respectively; in a t test $t = 7.16$, $P < 0.001$), which indicates a bias in favor of p60+ protoplasts on ZOG. Thus, although some protoplasts that lack the ability to use ZOG can obtain cytokinin from protoplasts that express Zm-p60.1, it is unlikely that the majority of p60+ protoplasts also obtained cytokinin from a small fraction of highly expressing or stably transformed protoplasts.
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Rice Prolamine Protein Body Biogenesis: A BiP-Mediated Process

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Rice prolamines are sequestered within the endoplasmic reticulum (ER) lumen even though they lack a lumenal retention signal. Immunochemical and biochemical data show that BiP, a protein that binds lumenal polypeptides, is localized on the surface of the aggregated prolamine protein bodies (PBs). BiP also forms complexes with nascent chains of prolamines in polyribosomes and with free prolamines with distinct adenosine triphosphate sensitivities. Thus, BiP retains prolamines in the lumen by facilitating their folding and assembly into PBs.

Rice seeds accumulate two types of storage proteins common to all seeds, glutelins (globulin-like) and prolamines. Glutelins are transported into vacuoles through the Golgi complex, whereas prolamines aggregate within the ER lumen (*1*). The mRNAs that encode glutelins and prolamines are localized to morphologically distinct ER membranes in endosperm cells, thus contributing to the asymmetric protein distribution (*1*, *2*). Prolamine transcripts are enriched on the ER that delimits the prolamine PBs, whereas glutelin mRNAs predominate on the cisternal ER (*2*).

Most proteins localized to the ER lumen have the amino acid sequence Lys(His)-Asp-Glu-Leu [(K(H)DEL] (or a related sequence) near their COOH-terminus, which serves to retrieve these proteins from an early Golgi compartment (*3*). Prolamines, however, lack this sequence. Prolamine PB formation may be a consequence of the high ionic strength environment of the ER lumen inducing aggregation of the hydrophobic protein (*4*). Alternatively, PB formation may be dependent on specific cellular factors. One candidate for such a factor is BiP, a cognate of the 70-kD heat shock protein (Hsp70) located in the ER lumen. BiP functions as a molecular chaperone to facilitate the proper folding or assembly of newly synthesized polypeptides (*5*), a prerequisite for subsequent protein transport through the secretory pathway (*6*). Here, we show that BiP retains prolamines within the ER lumen by promoting the folding and assembly of prolamines into PBs.

We used antibody to maize BiP to quantify amounts of BiP in subcellular fractions from developing rice seeds (*7*). A 72-kD protein, similar in size to the maize BiP, was detected in subcellular fractions enriched for cisternal ER and PBs (*2*). At least 90% of the BiP present in endosperm cells was observed in the PB fraction and accounted for 2 to 4% of the total protein in this fraction (*8*). Immunocytochemical localization studies revealed that BiP was associated with the surface of prolamine PBs and was rarely detected within the cisternal ER, even those connected to PBs (*8*). These quantification and localization results indicate that BiP is asymmetrically localized in the ER lumen and is primarily associated with prolamine PBs.

To determine if BiP interacts with prolamines during translocation of the nascent chains across the ER membrane and subsequent folding and assembly of mature polypeptides into PBs, we extracted the PBs with detergent (*9*, *10*) to remove the delimiting ER membrane (Table 1). Thirty percent of the BiP was released by the detergent treatment and presumably located within the lumen (Table 1). Mature prolamines (14 kD) and BiP in the detergent extract cosedimented in a sucrose density gradient (Fig. 1A), which suggests that these proteins were bound to one another. These complexes ranged in size from slightly larger than the monomeric BiP (72 kD) to about 400 kD (Fig. 1A). Incubation of

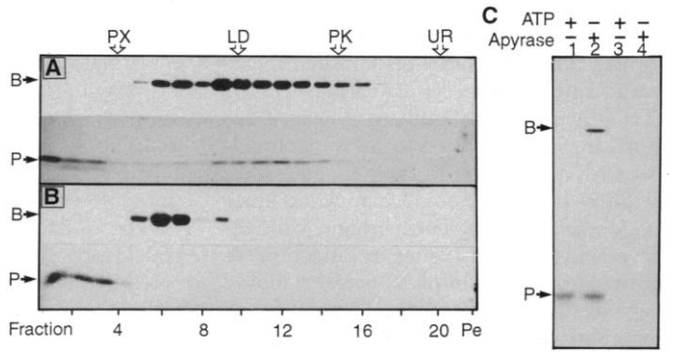

Fig. 1. (**A** and **B**) Cosedimentation of BiP (B) and prolamines (P). The detergent extract (*9*, *10*) was clarified of ribosomes by centrifugation at 200,000*g* for 2 hours. The resulting supernatant (5 mg of protein) was then incubated at 25°C for 20 min in the presence of 10 U of apyrase (A) or 5 mM ATP (B) and then centrifuged on a 5 to 20% sucrose density gradient at 200,000*g* for 23 hours at 4°C. Fractions (0.5 ml) were collected and then subjected to trichloroacetic acid precipitation followed by immunoblot analyses with anti-BiP and anti-prolamine. Peroxidase (PX, 40 kD), lactic dehydrogenase (LD, 137 kD), pyruvate kinase (PK, 237 kD), and urease (UR, 480 kD) were cosedimented with the rice samples; the locations of their enzyme activity peaks are indicated by the arrows. Pe, pellet. (**C**) Coimmunoprecipitation of BiP and prolamine. The postribosomal supernatant of the detergent extract (1 mg of protein) was incubated at 4°C for 4 hours with 25 μg of anti-prolamine (lanes 1 and 2) or preimmune serum (lanes 3 and 4), followed by agarose–protein A in the presence of 10 units of apyrase or 5 mM ATP, as indicated. Subsequent manipulations were as described (*21*). The precipitated protein was analyzed by immunoblot with anti-BiP and anti-prolamine.

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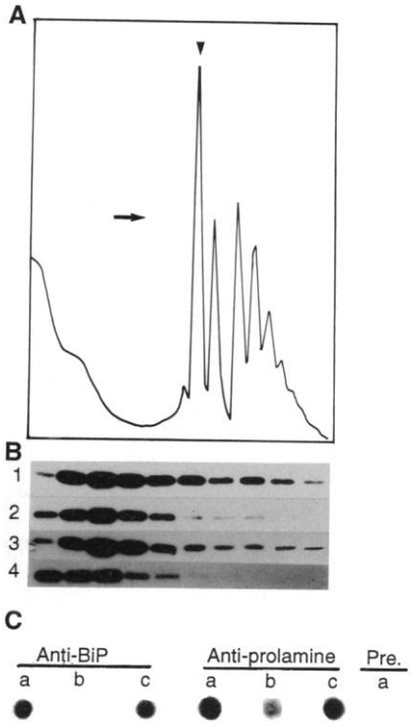
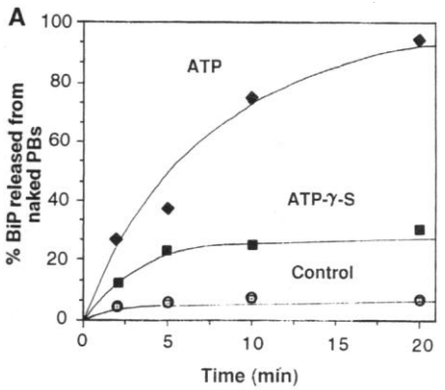


Fig. 2. (**A** and **B**) Cosedimentation of BiP and polyribosomes. The salt extract (previously frozen) was incubated at 25°C for 20 min with distilled H_2O [(A) and row 1 of (B)], 1 mM puromycin (row 2), 5 mM MgATP (row 3), or 0.1 mg bovine pancreatic ribonuclease (row 4), followed by sucrose density gradient centrifugation (*13*). Protein samples in each fraction were collected by trichloroacetic acid precipitation and then analyzed by immunoblot with anti-BiP (rows 1 through 4). Shown in (A) is the polyribosome profile of the mock control in (B), row 1. The arrow and arrowhead in (A) represent the direction of sedimentation and the location of monosomes, respectively. (**C**) Polyribosomes were immunoprecipitated (*14*) with antibody to maize BiP (anti-BiP), anti-prolamine, or preimmune serum (Pre.) in the absence (a) or presence of 1 mM puromycin (b) or 5 mM ATP (c). The RNA in the precipitate was extracted and subjected to dot blot analyses with a prolamine complementary DNA probe as described (*2*).

the detergent extract with adenosine triphosphate (ATP) before centrifugation eliminated the faster sedimenting complexes (Fig. 1B). In contrast, treatment with ATP-γ-S, the nonhydrolyzable analog of ATP, did not affect the sedimentation of BiP or prolamines (*8*). We presume that this reflects dissociation of the BiP-prolamine complexes catalyzed by the adenosine triphosphatase function of BiP (*3*). Antibody to prolamines coprecipitated both BiP and mature prolamines from the detergent extract in the absence, but not in the presence, of ATP (Fig. 1C). Together, these data indicate that BiP and mature prolamines formed ATP-sensitive complexes.

Detergent solubilization of PB membranes released only 20 to 25% of all polyribosomes in the crude PB fraction (*11*); the rest remained associated with the PB (Table 1). Puromycin did not dissociate these polyribosomes from the detergent-treated PBs, although nascent prolamine chains were released (*8*). These polyribosomes, however, were readily released from the PBs by solutions of moderate ionic strengths (*8*, *12*). As determined by sucrose density gradient centrifugation (*13*), BiP cosedimented with the polyribosomes (Fig. 2, A and B, row 1). A similar sedimentation pattern was observed when the fractions were analyzed with antibody to HDEL (*8*). Pretreatment with puromycin had no effect on the polyribosome profile, but the amount of BiP in complexes was reduced (Fig. 2B, row 2), which indicates that BiP interacted with the nascent peptides. Pretreatment with ATP had less effect on the amount of BiP (Fig. 2B, row 3) or on the polyribosome sedimentation profile (*8*). As a control, ribonuclease treatment completely disrupted polyribosomes and, consequently, eliminated the fast-sedimenting BiP species (Fig. 2B, row 4). We conclude that most of the BiP in the salt extract was associated with the polysomal nascent polypeptide chain through an ATP-insensitive interaction.

To determine whether BiP interacted with the prolamine nascent polypeptide chain, we incubated the purified polyribosomes with antibody to BiP (anti-BiP), antibody to prolamine (anti-prolamine), or control antiserum (*14*). The RNA in the immunoprecipitate was extracted and then analyzed by Northern (RNA) blot for the presence of prolamine mRNA. The BiP- or prolamine-specific antiserum precipitated about 50% of prolamine polyribosomes (*15*) even in the presence of ATP (Fig. 2C). The inhibitory effect of puromycin on the immunoprecipitation of prolamine polysomes by anti-BiP (Fig. 2C) also indicated that BiP was complexed to the nascent prolamine polypeptide.



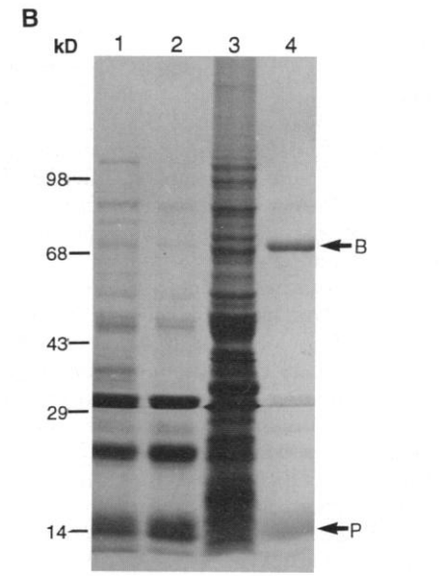


Fig. 3. Time course of BiP release from cell-free (naked) PBs. The PB samples (5 mg of protein) were incubated at 25°C in the presence or absence of 0.1 mM ATP or ATP-γ-S, followed by centrifugation at 12,000*g* for 10 min. (**A**) The amount of BiP released into the supernatant at various time points as quantified by immunoblot (*7*). (**B**) Protein profiles. The protein (10 μg) released by the 45-min ATP treatment of naked PBs was resolved on an SDS-polyacrylamide gel and then stained by Coomassie blue (lane 4). For comparison, 50-μg extracts of total seed (lane 1), PBs (lane 2), or microsomal membranes enriched in cisternal ER (lane 3) were also included. B and P are as in Fig. 1.

Table 1. Distribution and properties of BiP in PB fractions. Crude PB (containing both BiP and polysomes) was sequentially extracted with Triton X-100 (TX-100) and salt, resulting in three subfractions (*9*, *10*). The amount of BiP was estimated by immunoblot (*7*), whereas polysomes were isolated and quantified as described (*11*).

Subfractions	Distribution (%)		BiP complex with	Location	ATP sensitivity
	BiP	Polysome			
TX-100 extract of crude PB	30 ± 5	22.5 ± 2.5	Mature prolamine	Lumen	Yes
Salt extract of TX-100–treated PB	45 ± 5	77.5 ± 2.5	Nascent prolamine	Polysomes	No
Cell-free PBs	25 ± 5	0.0	PB	PB surface	Yes

Although a majority of the BiP in the enriched PB fraction was released by detergent and salt, about 25% remained associated with the protein granules (Table 1). The ATP (but not ATP-γ-S) released almost all of this tightly bound BiP (Fig. 3A). Two major polypeptides with electrophoretic mobilities identical to BiP and prolamine (*16*) were evident in ATP-released products (Fig. 3B). The ATP-dependent dissociation of BiP from the PBs suggests that BiP has an essential role in the assembly of prolamines onto the PB and would account for the localization of rice BiP on the surface of, but not within, the PBs (*8*).

The tight binding of BiP-prolamine complexes is likely due to the preference of BiP for aliphatic amino acids (*17*), residues enriched in prolamines (*18*). The higher affinity of BiP for prolamines than for glutelins may explain the localization of BiP to the prolamine PBs but not to the cisternal ER where glutelins are synthesized (*2*). However, BiP may help the folding and assembly of glutelins in the ER before they are transported to the Golgi complexes. The association of BiP with glutelins is probably transient.

We propose that prolamine PB formation is a sequential process mediated by BiP. BiP binds to the nascent prolamine peptide as it emerges through the ER membrane. This initial complex, which resembles the interaction between cytosolic Hsp70 and nascent polypeptides (*19*), maintains the polypeptide chain in a competent state for subsequent assembly onto the PBs. The completion of protein synthesis results in the release of the prolamine-BiP complexes into the ER lumen. These protein complexes then interact with the PB surface, and prolamine molecules are dissociated from BiP and assembled onto the PB surface at the expense of ATP. The dissociated BiP is then recycled. The PB formation would be initiated when a critical concentration of BiP-prolamine complexes is accumulated in the ER lumen and BiP-mediated aggregation of the prolamine polypeptides begins. This involvement of BiP in prolamine PB formation is also supported by its elevated amounts in the PBs of maize mutants defective in zein accumulation (*20*).

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13. The salt extract (4 absorbance units at 260 nm) was loaded onto a 4.5-ml sucrose density gradient (15 to 60%) in PB buffer (*17*) and centrifuged at 240,000*g* for 3 hours followed by fractionation. Polyribosome profiles were monitored at 254 nm.
14. Twenty micrograms of antiserum was incubated with 50 μl of agarose–protein A overnight at 4°C in 50 mM tris-HCl (pH 7.2), 500 mM NaCl, and 0.1% Tween 20 (TBST). The resulting agarose beads were washed thoroughly with TBST, then with PB buffer containing 1% Tween and 1% bovine serum albumin (buffer Q), followed by suspension in 0.5 ml of buffer Q. After addition of purified polyribosomes (0.1 absorbance unit at 260 nm), the mixture was incubated overnight at 4°C and the beads washed thoroughly with buffer Q. The bound polyribosomes were released by an incubation at 25°C for 10 min in 200 mM acetate.
15. Northern blot analyses showed that prolamine transcripts predominated in the salt extract (*8*), which indicates that these transcripts represented the principal mRNA species on the prolamine PB membrane (*2*).
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Formation and Hydrolysis of Cyclic ADP–Ribose Catalyzed by Lymphocyte Antigen CD38

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CD38 is a 42-kilodalton glycoprotein expressed extensively on B and T lymphocytes. CD38 exhibits a structural homology to *Aplysia* adenosine diphosphate (ADP)–ribosyl cyclase. This enzyme catalyzes the synthesis of cyclic ADP–ribose (cADPR), a metabolite of nicotinamide adenine dinucleotide (NAD^+) with calcium-mobilizing activity. A complementary DNA encoding the extracellular domain of murine CD38 was constructed and expressed, and the resultant recombinant soluble CD38 was purified to homogeneity. Soluble CD38 catalyzed the formation and hydrolysis of cADPR when added to NAD^+. Purified cADPR augmented the proliferative response of activated murine B cells, potentially implicating the enzymatic activity of CD38 in lymphocyte function.

CD38 has been used predominantly as a phenotypic marker of different subpopulations of T and B lymphocytes (*1*, *2*). Antibodies to CD38 (anti-CD38) can stimulate the growth of lymphocytes in the presence of other stimuli such as interleukin-4 (IL-4) (*3*, *4*) or IL-2 and accessory cells (*2*). Anti-CD38–induced proliferation of murine B lymphocytes is preceded by a rapid Ca^{2+} influx that can be distinguished from the inositol triphosphate (IP_3)–dependent Ca^{2+} influx induced by stimulation of the same cells with antibody to immunoglobulin M (anti-IgM) (*3*). Sequence analysis of the complementary DNAs (cDNAs) encoding murine CD38 (*4*), human CD38

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