Isolation of a cDNA Clone (Accession No. U57638) Encoding a Type 2 Metallothionein-like Protein from Rice Embryo

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Metallothioneins (MTs) are low molecular weight, cysteine-rich metal-binding proteins which have been identified throughout the animal and plant kingdoms. Based on the contents and distribution of cysteine residues, and on structural relationships among various MTs, Kagi and Schaffer (1988) divided them into three classes. Class I and class II MTs are gene-encoded polypeptides, whereas class III MTs are phytochelatins that are enzymatically synthesized by gamma-glutamylcysteinyl dipeptidyl transpeptidase. Most MT-like genes isolated from plants have shown sequence similarity to the class I MTs, excepted the wheat Ec protein gene (Kawashima et al., 1992) that was classified as class II MTs (Robinson et al., 1993). The class I plant MTs have been further divided into two types (type 1 and type 2) on the basis of the arrangement of cysteine residues in the encoded proteins (Robinson et al., 1993). In type 1 there are exclusively Cys-x-Cys motifs, whereas in type 2 there is a Cys-Cys and a Cys-x-x-Cys pair within the N-terminal domain in addition to the Cys-x-Cys motif. All type 1 MTs express primarily in roots, while type 2 MTs express primarily in the aerial tissues (Hsieh et al., 1995).
We have isolated and sequenced a rice embryo-specific cDNA clone, Ose712. The cDNA fragment of Ose712 is 645 nucleotides in length, consisting of a 57-nucleotide 5' untranslated region, an open reading frame of 255 bp, and a 333-nucleotide 3' untranslated region including two putative polyadenylation signals, AATAAA and AATATA, at 459-461 and 604-609, respectively. The deduced translation product consists of 84 amino acids including 17 cysteine residues distributed in the N- and C-terminal regions of the polypeptide. All three types of cysteine motifs (Cys-Cys, Cys-x-Cys, Cys-x-x-Cys) that are suggested to bind metals are present in these two terminal domains. The arrangement of the cysteine residues in the deduced polypeptide of Ose712 is similar to the type 2 MTs (Hsieh et al., 1995; Robinson et al., 1993). Also consistent with classification as a type 2 MT is the absence of detectable mRNA expression in roots. However, contrary to previously characterized type 2 MTs, Ose712 mRNA was predominantly expressed in the embryo and was very low in leaves, suggesting that it is a new type 2 MT.

The role of MT-like proteins has not been clearly established in plants, although, by comparison with animal MTs, they have been suggested to play a role in metal detoxification or metal ion homeostasis (Robinson et al., 1993). Several lines of evidence have indicated that the plant MT-like proteins bind metals (Evans et al., 1992; Robinson et al., 1993) and the expression of some MT-like genes could be induced by various stress conditions including metal stress (Hsieh et al., 1995; Snowden and Gardner, 1993). Nevertheless, even without stress, the expression of several MT-like genes was observed during embryo or fruit development (Klemsdal et al., 1991; Kawashima et al., 1992; Ledger and Gardner, 1994), and could also be identified in various tissues, like rice endosperm and scutellum derived callus (Liu et al., 1995; Sasaki et al., 1994). Expression of MT-like genes in such diverse plant tissues suggests that a fundamental role of these MT-like proteins must exist. The MT-like gene, Ose712, isolated in this study, was predominantly expressed in the rice embryo in the absence of metal stress. Therefore, a role other than metal detoxification for this MT-like gene is suggested. What role of this MT-like gene may play during the growth and development of rice embryo will be the topic of further investigation.

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**Table 1. Characteristics of rice cDNA clone Ose712**

**Organism:**
Rice (*Oryza sativa* L.; cv. Lomello)

**Gene Product:**
A full-length cDNA clone that codes for a metallothionein-like protein. The nucleotide sequence shares a high degree (>90%) of similarity to several rice ESTs (Accession No. [D29998](https://www.ncbi.nlm.nih.gov/nuccore/D29998), [D21111](https://www.ncbi.nlm.nih.gov/nuccore/D21111) and [D23569](https://www.ncbi.nlm.nih.gov/nuccore/D23569)) and a rice complete cDNA sequence ([U77294](https://www.ncbi.nlm.nih.gov/nuccore/U77294)) that have been deposited in the GenBank. The amino acid sequence shares 71.2% identity to that of barley B22E (Klemsdal *et al.*, 1991) and is identical to [U77294](https://www.ncbi.nlm.nih.gov/nuccore/U77294).

**Source:**
cDNA library in Lambda UniZAP XR vector (Stratagene, USA) constructed using poly(A) mRNA isolated from rice embryos 10 days after pollination.

**Techniques:**
Differential screening; in vivo pBluescript plasmid excision; restriction fragment subcloning; dideoxy sequencing of both strands; Northern blot hybridization.

**Methods of identification:**
Nucleotide sequences of Ose712 and the deduced translation product were compared with the sequence data in GenBank through the BLAST algorithm. The Ose712 was identified as a MT-like protein based on the characteristic arrangement of cysteine residues at the two terminal domains of the polypeptide.

**Features of the deduced proteins:**
The deduced protein has a calculated molecular mass of 7.969 kD and an estimated PI value of 5.0. This protein, similar to the characteristic structural domains of plant type 2 MTs (Hsieh *et al.*, 1995; Robinson *et al.*, 1993), contains seventeen cysteine residues arranged as CCxxxCxCxxxCxxxCxxC at the N-terminus and CxCxxCxxxCxxxCxxC at the C terminus. These two domains are separated by 40 intervening amino acid residues.

**Expression characteristics:**
Embryo-specific expression. The size of the transcript for Ose712 is about 0.8 kb, measured by RNA gel hybridization assay.

**Literature Cited:**


