Transport of Seed Storage Protein RNAs in Rice

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Objectives

Localizing RNAs are targeted to destination by thier own *cis*-acting factor (zipcode) and protein complex as well as cytoskeletal elements. To extend our knowledge as to the mechanism of plant RNA transport, we investigate the zipcode-binding site on rice prolamine RNA and acto-myosin elements.

Materials and Methods

Rice (*O. sativa* cv. Kitaake) was grown to maturity and its mid-developing seeds were used for *in situ*-RT PCR, immunofluorescence, and various cytoskeleton-disrupting chemicals. For analysis of *cis*-acting factors that play roles on prolamine RNA localization, truncated prolamine RNAs were expressed in rice seeds after transformation using *A. tumefaciens* AGL1. For the tracking down of prolamine RNA, two-gene system containing prolamine RNA-GFP protein fusion was expressed in endosperm cells. Confocal microscopic observations was made throught the experiments for the detection of fluorescence including GFP signal in tissue sections.

Results and Discussion

mRNA localization is an efficient way to target the gene products to specific subcellular compartments or to specific tissues in organisms. It occurs in a variety of organisms including yeast, insect, animal and plant system. In plants, RNAs are generally known to travel long-distance through phloem to play a role as a non-cell-autonomous signal in target site, however some RNA species are known to target within a cell to rapidly provide the gene products in a limited time period. In this presentation, an intracellular trafficking of RNA in plants, *e.g.*, RNAs of rice storage proteins, is presented.

Rice synthesizes and accumulates two major classes of storage proteins, prolamines and glutelins, which are stored in distinct subcellular compartments of the secretory pathway. Prolamines are deposited directly as spherical protein granules within the ER lumen, whereas glutelins are transported from the ER to the Golgi where they are eventually packaged in protein storage vacuoles (PSV). It has been previously shown that prolamine RNAs are targeted to the ER surface of spherical protein bodies (PB-ER) as a RNA dependent manner. Further deletion analysis indicated that prolamine RNA has at least two *cis*-acting domains for its proper targeting, and that two RNA transport pathways are necessary for rice storage RNA transport.

To demonstrate the transport of prolamine RNAs to PB-ER in living endosperm cells, a two-gene system was employed that is consisted of GFP fused to the viral RNA binding protein MS2 and a hybrid prolamine RNA containing tandem MS2 RNA binding sites. Microscopic observation showed that the GFP-labeled prolamine RNAs are transported as particles which move at an average speed of 0.3-0.4 m/sec. These prolamine RNA transport particles moved via the actomyosin system as particle movement is rapidly inhibited by the actin filament disrupting drugs, cytochalasin D and latrunculin B and the myosin inhibitor 2,3-butanedione monoxime.

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