THE SIGNAL PEPTIDE CLEAVAGE SITE OF A B1 HORDEIN DETERMINED BY RADIOSEQUENCING OF THE IN VITRO SYNTHESIZED AND PROCESSED POLYPEPTIDE

by

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The major storage proteins which accumulate in the endosperm of the developing barley grain are the hordein polypeptides. They are synthesized on membrane-bound polysomes and co-translationally transported into the lumen of the ER and are ultimately deposited in the vacuole. The processing step, which accompanies transport across the ER membrane, was studied in vitro with the B1 hordein gene, λ hor2-4 in the expression plasmid pDS6. Microsomes were prepared from barley endosperm which transported and processed the B1 hordein polypeptide to a mature form with a high degree of efficiency. Because the B1 hordein was previously found to be NH₂-terminally blocked, the site of processing was unknown. The in vitro synthesized precursor and processed B1 hordein polypeptides were subjected to N-chlorosuccinimide peptide mapping and NH₂-terminal radiosequencing. The signal peptide cleavage site, predicted from the deduced amino acid sequence, was confirmed.

1. INTRODUCTION

The major storage proteins found in the endosperm tissue of barley seeds are the alcohol-soluble prolamins, hordein. Hordein is composed of at least 4 classes of proteins, namely B, C, D and γ hordein, which are coordinately synthesized during endosperm development (15, 30). The isolation and characterization of genomic clones encoding members of B (6), C (12) and γ hordein (8) polypeptide classes have made it possible to examine the synthesis, processing and structure of these storage proteins in greater detail.

The hordein polypeptides were first shown to be synthesized on membrane-bound polysomes, with primary translation products approximately 2 kd larger than the mature proteins, by BRANDT and INGVERSEN (4, 5). In vitro translation of isolated membrane-bound polysomes from barley endosperm, reconstituted with stripped microsomal membranes, revealed that hordein polypeptides are co-translationally processed and transported into the lumen of the endoplasmic reticulum (31).

In order to examine the co-translational transport and processing events in greater detail the

Abbreviations: DTT = dithiothreitol; kD = kilodalton; NCS = N-chlorosuccinimide; PK = proteinase K; PMSF = phenylmethylsulfonylfluoride; PVDF = polyvinylidene difluoride; RER = rough endoplasmic reticulum; RM = rough microsomes; RM-N = rough microsomes - nuclease treated; SDS-PAGE = sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

technique of coupled transcription-translation, with the *in vitro* synthesis of a single protein, was exploited. The B1 hordein gene λ hor2-4, characterized by BRANDT et al. (6), has been cloned in the expression vector pDS6 (29). The first 19 amino acids in the deduced amino acid sequence from the B1 hordein gene have the common features of a signal peptide, namely a charged lysine adjacent to the initiator methionine and a hydrophobic core sequence (17). However, the absence of NH₂-terminal sequence data for mature B1 hordein, due to NH₂-terminal blocking (26), leaves the predicted cleavage site unconfirmed. In the present work the B1 hordein gene, transcribed and translated *in vitro*, was shown to encode a precursor with a 19 amino acid signal peptide which was co-translationally processed by barley RER membranes to a mature form. The cleavage of the signal peptide was shown by N-chlorosuccinimide peptide mapping. The NH₂-terminus of the mature B1 hordein was determined by radiosequencing, taking advantage of new techniques in SDS-PAGE and protein sample preparation which reduce the occurrence of NH₂-terminus blocking reactions (22).

2. MATERIALS AND METHODS

2.1. Plant Material

Barley plants (*Hordeum vulgare* L.) cv. Bomi were grown in the field. Spikes were harvested 20 days after anthesis, immediately frozen in liquid nitrogen, and stored at -80 °C.

2.2. Isolation and translation of poly A⁺RNA

Total RNA was prepared from developing barley endosperms according to AVIV and LEDER (2) with minor modifications. The frozen ground tissue was extracted in 0.1 M Tris-HCl pH 9.0, 10 mM EDTA, 25 mM DTT and 1% SDS together with an equal volume of phenol, and the RNA precipitated from the aqueous phase with 2 M LiCl at -20 °C.

Poly A⁺RNA was isolated from the total RNA preparation by Poly(U)-Sephacrose 4B (Pharmacia) affinity chromatography and translated in a wheat germ extract (24), as described by BRANDT and INGVERSEN (5).

2.3. Immune precipitation of hordein polypeptides from cell-free translations

Polyclonal IgG antibodies raised against hordein from *Hordeum vulgare* cv. Bomi by DIERKS-VENTLING and COZENS (11) were a gift. Immunoprecipitation of hordein polypeptides from poly A⁺RNA translations in the wheat germ system was performed according to ANDERSON and BLOBEL (1). Translation assays of 25 μ l, solubilized in 1% SDS, were incubated with 50 μ g anti-hordein IgG and the antigen-IgG complex coupled to the immunoabsorbant Protein A-Sepharose CL-4B (Pharmacia) as described (1). The antigens were subsequently released by boiling the Protein A-Sepharose beads in 20 μ l of 125 mM Tris-HCl pH 6.8, 2.5% SDS, 10% glycerol and 50 mM DTT and then acetylated with 70 mM iodoacetamide at 37 °C. The translation products were analysed by 10-15% gradient SDS-PAGE (19) and fluorography (10).

2.4. Preparation of barley microsomal membranes

Rough endoplasmic reticulum (RER) was isolated from developing barley endosperms according to a rapid procedure developed for maize endosperm tissue by BURR and BURR (7). The homogenized endosperm tissue, after a 10 min centrifugation at 3000 $\times$ g to remove cell debris and starch, was layered on a 35-70% sucrose gradient and centrifuged at 160,000 $\times$ g for 2 hours. The RER banded in the density gradient at 1.37 M sucrose and was pelleted as described (7).

The RER microsomes (RM), suspended in 10 mM Hepes pH 7.6, 1 mM CaCl₂, 20% glycerol and 1 mM DTT, were then treated with 15 U/ml of nuclease S7 (Boehringer Mannheim) for 10 min at 23 °C. Nuclease activity was terminated by addition of EGTA to 4 mM and the microsomes (RM-N) pelleted by a 30 min centrifugation at 100,000 $\times$ g. Microsomes (RM and RM-N) were suspended in 10 mM Hepes pH 7.6, 1 mM EGTA and 1 mM DTT, at 100A₂₆₀ U/ml and stored at -196 °C in aliquots for single use.

2.5. Plasmid constructs and preparation of plasmid DNA

The B1 hordein gene, λ hor2-4 (6), was cloned into the expression plasmid pDS6 between the EcoRI and HindIII sites of the polylinker (S.M. MADRID, manuscript in preparation). The plasmid (pDS-hor2-4) was propagated in the *E. coli* host DH5 α (16) in medium selective for ampicillin resistance. Plasmid DNA was prepared according to BIRNBOIM and DOLY (3), followed by CsCl/ethidium bromide equilibrium centrifugation (23).

2.6. Coupled transcription-translation

The B1 hordein gene in pDS6 was transcribed with *E. coli* RNA polymerase (Promega) in the presence of 7mGpppA to give capped mRNA and subsequently translated in the wheat germ system (24) as described by STUEBER et al. (29). The wheat germ extract was given an additional 30,000 $\times$ g centrifugation for 10 min immediately prior to the translation assay and the amount added was carefully titrated to minimise polypeptide processing and transport activity of wheat germ origin. With the exception of translations performed for radiosequencing, the polypeptides were synthesized with 20 μ C of ³⁵S methionine (sp. act. 1100 Ci/mmol). RM-N microsomes were included in the translations where indicated at a final concentration of 7 A₂₆₀ U/ml.

The reticulocyte lysate and dog pancreas microsomes were purchased from Amersham and translation of B1 hordein mRNA was performed according to Amersham protocols.

Post-translational proteinase K (PK) digestions were performed on 10 μ l aliquots of each 25 μ l translation assay, by addition of 1 μ l of PK (3 mg/ml) and a 45 min incubation on ice. The digestions were terminated by addition of 1 μ l of 2% phenylmethylsulfonylfluorid (PMSF) in isopropanol.

The translation products (3 μ l aliquots) were analysed by 18% SDS-PAGE in the high-molarity Tris buffer system (14) on 0.75 mm thick Mini-PROTEAN II slab gels (Bio-Rad). The gels were subsequently fixed and prepared for fluorography (10).

2.7. Peptide mapping with N-chlorosuccinimide

B1 hordein polypeptides, synthesized by coupled transcription-translation of the B1 hordein gene (see 2.6), were separated on a 0.75 mm 10% high-molarity Tris SDS-PAGE, and cut out from the dried gel, after localization by autoradiography. The gel slices were incubated at room temperature by shaking with the following solutions for N-chlorosuccinimide (NCS) cleavage (20): 1. Two 10 min washes in 10 ml H₂O, 2. Two 10 min washes in 10 ml urea/H₂O/CH₃COOH (1g/1ml/1ml), 3. 30 min wash in 5 ml 0.015 M NCS in urea/H₂O/CH₃COOH, 4. Two 10 min washes in 10 ml H₂O, 5. Three 30 min equilibrations in 10 ml electrophoresis sample buffer: 0.0625 M Tris-HCl pH 6.8, 3% SDS, 10% glycerol and 15% β -mercaptoethanol.

The NCS cleavage products were separated on an 18% high-Tris SDS-PAGE, loading the gel slices into the slots of a 1 mm thick Mini-PROTEAN slab gel. The gel was incubated in 1 M sodium salicylate in 50% methanol and dried for autoradiography.

2.8. B1 hordein radio-sequencing

Precursor and mature B1 hordein polypeptides were synthesized in vitro (see 2.6) in the wheat germ system with or without barley microsomes. The translation products were labelled with 20 μ C of ³H isoleucine (sp. act. 110 Ci/mmol) per 25 μ l assay. The B1 hordein polypeptides were purified for NH₂-terminal sequencing using procedures, described below, to avoid NH₂-terminal blockage resulting from the conversion of glutamine to pyroglutamate (6), and to attain high peptide recovery for radio-sequencing (22). The polypeptides were first separated by Tricine-SDS-PAGE as described by SCHAGGER and VON JAGOW (27).

10 μ l aliquots of the translation products were incubated for 30 min at 40 °C with sample buffer: 50 mM Tris-HCl pH 6.8, 12% glycerol, 4% SDS, 2% β -mercaptoethanol and 0.01% Serva Blue G. The slab gels (1 mm thick) comprising a 15 cm separating gel (7.5% T, 3% C) and a 3 cm stacking gel (4% T, 3% C), were stored for 24 hours at room temperature to maximise acrylamide polymerization. Acryl-

amide stock solutions were stored over mixed-bed ion exchange resin (Amberlite MB1 BDH) and the SDS was re-crystallized (18). The gel was pre-electrophoresed for 12 hours at 30 mA in separating gel buffer (0.5 M Tris-HCl pH 8.45, 0.1% SDS) in the presence of sodium thioglycolate, a charged nucleophilic scavenger, to eliminate residual acrylamide monomer (18, 22). Pre-electrophoresis is possible since the stacking and separating gels have the same Tris buffer.

Electrophoresis was performed at room temperature with 1 mM sodium thioglycolate in the cathode buffer. The samples were run into the stacking gel at 30V and then run at 30mA for 16 hours. The B1 hordein polypeptides were transferred to the chemically inert polyvinylidene difluoride (PVDF) membrane (Immobilon, Millipore) by semi-dry electroblotting (JKA Biotek, Denmark), according to (22). The low % acrylamide separating gel was selected to enhance the transfer efficiency. The gel was washed for 10 min in transfer buffer, 50 mM borate pH 8.4, 10% MeOH. The PVDF membrane was wetted in 100% MeOH prior to electrotransfer for 2 hours at 4 °C with 0.4mA/cm². The PVDF membrane was rinsed in H₂O and dried under a stream of nitrogen. The hordein polypeptides were located by autoradiography and the excised areas of the PVDF membrane taken for sequencing on an Applied Biosystems sequencer model 477A. The sequencing runs were performed by Dr. KAREN G. WELINDER and ANNE BLICHER. The products of 14 cycles of NH₂-terminal Edman degradation were then counted in a scintillation counter.

3. RESULTS

3.1. In vitro synthesis and processing of hordein polypeptides

Poly A⁺RNA prepared from developing Bomi endosperm was translated in the wheat germ

system with ³⁵S methionine, and the anti-hordein IgG immune precipitates analysed by SDS-PAGE (Fig. 1, lane 2). The primary hordein translation products have a mobility similar to the B, C and D hordein polypeptides characteristic of Bomi endosperm (25). However, as previously demonstrated (5), the hordein polypeptides are synthesized as larger-sized precursors, which are co-translationally processed to a mature form in the presence of the E.R. (e.g. Fig. 1, lane 11). The molecular weight shift is most apparent for the C hordein polypeptides. To examine the processing event in greater detail, rough and stripped microsomes were prepared according to BURR and BURR (7). The rough microsomes (RM) were first shown to be translationally active (Fig. 1, lane 11) and then stripped of most of their endogenous mRNA activity (RM-N) by micrococcal nuclease treatment (lane 7, 9). Reconstitution of these microsomes with endosperm poly A⁺RNA results in the synthesis of mature hordein polypeptides (lanes 3, 5). The hordein polypeptides are co-translationally transported into the lumen of the microsomes and have thereby become resistant to proteinase K digestion (lanes 4, 6, 12). The translation activity of the RM assay (lane 11) was only 30% of that of the poly A⁺RNA with RM-N assay (lane 5), as judged by TCA precipitable counts, and hence the amount of protected hordein polypeptides was proportionally less (lane 12). The addition of microsomes to the assay was generally seen to be inhibitory to translation.

3.2. Coupled transcription-translation of a B1 hordein gene

The coding region of a B1 hordein gene, λ hor2-4 (6) was cloned into the expression plasmid pDS6 (see section 2.5). The cloned

Figure 1. Hordein in vitro translation products of barley endosperm poly A⁺RNA and processing by ER microsomes. Poly A⁺RNA (0.06 A₂₆₀U) was translated in a 25 μ l wheat germ assay (lane 2). Barley RM (0.23 A₂₆₀U) were similarly translated in vitro (lanes 11, 12) and after nuclease S7 treatment as RM-N (0.35 A₂₆₀U, lanes 7, 8; 0.17 A₂₆₀U, lanes 9, 10). Poly A⁺RNA was translated after reconstitution with RM-N (0.17 A₂₆₀U, lanes 3, 4; 0.35 A₂₆₀U, lanes 5, 6). A wheat germ translation without template is included (lane 1). Post-translational proteinase K treatment of aliquots from each assay is as indicated. The hordein polypeptides in each assay were immunoprecipitated and analyzed by 10-15% SDS-PAGE and fluorography. The film exposure for lanes 3-12 was approximately 3 fold longer than lanes 1 and 2.

Figure 2. A B1 hordein polypeptide synthesized *in vitro* and processed by barley microsomes. A. mRNA, transcribed from 0.5 μ g pDS-hor2-4, was translated in the wheat germ system (lanes 1, 2) and with the addition of 7 A_{260} /ml of RM-N (lanes 3, 4). B. The concentration of RM-N added to the translation varied: 12 A_{260} /ml (lanes 1, 2), 6 A_{260} /ml (lanes 3, 4), 3 A_{260} /ml (lanes 5, 6). Aliquots of each assay, given a post-translational proteinase K treatment, are in A: lanes 2, 4 and B: lanes 2, 4, 6. The translation products were analyzed by 18% (A) or 20% (B) SDS-PAGE and fluorography.

fragment extends 14 bp 5' upstream of the ATG translation start from the HpaI site and 16 bp 3' downstream of the TAA termination codon at the XbaI site. Capped mRNA was transcribed as described in section 2.6 and translated in the wheat germ system. Precursor B1 hordein was the single major translation product (Fig. 2A, lane 1) and was sensitive to proteinase K diges-

tion (lane 2). Co-translational processing and transport of the mature form occurred in the presence of barley microsomes (lane 3) and led to proteinase K insensitivity (lane 4). Processing and transport of the B1 hordein polypeptide was dependent on microsome concentration in the assay (Fig. 2B), such that 12 A_{260} /ml RM-N gave 90% processing, based on the relative inten-

Figure 3. A B1 hordein polypeptide synthesized *in vitro* and processed by dog pancreas microsomes. A. mRNA, transcribed from 0.4 μ g pDS-hor2-4, was translated in a 30 μ l reticulocyte lysate assay (lanes 1, 2) and with the addition of 10 μ l of dog pancreas RM-N (lanes 3, 4). Aliquots of each assay were given a post-translational proteinase K treatment (lanes 2, 4). The translation products were analyzed by 18% SDS-PAGE and fluorography. B. Translation products, containing precursor and processed B1 hordein (A: lanes 1 and 3), were combined and replicate samples separated on an 18% SDS-PAGE containing a 0-8 M urea gradient.

sity of the two bands of approximately 31 and 29 kD, in the autoradiogram (lane 1, 2), while 3 A₂₆₀U/ml RM-N gave 50% processing to the mature form (lane 5, 6).

Dog pancreas microsomes were similarly able to co-translationally process and transport B1 hordein polypeptides synthesized in the reticulocyte lysate system (Fig. 3A). Processing to the

mature B1 hordein was 100% at microsome concentrations recommended by the manufacturer (lane 3, 4).

Electrophoretic conditions which might improve the separation of the precursor and the mature B1 hordein were investigated. Polyacrylamide concentrations of less than 18% (see Fig. 1) failed to resolve the two forms. The influence

Table I. Predicted N-chlorosuccinimide cleavage products of precursor and mature B1 hordein

of the denaturing agent urea, on their electrophoretic mobility, was analysed on a transverse 0-8 M urea gradient gel prepared in the high-Tris SDS-PAGE system with a 5 M urea sample buffer (14). Urea was not found to improve the resolution of the two forms (Fig. 3B).

3.3. B1 hordein processing examined by peptide mapping

The peptide fragments released by N-chlorosuccinimide (NCS) cleavage of in vitro synthesized B1 hordein were analysed to confirm the co-translational processing event. The B1 hordein gene in pDS6 was transcribed and translated in vitro in either the wheat germ system, with or without barley microsomes, or in the reticulocyte lysate with the addition of dog pancreas microsomes. The products were separated by 10% SDS-PAGE, which does not resolve precursor and mature B1 hordein, and located by autoradiography. The gel pieces containing the hordein polypeptides were treated with NCS according to LISCHWE and OCHS (20) and analysed by 18% SDS-PAGE and fluorography.

Under acidic conditions NCS specifically cleaves tryptophanyl peptide bonds by oxidative chlorination (28). Precursor B1 hordein, labelled with ³⁵S methionine, should be cleaved after the single tryptophan residue at position 61 to yield ³⁵S labelled peptides of 6.9 kD and 23.8 kD (Table I). The 6.9 kD peptide has a single

methionine residue at the NH₂-terminus. Signal peptide cleavage of the B1 hordein is predicted after residue 19 giving a 2 kD signal peptide and the mature B1 hordein of 28.8 kD. NCS cleavage of mature B1 hordein should yield an unlabelled N-terminal 4.9 kD peptide and a labelled 23.8 kD peptide. Since NCS cleavage of denatured proteins is partial (20), at least 50% of the precursor and mature B1 hordein polypeptides will remain uncleaved.

Analysis of the NCS cleavage products of precursor B1 hordein (Fig. 4 lanes 1, 5) showed the predicted 50% cleavage to 23.8 kD and 6.9 kD peptides [the latter migrating with an abnormally high mobility]. The relative labelling intensities of the products correlated with their methionine content (Table I). 40% to 60% processing of the precursor to mature B1 hordein by barley microsomes led to a reduced yield of the 6.9 kD peptide (lanes 2, 4), which was completely absent when the precursor had been digested with proteinase K (lane 3). Where in vitro processing of B1 hordein was 100%, as was the case with dog microsomes, the 6.9 kD peptide was also absent (lanes 6, 7).

3.4. Radiosequencing a B1 hordein polypeptide

Precursor and mature B1 hordein polypeptides were radiosequenced to determine the precise signal peptide cleavage site. Since purified B1 hordein was found to be blocked to Edman degradation (26), particular care was

taken to select protocols which minimized the occurrence of NH₂-terminal blocking of proteins. The B1 hordein gene in pDS6 was transcribed and translated in the wheat germ system, with and without barley microsomes. The polypeptides were synthesized with ³H-isoleucine to enable the N-terminal sequence of precursor and mature B1 hordein polypeptides to be distinguished. The translation products were separated by SDS-PAGE, blotted onto PVDF paper and subjected to 14 cycles of automated Edman degradation (Fig. 5). In the presence of barley microsomes processing was approximately 67%, although it was not possible to separate the precursor and mature forms for

sequencing. The radiosequence of these products (Fig. 5B) thus reflects a combination of precursor and mature B1 hordein polypeptides. The contribution of the precursor in Fig. 5B can be removed by proportional subtraction of Fig. 5A to show the amount of radioactivity due to the mature B1 hordein sequence (Fig. 5C). The radiosequence thus confirmed the predicted site of signal peptide cleavage.

4. DISCUSSION

When poly A⁺RNA from barley endosperm is translated in vitro, the co-translational transport of the hordein polypeptides across the microso-

Figure 4. N-Chlorosuccinimide cleavage of a B1 hordein polypeptide. mRNA, transcribed from 0.5 µg pDS-hor2-4, was translated in either the wheat germ system (lane 1) and with added barley RM-N (7 A₂₆₀U/ml, lanes 2, 3; 3 A₂₆₀U/ml, lane 4) or in the reticulocyte lysate (lane 5) and with added dog pancreas RM-N (lanes 6, 7). Post-translational proteinase K treatment of aliquots of some assays are included (lanes 3, 7). The translation products were separated by 10% SDS-PAGE and the gel fragments containing B1 hordein polypeptides were subjected to NCS cleavage and the products analyzed by 18% SDS-PAGE and fluorography. A longer film exposure of part of the gel is shown below.

mal membrane, and their consequent protection from protease digestion, can be clearly demonstrated. However a change in SDS-PAGE mobility, as a result of signal peptide cleavage, is clearly apparent only in the C hordein polypeptides. Characterization of a C hordein gene and comparison with the NH₂-terminal sequence of mature C hordein polypeptides, has confirmed the presence of a cleavable signal peptide (12).

In order to study the processing events which accompany the transport of B hordein across the ER, the B1 hordein gene *lhör 2-4* (6) cloned in the vector pDS6 was expressed *in vitro*. The major translation product, synthesized in either the wheat germ system or the reticulocyte lysate, was the B1 hordein polypeptide. Translation in the presence of either barley microsomes or dog pancreas microsomes gave a second form, protected from protease attack and of lower molecular weight.

Electrophoretic resolution of the precursor and processed forms was limited. A variety of denaturing and reducing reagents was tested, together with different acrylamide concentrations, without significantly improving their separation. Protein modification, which may accompany protein transport across the ER and

Figure 5. B1 hordein radiosequencing. pDS-hor2-4 was transcribed and translated *in vitro* in the wheat germ system and with added barley RM-N. The synthesized B1 hordein polypeptides, labelled with ³H-isoleucine, were separated by SDS-PAGE, electroblotted onto PVDF membrane and subjected to 14 cycles of Edman degradation. The NH₂-terminal radiosequence of the B1 hordein mRNA primary translation product is shown in A and the expected amino acid sequence given at the top of the panel. The position of the radioactive isoleucines is marked with asterisks. Panel B presents the radiosequence of a mixture of 67% processed translation product and 33% precursor. The two expected amino acid sequences and the position of the labelled isoleucine residues is given at the top of the panel. The signal peptide cleavage point is indicated with an arrow. By removing the contribution of the precursor sequence from the histogram of B by proportional subtraction, the mature B1 hordein sequence is derived and presented in panel C.

lead to an increase in molecular weight, includes the addition of oligosaccharide side chains to the protein backbone. In all eucaryotes, N-glycosylation occurs at asparagine residues in the consensus sequence Asn X Ser/Thr (13). Since this sequence is absent from the B1 hordein, the absence of a significant mobility shift of the processed B1 hordein cannot be attributed to this common type of glycosylation. One may conclude that SDS solubilized precursor and processed B1 hordein polypeptides have a rather similar electrophoretic mobility.

The location of the processing event was determined by N-chlorosuccinimide peptide mapping. NCS cleavage of ³⁵S methionine-labelled precursor B1 hordein released a 6.9 kd peptide labelled with a single NH₂-terminal methionine, as predicted from the deduced sequence. Signal peptide cleavage of the B1 hordein, as the co-translational processing event, was indicated by the disappearance of this labelled NH₂-terminal peptide. To determine the exact processing site, the precursor and processed B1 hordein polypeptides, synthesized in the wheat germ system and in the presence of barley microsomes, were subjected to NH₂-terminal radiosequencing. Although B1 hordein processing by barley microsomes was not 100% efficient and the proteins subjected to sequencing were a mixture of precursor and mature forms, it was considered desirable to analyse the products of an in vitro processing event which most closely resembled their in vivo synthesis. Comparison of the radiosequence data from precursor and processed B1 hordein translation products confirmed the presence of a 19 amino acid signal peptide with co-translational cleavage to the mature form.

The combination of in vitro transcription of the B1 hordein gene and subsequent translation and polypeptide transport into microsomes derived from barley endosperm ER, provides a system for examining the components which contribute to the transport and processing events. The presence of signal recognition particles, primary components in the recognition of signal peptides of secreted proteins and their subsequent association with the ER, has been demonstrated in plants (9). However, other membrane components, such as the signal

recognition particle receptor (21), have yet to be identified in plant membranes.

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