A Defective Signal Peptide Tethers the floury-2 Zein to the Endoplasmic Reticulum Membrane

Jeffrey W. Gillikin, Fan Zhang, Craig E. Coleman, Hank W. Bass, Brian A. Larkins, and Rebecca S. Boston

Department of Botany, North Carolina State University, Raleigh, North Carolina 27695 (J.W.G., F.Z., H.W.B., R.S.B.); and Department of Plant Sciences, 303 Forbes Building, University of Arizona, Tucson, Arizona 85721 (C.E.C., B.A.L.)

The maize (Zea mays L.) floury-2 (f2) mutation is associated with a general decrease in storage protein synthesis, altered protein body morphology, and the synthesis of a novel 24-kD α-zein storage protein. Unlike storage proteins in normal kernels and the majority of storage proteins in f2 kernels, the 24-kD α-zein contains a signal peptide that would normally be removed during protein synthesis and processing. The expected processing site of this α-zein reveals a putative mutation alanine→valine (Ala→Val) that is not found at other junctions between signal sequences and mature proteins. To investigate the impact of such a mutation on signal peptide cleavage, we have assayed the 24-kD f2 α-zein in a co-translational processing system in vitro. Translation of RNA from f2 kernels or synthetic RNA encoding the f2 α-zein in the presence of microsomes yielded a 24-kD polypeptide. A normal signal peptide sequence, generated by site-directed mutagenesis, restored the capacity of the RNA to direct synthesis of a properly processed protein in a cell-free system. Both the f2 α-zein and the f2 α-zein (Val→Ala) were translated into the lumen of the endoplasmic reticulum. The processed f2 α-zein (Val→Ala) was localized in the soluble portion of the microsomes, whereas the f2 α-zein co-fractionated with the microsomal membranes. By remaining anchored to protein body membranes during endosperm maturation, the f2 α-zein may thus constrain storage protein packing and perturb protein body morphology.

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* Corresponding author; e-mail boston@unity.ncsu.edu; fax 1-919-515-3436.

Abbreviations: BiP, binding protein; DAP, days after pollination; PDI, protein disulfide isomerase.
The phenotypic consequences of mutations that block signal peptide processing are not well known. Studies of proteins with signal peptides that were not processed by signal peptidase suggest that these proteins remain anchored to ER membranes (Schauer et al., 1985; Shatters and Miernyk, 1991). The fl2 mutant provides us with a means for probing in vitro the molecular events that contribute to its pleiotropic phenotype. Here we report that the Val at the −1 position of the fl2 gene product is sufficient to block cleavage of the signal peptide from the primary translation product. The uncleaved fl2 gene product is associated with microsomal membranes to which it remains anchored even after the membranes have been disrupted by alkaline lysis. Based on these results, we propose a model to explain the effects of the fl2 mutation on protein body synthesis and morphology during endosperm development.

**MATERIALS AND METHODS**

Maize (Zea mays L.) inbred W64A + and its near-isogenic mutant, fl2, were grown and self-pollinated at the North Carolina State University Research Unit I (Raleigh) and the Central Crops Research Station (Clayton, NC) in the summers of 1993 and 1994, respectively. Ears were harvested 18 DAP and rapidly frozen in liquid N₂. The frozen kernels were shelled onto dry ice and stored at −80°C.

**RNA Isolation**

Kernels (5–10 g) were ground to a powder in liquid N₂ with a mortar and pestle. RNA was extracted from the powdered tissue essentially by the procedure of Parish and Kirby (1966), except that Tris-buffered phenol was used for microsomal membranes to which it remains anchored even after the membranes have been disrupted by alkaline lysis. Based on these results, we propose a model to explain the effects of the fl2 mutation on protein body synthesis and morphology during endosperm development.

**Cloning and Mutagenesis**

The coding region of the fl2 α-zein(4,386),(986,994) was subcloned into the pALTER vector of an in vitro mutagenesis system (Altered Sites II, Promega; Coleman et al., 1995). An oligonucleotide (GTG GAA TAA TGA ATG CAT TTG TTG CGC) was used to introduce an Ala codon in the −1 position of the fl2 gene product. Zein sequences from the resulting plasmid (pCC515), a deletion construct lacking the signal peptide region, or a plasmid (pMZ22.3) encoding a normal 22-kD α-zein were subcloned into the poly(A)+ vector (pSP64, Promega) for use in producing transcripts with poly(A) tails (Marks and Larkins, 1982).

**Protein Body Purification**

Protein bodies were prepared by a modification of the method of Larkins and Hurkman (1978) from normal and mutant endosperm tissue harvested 18 DAP. Endosperm tissue (10 g) was homogenized in an extraction buffer containing 10 mM Tris-HCl, pH 8.5, at 25°C, 10 mM KCl, 5 mM MgCl₂, and 7.2% (w/v) Suc at a ratio of 1 g of endosperm to 2 mL of extraction buffer. The homogenate was subjected to centrifugation at 80g for 5 min at 4°C to remove starch and other cellular debris. The supernatant was fractionated through discontinuous Suc gradients composed of 3 mL of 2 M Suc and 3 mL of 1.5 M Suc, each in 10 mM Tris-HCl, pH 8.5, at 25°C, 10 mM KCl, and 5 mM MgCl₂. Samples were fractionated by centrifugation at 80,000g for 40 min at 4°C. Protein bodies were harvested from the 1.5/2 M Suc interface, solubilized in 1% (w/v) SDS, and quantified with a protein assay (BCA, Pierce) with BSA as a standard.

**Maize Microsome Preparation**

Kernels (60 g) harvested 18 DAP were powdered in a coffee mill with dry ice. One milliliter of protein body buffer (50 mM Tris-HCl, pH 8.0, at 25°C, 100 mM KCl, 30 mM MgCl₂, 1 mM EGTA-NaOH, 1 mM Na₂EDTA, 5 mM DTT, and 17% [w/v] Suc) was added per gram of powdered kernels. The slurry was homogenized for 3 min on ice. All subsequent steps were performed according to the method of Burr and Burr (1981).

Prior to protein synthesis in vitro, membranes were treated with micrococcal nuclease (300 units/mL) for 20 min at 25°C (Pelham and Jackson, 1976). The membranes were adjusted to 4 mM EGTA-NaOH and collected by centrifugation at 100,000g for 15 min. Pellets were resuspended in the reaction buffer (20% [v/v] glycerol, 3 mM Hepes-KOH, pH 7.6, and 1 mM magnesium acetate) to a final concentration of 100 A₂₈₀ units/mL and stored on ice.

**Translation/Translocation Assays in Vitro**

Protein synthesis reactions in either rabbit reticulocyte or wheat germ cell-free systems were programmed with poly(A)⁺ RNAs from kernels or synthetic RNAs, as noted in the figure legends. Co-translational processing was assayed in translation reactions supplemented with an aliquot of canine pancreatic microsomes (Promega) or maize microsomes equal to one-sixth of the reaction volume. All initial reaction volumes of 25 μL contained 12.5 μCi of [³H]Leu (171 Ci/mmol, Amersham).

To assay for translocation, we diluted 12.5-μL aliquots of the wheat germ translation reactions to 40 μL with 20%
probed with anti-zein antiserum. Molecular masses (in kD) of major a-zein subclasses are shown to the left. Figure 1A shows translation products synthesized from normal or fl2 RNAs. Primary alcohol-soluble translation products had apparent molecular masses of 21 and 24 kD (Fig. 1A, lanes 1 and 3). The addition of canine pancreatic microsomes to the translation reactions programmed with RNA from normal endosperm yielded polyepitides of 19 and 22 kD. These products corresponded to the processed forms of the 21- and 24-kD translation products, respectively (Fig. 1A, compare lanes 1 and 2; Burr and Burr, 1981). Translation of RNA from fl2 endosperm in the presence of canine pancreatic microsomes, however, resulted in the production of alcohol-soluble polyepitides of 19-, 22-, and 24-kD size classes (Fig. 1A, lane 4). The presence of a 19-kD polyepitide and corresponding disappearance of the 21-kD polyepitide indicated that the initial 21-kD translation product(s) had been converted to a 19-kD form(s). However, the presence of both 24- and 22-kD products suggested that some of the 24-kD polyepitides had not been processed or that some posttranslational modification had occurred. The profile of polyepitides synthesized with normal and fl2 RNAs resembled that of an immunoblot of normal and fl2 protein body proteins probed with a-zein antiserum (Fig. 1B). Previously, we showed that the 24-kD band from fl2 protein

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**RESULTS**

**Translation/Translocation Assays with Heterologous and Homologous Microsomes**

Poly(A)+ RNAs from W64A+ (normal) and W64A//fl2 (mutant) endosperm were used to transcribe in rabbit reticulocyte lysate cell-free translation/translocation reactions. Figure 1 shows translation products synthesized from normal or fl2 RNAs. Primary alcohol-soluble translation products had apparent molecular masses of 21 and 24 kD (Fig. 1A, lanes 1 and 3). The addition of canine pancreatic microsomes to the translation reactions programmed with RNA from normal endosperm yielded polyepitides of 19 and 22 kD. These products corresponded to the processed forms of the 21- and 24-kD translation products, respectively (Fig. 1A, compare lanes 1 and 2; Burr and Burr, 1981). Translation of RNA from fl2 endosperm in the presence of canine pancreatic microsomes, however, resulted in the production of alcohol-soluble polyepitides of 19-, 22-, and 24-kD size classes (Fig. 1A, lane 4). The presence of a 19-kD polyepitide and corresponding disappearance of the 21-kD polyepitide indicated that the initial 21-kD translation product(s) had been converted to a 19-kD form(s). However, the presence of both 24- and 22-kD products suggested that some of the 24-kD polyepitides had not been processed or that some posttranslational modification had occurred. The profile of polyepitides synthesized with normal and fl2 RNAs resembled that of an immunoblot of normal and fl2 protein body proteins probed with a-zein antiserum (Fig. 1B). Previously, we showed that the 24-kD band from fl2 protein

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**SDS-PAGE and Two-Dimensional PAGE**

Protein synthesis reactions were adjusted to 70% ethanol, incubated for 10 min at 60°C, and fractionated by centrifugation at 16,000g for 10 min. The supernatant was transferred to a fresh tube and lyophilized to dryness. Samples to be analyzed by one-dimensional PAGE were resuspended directly in a loading buffer (Laemmli, 1970). Samples to be analyzed by two-dimensional PAGE were washed with ice-cold H2O and collected by centrifugation for 5 min at 4°C. The pellet was solubilized and subjected to separation by two-dimensional PAGE as described previously, except that the ampholyte concentration was 0.67% (v/v) each of 6/8, 5/7, and 3/10 ampholytes (Bio-Lyte, Bio-Rad), and pipera-}

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**Fluorography and Immunoblot Analysis**

Following PAGE, SDS-polyacrylamide gels were fixed in 10% (v/v) methanol and 7.5% (v/v) glacial acetic acid for 30 min. Gels were then impregnated with En3Hance (Du-Pont), dried, and subjected to fluorography according to the manufacturer's instructions. For immunoblot analysis, proteins were electroblotted from 10% SDS-polyacrylamide gels to nitrocellulose using a semidry blotting apparatus (Trans-Blot SD, Bio-Rad) and the buffer system described by Bjerrum and Schafer-Nielsen (1986). Filters were probed with polyclonal antiserum (1:5000 dilution; Lending et al., 1988), and probed with anti-zein antiserum. Molecular masses (in kD) of major a-zein subclasses are shown to the left.
bodies is composed of at least two polypeptides (Coleman et al., 1995). One of them is the \( \text{fl2} \) \( \alpha \)-zein with an intact signal peptide and the other is a 19-kD \( \alpha \)-zein that is processed by signal peptidase but is also modified by core glycosylation (Coleman et al., 1995; J.W. Gillikin and R.S. Boston, unpublished results).

Two-dimensional PAGE was performed to determine the composition of the 24-kD band produced in the cell-free protein synthesis reactions. Two-dimensional gels of translation products from normal and \( \text{fl2} \) RNA are shown in Figure 2. Identical polypeptide patterns were observed when normal and \( \text{fl2} \) RNAs were translated in the absence of microsomes (Fig. 2, A and C). When microsomes from normal kernels were included in the translation reactions, RNA from normal maize directed the synthesis of 19- and 22-kD alcohol-soluble proteins (Fig. 2B). In contrast, \( \text{fl2} \) RNA translated in the presence of the normal microsomes resulted in the production of the expected 19- and 22-kD \( \alpha \)-zein proteins, as well as two distinct 24-kD polypeptides (Fig. 2D). The 24-kD polypeptides corresponded to the glycosylated 19-kD and \( \text{fl2} \)-specific 24-kD polypeptides observed in the \( \text{fl2} \) protein bodies (Coleman et al., 1995). These results, together with those shown in Figure 1, suggest that both normal maize microsomes and canine pancreatic microsomes lacked the capacity to process the \( \text{fl2} \) \( \alpha \)-zein.

Site-Specific Mutagenesis of the \( \text{fl2} \) Signal Peptide Restores Functional Activity

To test the hypothesis that processing of the \( \text{fl2} \) \( \alpha \)-zein was blocked because of the Val substitution at the –1 position of the signal peptide, we altered the coding sequence of the signal peptide so that the protein contained an Ala codon at that position. This construct pSP522R (encoding the Val-to-Ala revertant at the –1 position), and the control constructs pSP22.3 (encoding a normal \( \alpha \)-zein), pSP522 (encoding the \( \text{fl2} \) \( \alpha \)-zein), and pSP519 (encoding the \( \text{fl2} \) \( \alpha \)-zein lacking a signal peptide) were used as the templates for transcription in vitro of RNAs that could be translated and processed in wheat germ translation extracts. Figure 3 shows fluorographs of alcohol-soluble translation products produced from synthetic RNAs in the absence and presence of maize microsomes. Translation of synthetic pSP22.3, pSP522, and pSP522R RNAs in the absence of microsomes resulted in the synthesis of alcohol-soluble translation products with apparent molecular masses of 24 and 18 kD (Fig. 3, lanes 1, 5, and 9). The 24-kD translation products corresponded to unprocessed zein polypeptides. The 18-kD polypeptides most likely resulted from translation initiation at an internal Met (residue 45), because the polypeptides were not translocated into the microsomes (Fig. 3, lanes 3, 7, and 11). Protein products from synthetic transcripts of pSP22.3, a normal, 22-kD \( \alpha \)-zein clone, were processed when microsomes from normal endosperm were added, as evidenced by the shift in the apparent molecular mass of the translation products from 24 to 22 kD (Fig. 3, compare lanes 1 and 2). These proteins were also resistant to proteinase K digestion (Fig. 3, lane 3), so they had been fully translocated across the microsomal membranes. Inclusion of Triton X-100 to dissolve membranes during proteinase K treatment resulted in degradation of the polypeptides (Fig. 3, lane 4). Translation/translocation assays supplemented with microsomes from \( \text{fl2} \) endosperm yielded results similar to those obtained with normal microsomes (Fig. 3, compare A and B).

Translation of pSP522 (\( \text{fl2} \) \( \alpha \)-zein) mRNA in vitro resulted in the production of a 24-kD polypeptide in the presence or absence of microsomes (Fig. 3, lanes 5–7). The translation products were insensitive to proteinase K in the absence of Triton X-100 but were degraded in the presence of proteinase K and Triton X-100 (Fig. 3, compare lanes 7 and 8). This difference in proteinase sensitivity is consistent with the \( \text{fl2} \) \( \alpha \)-zein being translocated into the microsomes but not undergoing processing by signal peptidase. However, when the Val at position –1 of the signal peptide was converted to Ala (pSP522R), translation of the corresponding RNA in the presence of microsomes resulted in a 22-kD protein that was insensitive to proteinase K digestion (Fig. 3, lane 11). This product migrated through SDS-polyacrylamide gels identically to the normal zein (Fig. 3, compare lanes 3 and 11).

To determine whether pSP522R was processed to the correct size, we altered the \( \text{fl2} \) \( \alpha \)-zein such that it did not possess a signal peptide (pSP519). The translation product from synthetic pSP519 RNA had an apparent molecular mass of 22 kD (Fig. 3, lane 13). This translation product was identical in size to the processed form of pSP522R (Fig. 3, compare lanes 11 and 13). In addition, it was susceptible to proteinase K treatment (Fig. 3, lanes 15 and 16). Thus, this polypeptide was not translocated into the microsomes. Such a result was unexpected, because pSP522R has a functional signal peptide sequence.
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Figure 3. Processing of α-zeins in vitro. Fluorograph of alcohol-soluble products translated from synthetic mRNAs encoding α-zeins. A, Processing assays performed in the presence of microsomes from a normal maize line. B, Processing assays performed in the presence of microsomes from a fl2 maize line. Synthetic transcripts from pSP22.3, pSP522, pSP522R, and pSP519 were translated in the absence (lanes 1, 5, 9, and 13) or presence of maize microsomes and subsequently treated with proteinase K and Triton X-100, as indicated by − and + above the lanes. Molecular masses (in kD) of unprocessed and processed α-zeins are shown to the left.

These results clearly demonstrate that the fl2 α-zein gene encodes a protein with a signal peptide that could not be processed by microsomes isolated from normal or fl2 kernels. Furthermore, the presence of a Val residue at the −1 position of the signal peptide was responsible for the failure of the fl2 α-zein to be processed to its mature form.

The fl2 α-Zein Interacts Strongly with Microsomal Membranes

Retention of the signal peptide could have profound effects on the localization of proteins within the protein body. One possible consequence of signal peptide retention is the anchoring of the protein to the ER membrane. To assay for membrane association of the fl2 α-zein, we disrupted microsomes from translation reactions by treating them with alkaline sodium carbonate. The disrupted microsomal membranes were subjected to centrifugation on discontinuous Suc gradients. Soluble and membrane components from protein translocation reactions were then isolated and fractionated through SDS-polyacrylamide gels.

Figure 4 shows a fluorograph of soluble and membrane components isolated by discontinuous alkaline Suc centrifugation. Alkaline lysis of canine pancreatic microsomes containing radiolabeled alkaline phosphatase, a soluble protein, resulted in the release of the protein into the soluble fraction of the Suc gradient (Fig. 4, lanes 1 and 2). Small amounts of alkaline phosphatase were also detected in the membrane fraction. The residual membrane-associated alkaline phosphatase was most likely due to either nonspecific interactions with membrane proteins, incomplete lysis of the membranes, or contamination of the Suc overlays with low levels of soluble protein released from the microsomes. Examination of canine pancreatic microsomes containing the normal zein (pSP22.3) with a processed signal peptide revealed that the translation product was distributed approximately equally between the membrane and soluble fractions (Fig. 4, lanes 3 and 4). This result was consistent with the translation product being partially soluble under the experimental conditions. The co-fractionation of this polypeptide with membranes may have been due to the hydrophobic nature of zeins and the propensity of these proteins to aggregate. In contrast to both alkaline phosphatase and the normal zein, the fl2 α-zein was found predominantly in association with microsomal membranes (Fig. 4, lane 5 and 6). This pattern, which is clearly distinct from that of the normal zein, was suggestive of the fl2 α-zein being tightly associated with microsomal membranes and/or membrane proteins through its signal peptide. Alkaline lysis of microsomal membranes from immature fl2 kernels also resulted in complete retention of the fl2 α-zein in the membrane fraction (J.W. Gillikin and R.S. Boston, unpublished results). Alteration of the −1 position of the signal peptide by substitution of Ala for Val restored signal peptide processing and caused the protein to partition like the normal zein between soluble and membrane fractions (Fig. 4, compare lanes 3 and 4 with 7 and 8). Taken together, these results
strongly suggest that the fl2 α-zein was anchored to microsomal membranes by its signal peptide.

DISCUSSION

The fl2 α-zein possesses an unprocessed signal peptide (Coleman et al., 1995). In this study we used translation/translocation assays with heterologous and homologous microsomes, as well as site-directed mutagenesis, to investigate the molecular basis of the processing defect. Translation of synthetic fl2 α-zein mRNA in the presence of normal or fl2 maize microsomes or canine pancreatic microsomes (data not shown) showed that the fl2 α-zein was not processed by signal peptidase (Fig. 3). These results demonstrated that the inability of the mutant 22-kD α-zein protein to be processed by signal peptidase resided in the primary amino acid sequence of the α-zein and not in the fl2 processing machinery. Furthermore, the Ala-to-Val substitution at the -1 position of the signal peptide, not the other two amino acid alterations that occur in the fl2 α-zein, was responsible for the block in signal peptidase cleavage (Fig. 3; Coleman et al., 1995).

Coleman et al. (1995) proposed that the unprocessed signal peptide of the fl2 α-zein is inserted into the ER membrane and thereby serves as an anchor. Other examples of unprocessed signal peptides that perhaps anchor proteins to membranes have been reported (Schauer et al., 1985). In one case the uncleaved signal peptide of a 19-kD α-zein/alcohol dehydrogenase fusion protein anchored the majority of the fusion protein to canine pancreatic microsomal membranes (Shatters and Miernyk, 1991). Although the molecular basis for the processing block in the α-zein/alcohol dehydrogenase fusion protein is not known, the observation of membrane association is consistent with our findings that the fl2 α-zein partitions with canine pancreatic microsomal membranes and fl2 maize microsomal membranes when separated in alkaline Suc gradients (Fig. 4; J.W. Gillikin and R.S. Boston, unpublished results). Together, such data are strongly supportive of our previous hypothesis that the signal peptide is capable of anchoring the fl2 α-zein protein to the ER membrane in maize endosperm (Coleman et al., 1995).

Accumulation of the glycosylated 19-kD α-zein in fl2 protein bodies is not strictly linked to the fl2 mutation. This polypeptide also accumulates in other normal inbred lines of maize, namely B37 and A636 (J.W. Gillikin and R.S. Boston, unpublished results). The amino-terminal sequence of the glycosylated 19-kD α-zein shares 100% amino acid identity over the first 40 amino acids with the deduced amino acid sequence of the pMS2 zein clone (Langridge et al., 1985; GenBank accession no. X58700). That protein bodies from B37 and A636 contain the glycosylated 19-kD α-zein and appear identical to those of the normal W64A inbred suggests that the expression of the glycosylated 19-kD α-zein does not affect the assembly and packaging of zeins into protein bodies (F. Zhang and R.S. Boston, unpublished results).

The assembly and packaging of different classes of zeins during endosperm development results in highly organized protein bodies (Esen, 1986; Lending and Larkins, 1989). This process might easily be disrupted as a consequence of an α-zein that either is inserted into the membrane or is incapable of folding properly. In Figure 5, we present a model of protein-protein interactions that lead to the formation of normal (Fig. 5A) or fl2 (Fig. 5B) protein bodies. Both types of protein bodies appear to initiate from nuclei of β- and γ-zeins that contain little or no α-zein.
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(Lending and Larkins, 1989, 1992; Zhang and Boston, 1992). As normal protein bodies mature, aggregates of α-zeins would coalesce within the central region of the β- and γ-zein complex. In turn, the β- and γ-zeins would become localized at the periphery of the protein bodies (Lending and Larkins, 1989). In contrast, locules of α-zeins would appear not at the center but at the periphery of fl2 protein bodies, thereby displacing the β- and γ-zeins into the central region. This displacement would result in an alteration in the spatial distribution of the zein proteins within the protein body (Fig. 5B; Lending and Larkins, 1989; Zhang and Boston, 1992).

In addition to providing a plausible explanation for storage protein aggregation, this model also accounts for other features of the fl2 phenotype. The extent of protein body deformation is positively correlated with the number of gene doses of the fl2 allele (Lending and Larkins, 1992). Furthermore, in fl2-opaque-2 double mutants, which do not synthesize the 24-kD α-zein, only small protein bodies were observed, and these maintained a normal, spherical shape (Lopes et al., 1994).

Each of the phenotypic traits described above is consistent with our suggestion that the unprocessed 24-kD α-zein is responsible for the protein body morphology observed in fl2 kernels. The altered spatial distribution of the α-zeins within the protein body can be attributed to the presence of a membrane-anchored α-zein. Even though the three-dimensional structure of α-zeins has not been resolved, circular dichroism and molecular modeling suggest that α-zeins contain a high percentage of α-helical structure (Argos et al., 1982; Garratt et al., 1993). Garratt et al. (1993) suggested that each α-helix has six alternating hydrophobic and polar faces that interact with six neighboring helices. Such an arrangement would provide a framework for the oligomerization and dense packaging of zeins into protein bodies. It follows, then, that retention of unprocessed zeins at the ER membrane would provide foci for aggregation with other α-zeins and/or prevent subsequent internalization of these complexes into the protein body. The retention of α-zein aggregates at the periphery of the protein body would disrupt the normal spherical shape of protein bodies and alter the spatial distribution of the proteins within them. Thus, anchoring of the fl2 α-zein to the membrane provides a straightforward, albeit unproven, explanation of the protein body phenotype observed in the fl2 mutant.

In the fl2 mutant the α-zeins located at the periphery of the protein bodies would be exposed to the aqueous environment of the ER lumen and would adopt nonnative conformations. These α-zeins would normally be shielded from the aqueous environment of the ER lumen by a shell of β- and γ-zeins. We and others have observed elevated levels of BiP, GRP94, calnexin, calreticulin, and PDI in fl2 endosperm (R.L. Wrobel, J.W. Gillikin, and R.S. Boston, unpublished results; Fontes et al., 1991; Marocco et al., 1991; Li and Larkins, 1996). Numerous studies have shown that the induction of chaperones such as BiP and GRP94 is triggered by the presence of nonnative structures and/or misassembled proteins in the ER (Gething and Sambrook, 1992). The amount of BiP associated with protein bodies in fl2 endosperm is positively correlated with the number of copies of the fl2 gene (Zhang and Boston, 1992). Furthermore, BiP has been shown to be localized at the interface between the α-zeins and the ER membrane of fl2 protein bodies (Zhang and Boston, 1992), a location consistent with BiP being associated with nonnative protein structures exposed to the aqueous environment of the ER. By demonstrating that an unprocessed signal peptide is sufficient for membrane anchoring of an α-zein, we have provided a starting point for studies to identify signaling intermediates between aberrantly folded proteins and chaperone induction.

Changes in packing and arrangement of protein within protein bodies are not limited to the fl2 mutant. Defective endosperm B-30 and Mucronate, two other endosperm-specific mutants, have phenotypes similar to fl2 with respect to elevated molecular chaperone levels, altered protein body morphology, and dominance of mutant over normal alleles. These mutants also possess zeins with altered electrophoretic mobility through SDS-polyacrylamide gels (Salamin et al., 1983; J.W. Gillikin and R.S. Boston, unpublished results). Further characterization of the mechanism by which changes in structural proteins lead to generation of mutant phenotypes should increase our understanding of storage protein interactions as well as protein body biogenesis in seeds.

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