-

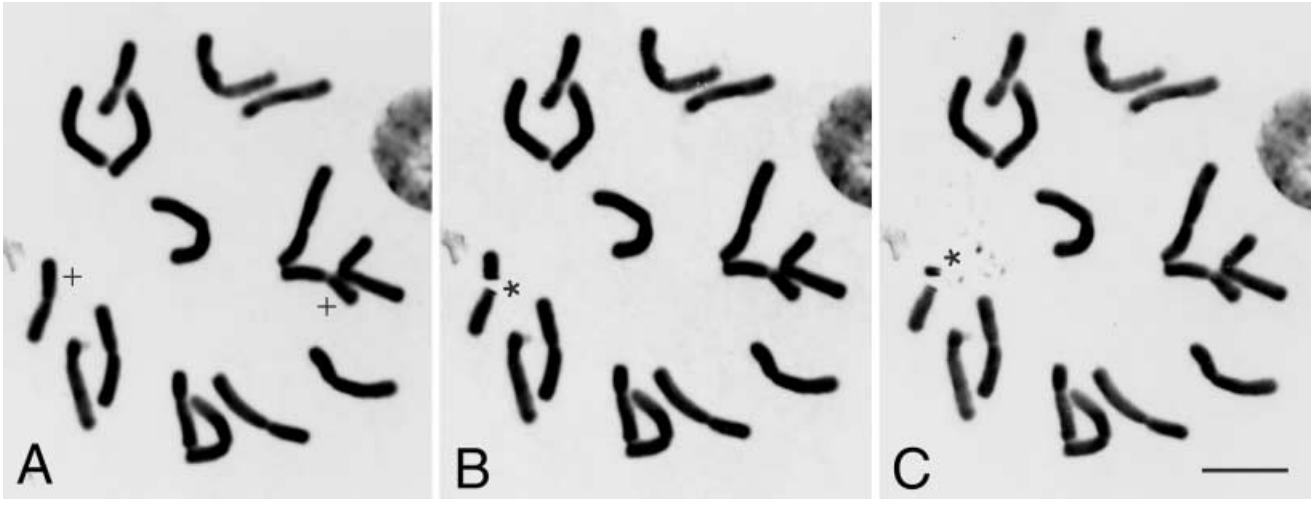

Fig. 2A–C Microdissection of segments of chromosome 7 of *A. cepa*. (**A**) Before dissection; (**B**) after dissection of the proximal segment of the short arm; (**C**) after dissection of the distal segment of the short arm; (+) short arm of chromosome 7; (*) area dissected with the micromanipulator. *Bar* = 10 μm

peat unit. We analyzed the chromosomal localization of each 5S rDNA unit and the evolution of the two different-sized repeat units in *A. cepa* and *A. schoenoprasum*.

Chromosomal localization of the two different-sized 5S rDNAs in *A. cepa*

The localization of 5S rRNA genes on chromosome 7 of *A. cepa* has been revealed by ISH (Hizume 1994) and by PCR investigation of aneuploid *A. fistulosum* monosomic addition lines carrying *A. cepa* chromosomes (Shigyo et al. 1996). The relationships between the two 5S rDNA units and their chromosome loci were analyzed by microdissection and subsequent PCR. Chromosome 7 is metacentric, and the shape and size of its short arm readily distinguish it from the other chromosomes (Fig. 2). Since the two 5S rRNA loci are separated by about 1.4 μm on the short arm of chromosome 7, the distal and proximal chromosome segments containing each locus can be isolated by microdissection with a glass needle using a manual micromanipulator (Fig. 2). PCR of the genomic DNA and of chromosome 7 resulted in a ladder pattern of two fragments of about 520- and 340-bp (lanes 1 and 2 in Fig. 3). The short fragment was amplified from the proximal segment of the short arm of chromosome 7 (lane 3 in Fig. 3). From the distal segment, the long 5S rDNA fragment was amplified predominantly along with a very small amount of the short fragment (lane 4 in Fig. 3). The PCR product from the chromosome segment was a monomer, not an oligomer, and it is conceivable that the template DNA was too little amount. In some experiments, the PCR products were only weakly detectable or were not detectable at all on agarose gels. This difficulty was overcome by using

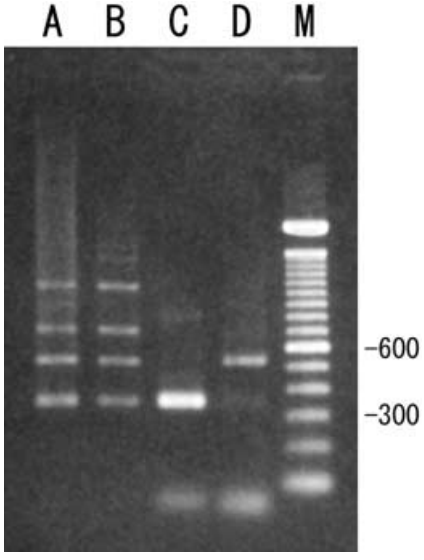

Fig. 3A–D Agarose-gel electrophoresis of PCR products from *A. cepa* templates. The templates consisted of (**A**) genomic DNA, (**B**) chromosome 7, and the (**C**) proximal and (**D**) distal segments of the short arm of chromosome 7. (*M*) contains a 100-bp ladder marker

an aliquot of the first PCR product mixture as a template for a second round of PCR, which successfully amplified the fragments. The results indicate that the short and long 5S rDNA units are primarily located at the proximal and distal loci, respectively. The distal locus seems to contain a few copies of the short repeat unit in addition to many copies of the long repeat unit. As shown in Fig. 1, the PCR product of 5S rDNA from *A. cepa* also included a 900-bp fragment. The fragment size suggests that this fragment contains a pair of short and long units. This supports the co-localization of the short and long repeat units at the distal 5S rDNA loci. We named the short and long 5S rDNA units as the Ac5SS and Ac5SL units, respectively.

The nucleotide sequences of four Ac5SL unit clones (515–519 bp) show 91–99% homology to each other. On

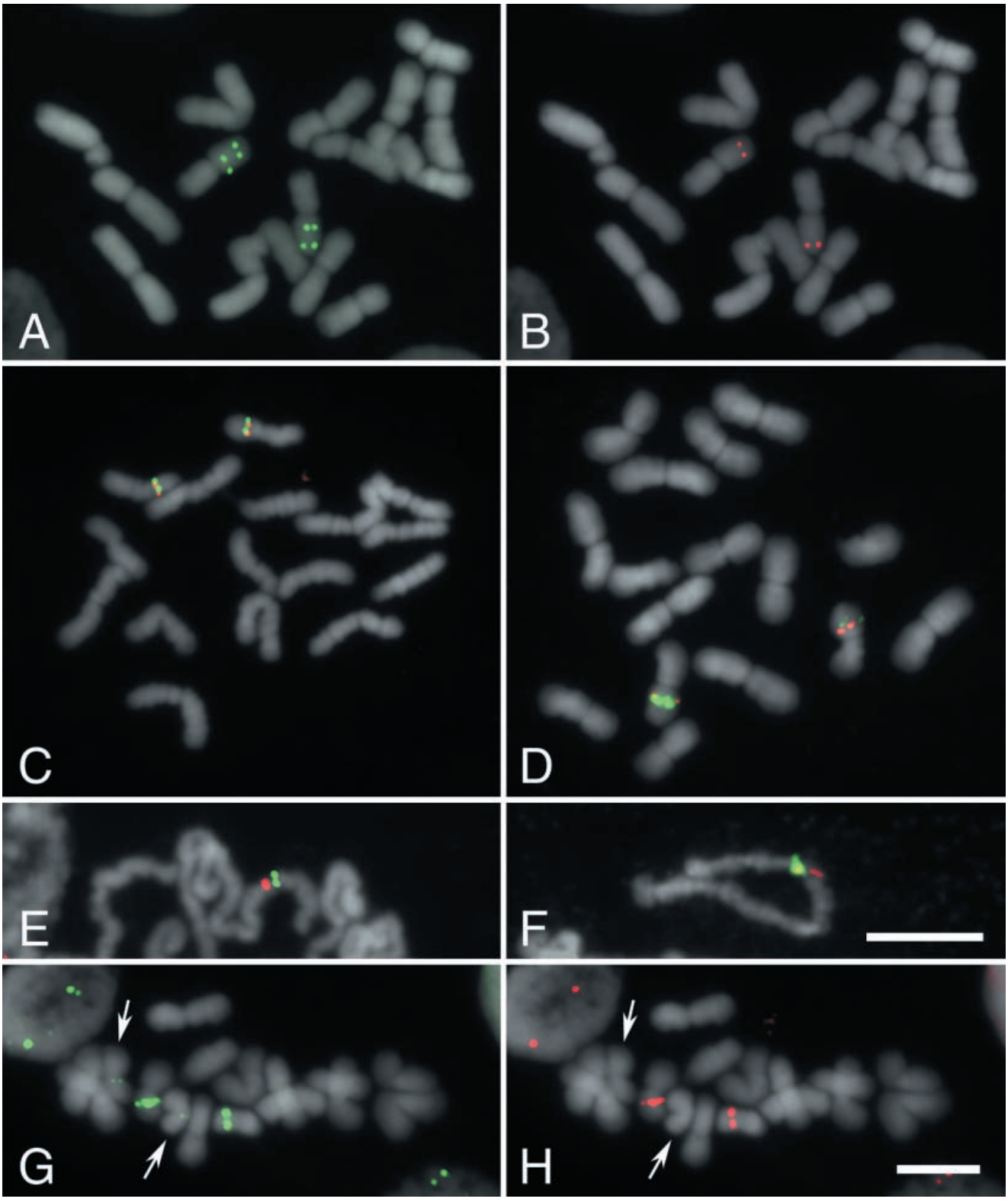

Fig. 4A–H FISH of 5S rDNA sequences to chromosomes of *A. cepa* and *A. schoenoprasum*. (**A, B**) FISH image of metaphase plate *A. cepa*, probed with the 5S rRNA gene sequence of *R. acetosa* (**A**) and the specific sequence of the Ac5SL unit (**B**). (**C**) FISH image of a metaphase plate of *A. schoenoprasum* probed with As5SL (*red*) and As5SS (*green*). (**D**) FISH image of a metaphase plate *A. schoenoprasum* var. *foliosum* probed with As5SL (*red*) and As5SS (*green*). (**E**) FISH image of prophase chromosomes of *A. schoenoprasum* probed with As5SL (*red*) and As5SS (*green*). (**F**) FISH image of prophase chromosome *A. schoenoprasum* var. *foliosum* probed with As5SL (*red*) and As5SS (*green*). In the FISH shown in **C**–**F**, the coding region of the 5S rDNA of *R. acetosa* was used as blocking DNA. FISH image of a metaphase plate of *A. schoenoprasum* probed with the coding region of 5S rDNA of *R. acetosa* (**G**) and As5SL plus As5SS (**H**). The *arrows* indicate chromosome 7. **A, B, C, D, G and H** same magnification. **E and F** same magnification. *Bar* = 10 μm

the other hand, the nucleotide sequences of seven Ac5SS unit clones (329–349-bp) have various NTSs and show 79–98% homology to each other. The Ac5SL and Ac5SS units show about 80% homology among fragments of the same length (nt 1–186 in clone pAc5S-16 to 1–185 in clone pAc5S-24, and nt 102–349 in clone pAc5S-16 and nt 274–516 in clone pAc5S-24, the shaded regions in Fig. 5A and B). The Ac5SL unit contains the entire Ac5SS unit sequence. The NTS of the Ac5SL unit contains duplicated sequences (nt 98–185 and 274–369, clone pAc5S-24, underlined in Fig. 5B). A sequence homologous to this region is present in the Ac5SS unit (nt 102–186, clone pAc5S-16, underlined in Fig. 5A). In the NTS of the Ac5SL unit, a unique 87-bp sequence is present between the duplicated regions (nt 187–273, clone pAc5S-24). This sequence does not appear in the Ac5SS unit. The nucleotide sequences suggest that the Ac5SL unit might be derived from the Ac5SS unit by duplication of the NTS and insertion of the unique sequence. The fact that Ac5SL nucleotide sequences show high homology among clones indicates that the Ac5SL unit was recently derived from a single Ac5SS unit. If the Ac5SL units had been derived from several Ac5SS units, then the Ac5SL units should show as much variation in the NTS nucleotide sequences as seen in the Ac5SS units.

Finally, the chromosomal localization of the 87-bp sequence unique to Ac5SL was determined by FISH using probes consisting of the 87-bp sequence and heterogeneous 5S rDNA (120-bp). The FISH signal of the unique Ac5SL sequence appeared distally on chromosome 7 (Fig. 4A, B), demonstrating that the Ac5SL unit is only localized distally. Based on microdissection followed by PCR, sequencing, and FISH using probes specific to the Ac5SL unit, we concluded that the long 5S rDNA was only distally localized and the short 5S rDNA was predominantly localized proximally.

Chromosomal localization of the two different-sized 5S rDNAs in *A. schoenoprasum*

In *A. schoenoprasum* and *A. schoenoprasum* var. *foliosum*, two closely located 5S rDNA loci were observed in the interstitial region of the short arm of chromosome 6 (Fig. 5C, D). *A. schoenoprasum* also had a weak signal near the centromeric region of chromosome 7 (Fig. 5G).

Repeat units of 5S rDNA were amplified by PCR from genomic DNA of *A. schoenoprasum* var. *foliosum* and *A. schoenoprasum*. The PCR products contained five different sized fragments: 350, 530, 700, 900 and 1,100 bp (lanes B and C in Fig. 1). The two 5S rDNA units are very similar to those of *A. cepa* (lane A in Fig. 1). These 5S rDNA units are about 350- and 530-bp long. The 700- and 1,100-bp fragments are dimers of the respective repeat unit. This double-ladder pattern suggests that the two 5S rDNA units are organized tandemly at separate loci in the genome. We named the 530-bp 5S rDNA unit the As5SL unit and the 350-bp 5S rDNA unit as the As5SS unit. Each unit was cloned and sequenced.

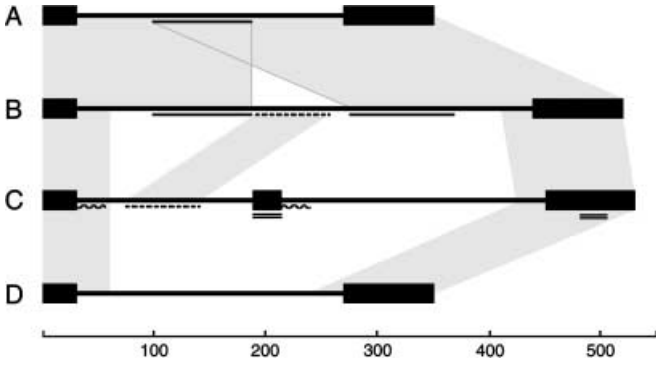

Fig. 5A–D Schematic illustration of the short and long 5S rDNA sequences of *A. cepa* and *A. schoenoprasum*. The *boxed region* indicates coding region. The *shaded and underlined areas with the same lines* have sequence homology. (**A**) Ac5SS unit; (**B**) Ac5SL unit; (**C**) As5SL unit; (**D**) As5SS unit

No major differences were observed in the nucleotide sequences from the two taxa. As5SS unit clones show 85–99% homology with each other and the As5SL unit clones show 95–100% homology with each other. In the As5SL unit a 26-bp sequence (nt 187–212 in clone pAs5Sa7) at the center of the NTS is common to the coding region (nt 480–505 of clone pAs5Sa7, double underlined in Fig. 5C) and a 27-bp sequence (nt 214–240 in clone pAs5Sa7) is common to the NTS sequence located next to the coding region (nt 29–55 in clone pAs5Sa7, wavy underline in Fig. 5C). Unlike *A. cepa*, the As5SL and As5SS units have quite different NTSs except for about 30-bp preserved NTS sequences located next to the coding region (Fig. 5).

Clones containing the As5SL and As5SS units were used as respective FISH probes, and hybridization to the 5S rRNA coding region was blocked by the unlabeled 5S rRNA gene sequence of *R. acetosa* (Fig. 4C–F). The signals of the two 5S rDNA units were closely mapped on chromosome 6 in the metaphase plate (Fig. 4C and D) and were clearly separated in the prophase chromosome (Fig. 4E and F). The As5SS unit was located distally and the As5SL unit proximally. However, the 900-bp sequence (clone pAs5Sc900-8) shows 85–98% homology to the As5SS unit clones at nt 1–341 and 96–98% homology to the As5SL unit clones at nt 346–872. This 900-bp fragment indicates that some As5SL and As5SS units were co-localized in one region.

In *A. schoenoprasum*, the As5SL and As5SS probes did not detect a 5S rDNA locus near the centromere of chromosome 7 (Fig. 4G and H). This indicates that the 5S rDNA located on chromosome 7 has no homology with the NTS of the As5SL or As5SS units. Hence, *A. schoenoprasum* possesses at least three kinds of 5S rDNA with different NTS sequences.

Comparison of the 5S rDNA sequences of *A. cepa* and *A. schoenoprasum*

The nucleotide sequences of the NTS of 5S rDNA units of *A. cepa* and *A. schoenoprasum* are quite different,

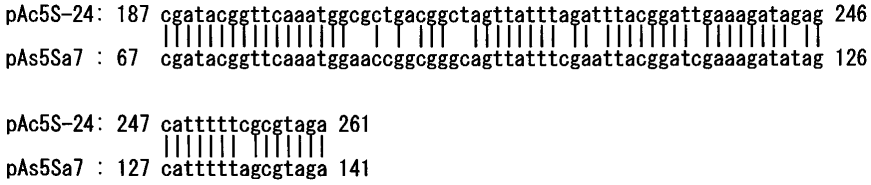

Fig. 6 The 75-bp homologous region in NTS of the long 5S rDNA unit of *A. cepa* (clone pAc5S-24) and *A. schoenoprasum* (clone pAs5Sa7)

suggesting that their phylogenetic relationship is not close. All taxa with the 350-bp repeat unit belong to the subgenus *Rhizirideum*. The 350-bp repeat unit is primitive in the subgenus *Rhizirideum* and the 520-bp repeat unit seems to have evolved later (Fig. 1). The 530-bp repeat unit observed in *A. cepa* and *A. schoenoprasum* might have been derived from the 350-bp repeat unit at or after species differentiation. Each 530-bp 5S rDNA unit evolves independently in each species. Two 5S rDNAs of different sizes have been reported in several species, including *Pisum sativum*, *Eruca sativa* and *Camellia sinensis* (Ellis et al. 1988; Singh et al. 1994; Singh and Singh 2001). In *P. sativum*, the difference in NTS length resulted from duplication of part of the NTS sequence (Ellis et al. 1988). In *E. sativa*, the long repeat unit arose from a mutation at an *Eco*RI site in the coding region (Singh et al. 1994). In the case of *A. cepa* and *A. schoenoprasum*, the appearance of the long rDNA unit did not result from such a simple mechanism. An interesting common sequence is found in the NTSs of both the Ac5SL and As5SL units. This common sequence is 75-bp long and is located at nt 187–261 in Ac5SL (clone pAc5S-24, broken line in Fig. 5B, Fig. 6) and at nt 68–142 in As5SL (clone pAs5Sa7, broken line in Fig. 5C, Fig. 6). This sequence shows about 82% homology between the Ac5SL and As5SL units, and in Ac5SL this sequence is part of the unique Ac5SL sequence. This sequence does not exist in the 350-bp 5S rDNA unit of *A. cepa* or *A. schoenoprasum*, or in other nucleotide sequences registered in the DNA data bank. In *A. cepa* and *A. schoenoprasum*, the sequence might have been added when the long 5S rDNA unit appeared. In *A. cepa*, the 75-bp sequence is inserted between the duplicated regions. In *A. schoenoprasum*, the 75-bp sequence is located near the duplicated partial gene sequences located in the NTS. We think that this 75-bp sequence is the remains of some kind of transposable element, which resulted in changes in the length of the 5S rDNA in *A. cepa* and *A. schoenoprasum*. In *A. cepa*, a transposable element is found in tandemly arranged nucleotide sequence regions, such as the 45S rDNA loci and the terminal repetitive sequence region (Pearce et al. 1996; Pich and Schubert 1998). The 5S rDNA loci should contain transposable elements, as do other tandemly arranged regions, and it is possible that these sequences caused the nucleotide sequence rearrangement in 5S rDNA.

Acknowledgements The authors sincerely thank members of the Center for Gene Research, Ehime University, Matsuyama, Japan; Dr. T. Kondo, of the Forest Tree Breeding Center, Ibaraki, Japan, for sequencing the 5S rDNAs; and Dr. H. Shibata and Mrs. F. Shibata of Shinshu University, Nagano, Japan, for collecting *A. schoenoprasum*. This work was partially supported by a Grant-in-Aid of Scientific Research (No. 07454254) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

References

Do GS, Seo BB (2000) Phylogenetic relationships among *Allium* subg. *Rhizirideum* species based on the molecular variation of 5S rRNA genes. Korean J Biol Sci 4:77–85

Ellis THN, Lee D, Thomas CM, Sympson PR, Cleary WG, Newman MA, Burcham KWG (1988) 5S rRNA genes in *Pisum*: sequence, long range and chromosomal organization. Mol Gen Genet 214:333–342

Gottlob-McHugh SG, Levesque M, MacKenzie K, Olson M, Yarosh O, Johnson DA (1990) Organization of the 5S rRNA genes in the soybean *Glycine max* (L.) Merrill and conservation of the 5S rDNA repeat structure in higher plants. Genome 33:486–494

Hanelt P (1990) Taxonomy, evolution, and history. In: Rabinowitch HD, Brewster JL (eds) Onions and allied crops, vol. I. CRC press, Boca Raton, Florida, pp 1–26

Hizume M (1993) Chromosomal localization of 5S rRNA genes in *Vicia faba* and *Crepis capillaris*. Cytologia 58:417–421

Hizume M (1994) Allodiploid nature of *Allium wakegi* Araki revealed by genomic in situ hybridization and localization of 5S and 18S rDNAs. Jpn J Genet 69:407–415

Lee SH, Seo BB (1977) Chromosomal localization of 5S and 18S-26S rRNA genes using fluorescence in situ hybridization in *Allium wakegi*. Korean J Genet 19:19–26

Lee SH, Do GS, Seo BB (1999) Chromosomal localization of 5S rRNA gene loci and the implications for relationships within the *Allium* complex. Chrom Res 7:89–93

Murray MG, Thompson WF (1980) Rapid isolation of high-molecular-weight plant DNA. Nucleic Acids Res 8:4321–4325

Pearce SR, Pich U, Harrison G, Flavell AJ, Heslop-Harrison JS, Schubert I, Kumar A (1996) The *Ty1-copia* group retrotransposons of *Allium cepa* are distributed throughout the chromosomes but are enriched in the terminal heterochromatin. Chrom Res 4:357–364

Pich U, Schubert I (1998) Terminal heterochromatin and alternative telomeric sequences in *Allium cepa*. Chrom Res 6:315–321

Shibata F, Hizume M, Kuroki Y (2000) Differentiation and the polymorphic nature of the Y chromosomes revealed by repetitive sequences in the dioecious plant, *Rumex acetosa*. Chrom Res 8:229–236

Singh D, Singh M (2001) Organization of 5S ribosomal RNA genes in tea (*Camellia sinensis*). Genome 44:143–146

Singh K, Bhatia S, Lakshmikumaran M (1994) Novel variants of 5S rRNA genes in *Eruca sativa*. Genome 37:121–128

Shigyo M, Tashiro Y, Isshiki S, Miyazaki S (1996) Establishment of a series of alien monosomic addition lines of Japanese bunching onion (*Allium fistulosum* L.) with extra chromosomes from shallot (*A. cepa* L. *Aggregatum* group). Genes Genet Syst 71:363–371