Rice glutelin RNAs are localized to the cisternal endoplasmic reticulum (ER) by a regulated RNA transport process requiring specific cis-localization elements. We set out to identify these glutelin sequences by their dominant character of being able to re-direct the normal protein body ER localization of a maize 10 kDa δ-zein RNA to the cisternal ER. In situ RNA localization analysis showed that the glutelin RNA contains multiple cis-localization elements; two located at the 5′ and 3′ ends of the coding sequences and a third located within the 3′-untranslated region. These three regions contain two conserved sequences, suggesting that these RNA recognition signals may be sequence based.

Keywords: Cis-localization element • Endoplasmic reticulum • Glutelin • In situ RT-PCR • Rice • RNA targeting.

Abbreviations: ER, endoplasmic reticulum; PB-ER, protein body ER; PSV, protein storage vacuole; UTR, untranslated region; nos, nopaline synthase; in situ RT–PCR, in situ reverse transcriptase-mediated PCR.

The targeting of proteins to specific subcellular locations (e.g. mitochondria, vacuole, etc.) is directed by a variety of peptide signal determinants. In addition to peptide sorting signals, it is also well established that the mRNA itself may have signals that direct it to discrete locations within the cell (St Johnston 2005, Czapinski and Singer 2006, Jambhekar and DeRisi 2007). One RNA targeting system that has been well studied in plants is the transport and localization of the seed storage protein RNAs in rice developing endosperm cells. Prolamine RNAs are targeted to the spherical protein body ER which bounds newly assembled prolamine polypeptides, whereas glutelin RNAs are enriched on the adjacent cisternal ER (Li et al. 1993, Choi et al. 2000, Hamada et al. 2003a, Hamada et al. 2003b). These ER subdomains are predominantly located in the cortical region, just underneath the plasma membrane, of the developing endosperm (Muench et al. 2000). Following translocation into the ER lumen, glutelins are transported to the protein storage vacuole (PSV) via the Golgi complex (Krishnan et al. 1986). This relationship between the location of RNAs on the cortical ER and the site of deposition of the coded protein within the endomembrane system is also conserved in maize (Washida et al. 2004). The cis-localization elements of the rice prolamine RNA that direct it to the PB-ER are located at the 5′ end of the coding sequence and the proximal end of the 3′-untranslated region (UTR) (Hamada et al. 2003b).

The rice glutelin RNAs are localized to the cisternal ER (Li et al. 1993, Choi et al. 2000, Hamada et al. 2003a, Hamada et al. 2003b). Although foreign RNAs that lack cis-localization elements are also targeted to the non-PB-ER by a constitutive transport pathway, glutelin RNAs are transported by a regulated process requiring cis-localization elements. The glutelin cis-localization elements are dominant to those which target RNAs to the PB-ER as evident by their unique capacity to re-direct the localization of rice prolamine (Choi et al. 2000) and maize 10 kDa δ-zein (Hamada et al. 2003b) RNAs from the PB-ER to the cisternal ER.

We exploited these properties to identify the cis-localization elements of rice glutelin mRNA by cloning various glutelin RNA sequences as 3′ UTRs between the maize 10 kDa δ-zein-coding sequence and the nopaline synthase (nos)-transcriptional terminator. The various 10 kDa δ-zein–glutelin gene constructs were then expressed in developing endosperm cells under the control of endosperm-specific
glutelin GluB-1 promoter (Wu et al. 1998). We had previously demonstrated that hybrid genes containing glutelin or prolamine sequences at the 3’ UTR were faithfully transcribed, resulting in RNAs of the correct size (Hamada et al. 2003b). Transgenic plants expressing 10 kDa δ-zein, identified by immunoblot analysis (data not shown), were then examined for the distribution pattern of 10 kDa δ-zein RNAs. Sections from developing rice endosperm 10–15 d after pollination were subjected to in situ reverse transcriptase-mediated PCR (RT–PCR) in the presence of Oregon Green 488 dUTP and δ-zein-specific primers. The endosperm sections were then post-stained by rhodamine B hexyl ester to distinguish the prolamine-containing PB that was surrounded by the PB-ERs (Muench et al. 1997).

**Fig. 1** shows the schematic representation of the 10 kDa δ-zein transgenes containing various segments of the glutelin transcript sequences as well as their spatial distribution to the PB-ER and cisternal ER as viewed by in situ RT–PCR using the 10 kDa δ-zein-specific primers. In the absence of glutelin sequences, the 10 kDa δ-zein RNA is targeted to the PB-ER in transgenic rice endosperm cells (Hamada et al. 2003b). The presence of intact glutelin transcript sequences as part of the 3’ UTR resulted in re-directing RNA targeting to the cisternal ER. Hybrid RNAs lacking the glutelin 5’ UTR, CDS1, CDS2, CDS3 and CDS4, retained their localization to the cisternal ER, indicating that the remaining 3’ coding sequences and 3’ UTR have one or more cis-localization elements for cisternal ER targeting (construct 4). Those observations are consistent with our previous results where
these 3’ glutelin sequences redirected prolamine RNAs to the cisternal ER (Choi et al. 2000, Hamada et al. 2003b).

Closer examination of these 3’ glutelin sequences showed that CDS6 by itself was able to retain cisternal ER targeting of 10 kDa δ-zein (construct 6) but that the 3’ UTR was only partially functional in directing 10 kDa δ-zein RNAs to both the cisternal ER and PB-ER (construct 7). Additional deletion studies of the 3’ UTR showed that the putative cis-localization element was contained in or overlapped nucleotides 1,612–1,692 of the glutelin RNA (constructs 7–11).

Cisternal ER targeting of hybrid RNAs was also observed in 3’ deletion series (constructs 12–19), indicating that CDS1 which includes the signal peptide coding sequences has one or more cis-localization elements for cisternal ER targeting. CDS3 and CDS4 RNA sequences appeared to lack any cis-localization elements as the hybrid 10 kDa δ-zein RNAs containing these glutelin sequences were retained on the PB-ER (constructs 20–22). Overall, our in situ RNA localization results indicate that the glutelin RNA possesses at least three regions which contain putative cis-localization elements located between nucleotides 1 and 110, 1,292 and 1,500, and possibly 1,612 and 1,692, the latter only partially functional in re-directing 10 kDa δ-zein RNAs to the cisternal ER.

A direct comparison of the RNA sequences within CDS1, CDS6 and the 3’ UTR indicates the presence of two conserved sequences (Fig. 2). A type 1 sequence motif is shared between the proximal end of CDS1 (nt 23–44) and the putative cis-localization element located near the 3’ UTR end (nt 1,618–1,640). The type 1 motif shows some homology to the prolamine 3’ UTR UA motif at its distal end (nt 88–110), a sequence motif which is prevalent in CDS6. This region contains two intact type 2 sequences (nt 1,376–1,398 and 1,439–1,463) and a third abbreviated form (nt 1,409–1,423). Although we have not excluded the possible presence of cis-localization elements in the 5’ UTR, CDS2 or CDS5, these regions lack these conserved type 1 and type 2 sequences.

The presence of redundant type 1 and type 2 conserved sequences would explain the differences in the capacity of the CDS1, CDS6 and 3’ UTR to re-direct 10 kDa δ-zein RNAs from the PB-ER to the cisternal ER. CDS1 and CDS6 are fully capable of re-directing 10 kDa δ-zein RNAs to the cisternal ER, whereas the 3’ UTR is only partially functional. The redundancy of three type 2 sequence elements in CDS6 may account for its dominant properties. While CDS1 has only a single type 2 sequence element, it also contains a type 1 sequence element. The type 1 sequence element may be functionally equivalent to the type 2 sequence element as suggested by the partial dominance of the distal 3’ UTR sequences in re-directing a portion of the 10 kDa δ-zein RNAs to the cisternal ER.

Overall, the glutelin RNA contains at least three regions which contain cisternal ER cis-localization elements composed of two conserved sequence elements. The type 1 sequence shares some sequence similarity to the prolamine UA cis-localization element while the type 2 sequence is unique. The presence of redundant conserved sequence elements in CDS1 and CDS6 but not in the 3’ UTR supports the view that two sequence elements may be required to dominate the function of PB-ER cis-localization elements.

![Fig. 2](https://academic.oup.com/pcp/article-abstract/50/9/1710/1853909) putative glutelin cis-localization element sequences responsible for cisternal ER RNA localization. A schematic representation of the glutelin RNA and location of the three cis-localization elements are depicted (A). The three cis-localization elements contained two conserved sequence motifs, type 1 and type 2. CDS1 contains a type 1 and type 2 motif, CDS6 contains three type 2 motifs, with one being an abbreviated form, while the 3’ UTR contains a single type 1 motif.
Materials and Methods

The core vector (pTO158) contained a rice glutelin GluB-1 promoter, maize 10 kDa δ-zein and the nopaline synthase (nos) 3′ transcriptional terminator sequence (Hamada et al. 2003b). Various glutelin gt-2 cDNA sequences were obtained by PCR using primers containing an overhanging SacI site listed in Table 1. The fragment was then cloned into the corresponding site of pTO158 between 10 kDa δ-zein and the nos 3′ transcriptional terminator. The resulting plasmid was then digested with HindIII and cloned into the corresponding site of the T-DNA vector pCAMBIA 1301. In all plasmid constructions, the fidelity of the cloned PCR fragments was confirmed by DNA sequencing (data not shown).

Rice transformation and growth conditions were essentially the same as described previously (Hamada et al. 2003b). The expression of the introduced gene in endosperm cells was confirmed by Western blot using 10 kDa δ-zein antibodies (data not shown).

The procedure for cryosectioning, fixation and washing of 10- to 15-day-old developing rice seed sections that were prepared and subjected to in situ RT–PCR was described previously (Hamada et al. 2003b). Confocal microscopy was performed on a Zeiss 410 series laser scanning confocal microscope (Jena, Germany) or a Bio-Rad view Scan DVC-250 laser scanning microscope using fluorescein and rhodamine filter sets. Image processing was performed using Adobe Photoshop (Mountain View, CA, USA).

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References


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