Rapid Report

MROS1, a Male Stamen-Specific Gene in the Dioecious Campion Silene latifolia Is Expressed in Mature Pollen

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The MROS1 gene, one of the genes that are expressed specifically in male reproductive organs of a dioecious campion Silene latifolia, was predicted to encode only 36 amino acids but have an intron. In situ hybridization revealed that MROS1 mRNA was localized in mature pollen grains.

Key words: Dioecious plant — In situ hybridization — Pollen — Sex chromosome — Short open reading frame.

The dioecious campion Silene latifolia Poiret ssp. alba [= Melandrium album (Miller) Garcke] has X and Y sex chromosomes (Ono 1939, Warmke and Blackshear 1939). The sex chromosomes account for about 16% of the total DNA in the male genome (Matsunaga et al. 1994). The early male and female flower buds have both sex organs (Ye et al. 1991, Grant et al. 1994). With the maturation of the flower buds, however, the differentiation of the gynoecium in the male flower bud and that of the stamen in the female flower bud are suppressed and, finally, male and female unisexual flowers are formed.

In order to analyze the molecular mechanism of male-specific differentiation in this dioecious plant, we had isolated and characterized four male reproductive organ-specific genes (MROS genes) from a cDNA library prepared from male flower buds (Matsunaga et al. 1996). Four MROS genes were independently expressed at different stages of the development of male flower buds. The transcript of MROS2, which encodes a glycine-rich protein, accumulated in the mature buds (Matsunaga et al. 1996). The site of accumulation of MROS2 mRNA in anthers changed from the epidermis and the endothecium near the stoma to the mature pollen grains with the maturation of floral organs. The transcript of MROS3 was localized specifically in the mature tapetal cells. Northern blot analysis revealed that the transcript of MROS1 accumulated specifically at the late stages of development of male flower buds. It was detected only in stamens of open male flowers and not in male and female vegetative organs or in female reproductive organs, for example, the styles and ovaries (Matsunaga et al. 1996). Sequence analysis of an MROS1 clone of 0.4 kbp in length revealed the absence of a long ORF. In general, short ORFs in genomes are considered likely not to be translated. However, it was shown recently that very short ORF encoding 10 amino acids in the ENOD40 gene, which is expressed during the early stages of development of legume nodules, is translated and the corresponding peptide acts to modulate the action of auxin (van de Sande et al. 1996). Therefore, we isolated the full-length cDNA and the genomic MROS1 gene and examined the pattern of accumulation of the transcript in male anthers by in situ hybridization.

We first rescreened 1.2 × 10⁵ plaques in the cDNA library prepared from male buds using the 0.4 kbp clone as the probe and the ECL direct nucleic acid labelling and detection system (Amersham International plc, Buckinghamshire, U.K.) and we isolated four clones. The inserts in two of them were longer than the probe and were 0.45 kbp and 0.55 kbp in length, respectively. All nucleotide sequences were determined by the dideoxynucleotide chain-termination method using an automated DNA sequencing system (373S; Perkin-Elmer, CA, U.S.A.). Sequencing data were analyzed with DNASIS software (Hitachi Software Engineering, Yokohama, Japan). Analysis of the nucleotide sequences revealed that the two inserts were identical to MROS1. The length of the longer insert excluding its poly-A tail was 537 bp, and it corresponded to an almost full-length MROS1 cDNA since the transcript of MROS1 was about 600 nucleotides long (Matsunaga et al. 1996). The longest ORF encoded 36 amino acids, which corresponds to a polypeptide of 3.7 kDa. Some short functioned peptides have been reported, for example, an inducer of the synthesis of the proteinase inhibitor in tomato. This inducer, known as systemin, is composed of 18 amino acids (Pearce et al. 1991). Moreover, pet M encodes a 4-kDa poly-

Abbreviations: ORF, open reading frame; DIG, digoxigenin.

The nucleotide sequences reported in this paper have been submitted to the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under accession numbers: D89818 for the cDNA sequence of MROS1 and AB000108 for the genomic sequence of MROS1.

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peptide of the chloroplast cytochrome b/f complex in *Chlamydomonas reinhardtii* (de Vitry et al. 1996). These peptides were synthesized as inactive precursors, and the mature short peptides are generated by proteolytical cleavage from the precursors (McGurl et al. 1992). By contrast, a nodule-specific protein in tobacco, ENOD40, is translated directly from a short ORF (van de Sande et al. 1996). It is possible that the short ORF of *MROS1* encodes a small peptide. However, it is unknown whether this putative short polypeptide of *MROS1* is actually translated. Untranslatable genes in plants, *CR20* and *AtCR20-1*, are isolated from cucumber and *Arabidopsis thaliana*, respectively (Teramoto et al. 1996). These genes have conserved regions and form stable secondary structures. However, *MROS1* does not have such features. A recent search of databases revealed that a male flower-specific gene in *S. latifolia, Men-1* (accession number, Y08773; Scutt et al. submitted data) exhibits 98.7% and 100% homology at the cDNA nucleotide level and the amino acid level to *MROS1*, respectively. Other genes and proteins in databases do not show any significant homology to *MROS1*.

In order to examine the nucleotide sequence of *MROS1*, in further detail, we amplified genomic clones of *MROS1* from four male and two female individual genomic DNAs by PCR. PCR was performed in 50 μl of a reaction mixture that contained 1 × Ex Taq buffer (Takara Shuzo, Otsu, Japan), 200 μM dNTP, 10 pmol each primer and 5 units of Ex Taq polymerase (Takara). The mixture was incubated for 30 cycles at 94°C for 1 min, at 57°C for 1 min and at 72°C for 2 min. When the 20-mer primers 1 (5'TAATCGTCAAAATGGCCTC3') and 2 (5'GGATGATGTGTAACAC3') were used for amplification, a single fragment of 1.2 kbp was obtained from both male and female DNAs. Figure 1 shows the nucleotide sequence of a fragment which was amplified from a male DNA. The nucleotide sequence outside two primers in Figure 1 shows the nucleotide sequence of the longest *MROS1* cDNA. The *MROS1* genomic fragment had an intron of 708 bp at position 114 (Fig. 1). This predicted intron conformed to the GT-AG rule, whereby an intron begins with GT and ends with AG. The nucleotide sequence of the two putative exons completely matched that of *MROS1* cDNA.

Figure 2 shows the pattern of genomic Southern hybridization with male and female genomic DNAs after

![Fig. 1 Nucleotide and deduced amino acid sequence of the genomic fragment of *MROS1*. The nucleotide sequences of putative exons are shown in capital letters and that of the predicted intron is shown in small letters. The amino acid sequence deduced from the longest ORF in the exon is shown under the respective codons. A possible polyadenylation signal is underlined. The arrows indicate the direction of DNA synthesis and the sequence of primers.](image-url)
digestion with HindIII and EcoRI. The MROSI cDNA without a 3'-poly-A tail as a probe was labeled with [α-32P]-dCTP by the Megaprime system (Amersham). Two strongly hybridizing bands were detected in both digested male and female DNA. The genomic fragment of MROSI contains an internal HindIII site. Although an EcoRI site was absent in the sequence of the genomic fragment of MROSI, two hybridizing bands were detected in EcoRI-digested DNA. The hybridizing band of female DNA was a doublet. It is possible that the two hybridizing bands in EcoRI-digested DNA reflect the restriction fragment length polymorphism (RFLP) between alleles of MROSI because our plant materials are not isogenic lines. In fact, analyses of genomic DNAs from several individuals indicated that differences in patterns of bands between males and females resulted from RFLP in our experimental population. The analysis by PCR and the genomic Southern blot analysis demonstrated that the MROSI gene was present in the both male and female genomes and suggested that the MROSI gene is not specifically located on the Y chromosome.

Figure 3 shows the localization of MROSI mRNA in a mature anther, as determined by nonradioactive in situ hybridization with a digoxigenin-labeled RNA probe. Mature anthers were collected from male buds at stage B5, when the transcript of MROSI accumulated at its highest level during the development of male buds (Matsunaga et al. 1996). Paraffin sections on glass slides for in situ hybridization were prepared as described by Takeda et al. (1995). RNA probes were produced from subclones of a fragment of MROSI cDNA without a 3'-poly-A tail in pBluescript SKII+ (Strategene, La Jolla, CA, U.S.A.). Antisense and sense RNA probes were synthesized and labeled by in vitro transcription by T3 and T7 RNA polymerases (Strategene)
with a DIG RNA labeling mixture (Boehringer Mannheim GmbH, Mannheim, Germany). In situ hybridization was performed as described by Matsunaga et al. (1996). When processed samples were observed under an inverted microscope (IMT-2; Olympus, Tokyo, Japan) equipped with a handmade slit on the condenser that changed the illumination to dark-field illumination (Matsunaga et al. submitted data), hybridization signals appeared bluish purple and cell walls appeared silvery white. At stage B5, just before the corolla opens and the locules of anthers are filled with mature pollen grains, the male buds are more than 21 mm long (Matsunaga et al. 1996). When the antisense probe was used, the product of hybridization was visible in pollen grains as bluish purple signals (Fig. 3A). Signals appeared in the cytoplasm that was extruded from pollen grains and weak signals were detected in pollen grains with a pollen cell wall. By contrast, the sense probe did not generate any signals above the background in anthers (Fig. 3B). The transcript of MROS2, which encodes a glycine-rich protein, was also detected in the mature pollen grains (Matsunaga et al. 1996). However, the patterns of expression of the two genes were different because the transcript of MROS1 was detected only in mature pollen grains whereas that of MROS2 was also found in the epidermis at a low level. Although numerous genes specific for mature pollen have been isolated (McCormick 1993), no pollen-specific gene with a short ORF such as MROS1 has previously been reported. Our results support a hypothesis that MROS1 is a novel pollen-specific gene that functions in the maturation of pollen grains.

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References


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