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Characterization of UMP Kinase cDNAs from *Oryza sativa* ([AF187062](#), [AF187063](#))

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▶ °° REPORT

UMP kinase catalyzed a phosphoryl transfer from ATP to either UMP or CMP to form ADP and UDP or CDP. Because all pyrimidine nucleotides within the cell arise from UMP, UMP kinase represents the first committed step of the various pyrimidine modification pathways leading to UTP, CTP and TTP. This enzyme has been well studied from a variety of bacterial sources (Valentin-Hansen, 1978, Yamanaka, et al., 1992, Serina, et al., 1995, Serina, et al., 1996), however it has been largely ignored in plants. The bacterial enzyme is allosterically regulated by both GTP and UTP (Serina, et al., 1995). Recently, the *Arabidopsis thaliana* cDNA was isolated, expressed in *E. coli*, and characterized (Zhou, et al., 1998, Zhou and Thornburg, 1998). The plant enzyme is insensitive to GTP and UTP (Zhou, et al., 1998) and therefore, must be regulated differently.

The plant enzyme shares high identity with the other eukaryotic UMP kinases that have been characterized from yeast, *Dictyostelium*, and mammals. Crystal structures for the yeast and *Dictyostelium* enzymes have been solved (Muller-Dieckmann and Schulz, 1994, Muller-Dieckmann and Schulz, 1995, Scheffzek, et al., 1996, permitting us to identify conserved features within the plant enzyme.

The eukaryotic UMP kinases all share a conserved glycine-rich sequence in the N-terminal region. This region is referred to as the "P-loop" or phosphate-binding loop. The crystal structures of UMP kinases from yeast and *Dictyostelium* suggest that this consensus sequence may play a role in ATP binding and/or enzyme catalysis (Muller-Dieckmann and Schulz, 1994, Muller-Dieckmann and Schulz, 1995, Scheffzek, et al., 1996). Site specific mutations in this conserved sequence of the *Arabidopsis* enzyme resulted in significant changes in catalytic activity (Zhou and Thornburg, 1998). Interestingly, mutations that showed reduced ATP binding showed increased UMP binding.

The expression of UMP kinase in plants has also received scant attention. The enzyme is known to be elevated during seedling development (Deng and Ives, 1972, Mazus and Buchowicz, 1972) and fruit ontogeny (Rudd and Fites, 1972, Deng and Ives, 1975), but its expression has not been characterized further.

We have had a long interest in pyrimidine metabolism in plants, focusing primarily on tobacco and Arabidopsis, two dicotyledonous species. Because of our interest in comparing the pyrimidine biosynthetic enzymes from dicots and monocots, we have characterized the cDNAs for UMP kinase from rice.

The MAFF (Japanese Ministry of Agriculture, Forestry and Fisheries) DNA database (rice genome project) was searched for clones that had identity to the Arabidopsis thaliana UMP kinase cDNA (Zhou, et al., 1998). Two clones were identified and each of these clones was kindly supplied to us by the MAFF DNA stock center. Sequencing reactions were performed using the Applied Biosystems Prism Dye-deoxy Cycle Sequencing Kit. The reactions were run on an Applied Biosystems Prism 377 DNA sequencer (Perkin-ElmerCorp.). Sequencing of the inserts was initiated from known vector sequences. Based on these sequencing runs, additional sequencing primers were designed to extend new sequence. Sequencing was continued until the gene was completely sequenced. The entire sequence was independently confirmed on the opposite strand. DNA sequences were performed in duplicate or triplicate for each run.

The inserts of each clone were completely sequenced and each contained a UMP kinase cDNA. These cDNAs have been named Os-ura6a and Os-ur2b based on the order that they were sequenced. The Os-ura6a clone corresponds to the MAFF clone R2392 (DDBJ locus RICR2392A; EMBL locus OSC23921A; & GenBank locus [D24696](#)) and the Os-ura6b clone corresponds to the MAFF clone S2089 (=DDBJ locus RICS2089A; EMBL locus OSS2089A; & GenBank locus [D40252](#)). The ura6a clone was isolated from a seedling root cDNA library, and the ura6b clone was isolated from an etiolated shoot cDNA library.

Both of these cDNAs were identical in nucleotide sequence. There was; however, a difference in the length of the cDNAs. The Os-ura6b clone was 7 nucleotides longer at the 5' end than was the Os-ura6a clone. There was also a single difference in the 3' untranslated region. The Os-ura6b clone also contained 7 extra nucleotides immediately preceding the poly A tail. There is no consensus poly adenylation signal located within the 3' untranslated region of either of these cDNAs. The closest candidate is CATAAG located 17 and 24 nucleotides upstream from the poly A sites of the respective cDNAs. There was also a single nucleotide difference within the coding region of the cDNAs. The ura6a clone contains a C at position 710 whereas the ura6b clone contains a T at this same position. This results in a single amino acid difference in the proteins; URA6a contains proline at position 191 whereas URA6b contains serine at this position.

Eukaryotic cDNAs encoding UMP kinases have been isolated from yeast (*Saccharomyces cerevisiae*, GenBank Accession # [M69295](#)), a slime mold (*Dictyostelium discoideum*, [M34568](#)), mammals (*Sus scrofa*, [D29655](#)) and from plants (*Arabidopsis thaliana*, [AF000147](#)). All of these enzymes share >45% identity; however, as expected the two plant enzymes share the highest degree of identity (79.8%). Both the rice and arabidopsis enzymes share roughly equivalent identity to the enzymes from yeast (45.5% and 49.7%), *Dictyostelium* (48.9% and 50.5%), and mammals (55.6% and 53.0%, respectively).

It has been previously demonstrated that a glycine-rich sequence near the N-terminus of the enzymes is important for binding of ATP (Walker, et al., 1982, Higgins, et al., 1988). Site specific mutations within this sequence of the Arabidopsis enzyme result in mutations that affect both ATP and UMP binding (Zhou and Thornburg, 1998). This sequence, GGPGSGKG, is completely conserved between the rice and Arabidopsis enzymes. The x-ray crystal structure of the yeast enzyme has permitted the functional assignment of other amino acid residues within the UMP kinase enzyme (Muller-Dieckmann and Schulz, 1994, Muller-Dieckmann and Schulz, 1995). This includes a group of amino acids that line the UMP binding site (Ala-47, Leu-51, Ile-75, Val-76, Thr-81, Phe-105, Arg-107, and Gln-111). With the single exception of an Asn substitution for Gln, these residues are completely conserved in both of the plant enzymes. The Asn for Gln substitution also occurs in the *Dictyostelium* enzyme.

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◦ ► °° TABLE I

Characteristics of the UMP kinase mRNA from *Oryza sativa*

Organism:

Oryza sativa var Nipponbare

Gene Product:

UMP kinase catalyzes the phosphoryl transfer from ATP to UMP or CMP to form ADP and UDP or CDP.

Characteristics of the UMP kinase cDNAs:

Each of the UMP kinase cDNAs was full length and encoded an identical 210 amino acid protein. Differences between the cDNAs were observed only in the 5' and 3' untranslated regions. These cDNAs have been named *ura6a* and *ura6b* based upon the order that they were sequenced. These clones have been deposited within the various databases with the following identification:

Os-*ura6a*

MAFF R2392
DDBJ RICR2392A2
EMBL OSC23921A2
GenBank [D24696](#)

Os-*ura6b*

MAFF R20892
DDBJ RICS2089A2
EMBL OSS2089A2
GenBank [D40252](#)

5' untranslated region:

The 5' untranslated regions of the two rice UMP kinase cDNAs were identical with the exception that the Os-*ura6a* 5' UTR was 5 nt shorter than that of Os-*ura6b*. The length of the 5' untranslated regions were 139 and 146 nucleotides for the two cDNAs, respectively. No significant palindromes, repeats or hairpins were identified within the 5' UTR.

Coding region:

630 nucleotides encode a 210 amino acid protein. The cDNAs terminate with an amber codon (TAG).

3' untranslated region:

The 3' untranslated regions of the two rice UMP kinase cDNAs were identical other than in length. Excluding the poly A tail, the length of the 3' untranslated regions were 355 nucleotides and 362 nucleotides for the two cDNAs, respectively. As with the 5' UTR, no significant palindromes, repeats or hairpin structures were identified within the 3' UTR. There is no consensus poly adenylation signal located within the 3' untranslated region of these cDNAs. The closest candidate is CATAAG located 17 and 24 nucleotides upstream from the poly A sites of the cDNAs.

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