TRANSFER OF IN VITRO SYNTHESIZED BARLEY ENDOSPERM PROTEINS INTO THE LUMEN OF THE ENDOPLASMIC RETICULUM

by

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Microsomes were prepared from 20 day old Bomi barley endosperm by sucrose density gradient centrifugation. Electron microscopy revealed the presence of ribosome-studded vesicles in the material banding at the 1.75-2.26 M sucrose interface. The isolated microsomes were active in the wheat-germ cell-free protein synthesizing system. Hordeins were present among the in vitro synthesized products and were identified by their solubility in 55% isopropanol and by their co-migration with native hordeins on SDS-polyacrylamide gels. The microsome- and polysome-directed, in vitro synthesized hordeins were analysed before and after chymotrypsin treatment by SDS-polyacrylamide gel electrophoresis. The hordeins were degraded when polysomes were used as a template. However, hordeins synthesized on microsomes showed significant protection from proteolysis. This protection could be abolished by treatment with membrane-solubilizing detergents. The reported experiments show that hordeins synthesized on microsomes were discharged vectorially into the lumen of the microsomes.

1. INTRODUCTION

A characteristic feature of endosperm development in cereal grains is the synthesis of hydrophobic storage proteins, which may comprise as much as 50% of the proteins present in the mature grain. Electron microscopy of endosperm tissue has revealed that the storage proteins are located in protein bodies, and zein- and hordein-rich bodies have been isolated and characterized from maize and barley respectively (7, 11). Techniques for the in vitro synthesis of proteins have recently been exploited in the synthesis of various plant proteins and in

particular the storage proteins, zein (13), hordein (4) and oat globulin (14). Bearing in mind the hydrophobic nature of many plant storage proteins and their confinement to membrane-bound vacuoles, it is significant that the main polysome fractions active in storage protein synthesis are found among the membrane-bound polysomes (4, 6, 13).

The synthesis and transport of proteins has become a subject of considerable interest, particularly in animal cells. Some proteins are transferred from the site of synthesis into intracellular organelles e.g. lysosomes, perox-

isomes and mitochondria, whereas other proteins such as hormones and immunoglobulins are excreted. It appears to be a general phenomenon that excreted proteins are synthesized on membrane-bound polysomes (1, 5, 18). The problems associated with transporting proteins across semi-permeable membranes have led to the hypothesis that proteins synthesized on membrane-bound polysomes are transferred across the membrane prior to the release of the nascent chain and assumption of its secondary and tertiary structure with the aid of a signal sequence of amino acids (1). The latter is then removed by site specific proteolysis.

Techniques to demonstrate the concurrent synthesis and transport of proteins in vitro in animal systems (1, 2, 12) have been utilized for the study of hordein synthesis in barley endosperm. Rough microsomes have been prepared, characterized and the in vitro synthesis of hordeins and their subsequent vectorial discharge into the microsomal lumen demonstrated.

2. MATERIALS AND METHODS

2.1. Chemicals

HEPES, ATP (disodium salt), GTP (sodium salt), creatine phosphate (disodium salt), creatine kinase (E. C. No. 2.7.3.2.), DTT, spermidine phosphate, α chymotrypsin and L-amino acids were purchased from Sigma Chemicals. (35 S)-Methionine (specific activity approx. 900 mCi/mmol) was obtained from Radiochemical Center, Amersham, U.K.

HKM buffer A 20 mM HEPES, pH 7.6, 100 mM-K acetate, 50 mM-Mg acetate, 0.2 M-sucrose and 2 mM-DTT

HKM buffer B 20 mM HEPES, pH 7.6, 100 mM-K acetate, 5 mM-Mg acetate and 2 mM-DTT

2.2. Plant material

Barley plants (*Hordeum vulgare* L.) cv. Bomi were grown in pots in a growth chamber under

a diurnal cycle of 16 h light, 16°C, and 8 h dark, 12°C at 65% relative humidity. Spikes were harvested 20 days after anthesis.

Fresh commercial wheat-germ was obtained from Ringsted Dampmølle, Denmark, and stored in polyethylene bags at -70°C.

2.3. Rough microsome preparation

Rough microsomes were prepared from 20 day old Bomi barley endosperm by a procedure adapted from that of BLOBEL and DOBBERSTEIN (1). Endosperms were squeezed out from 10-15 freshly harvested spikes and ground to a powder with a mortar cooled with liquid nitrogen. The ground endosperms were suspended in 20 ml HKM buffer A, and all subsequent operations were performed at 2-4°C. The suspensions were further homogenized three times for 30 seconds in a Potter-Elvehjem homogenizer operated at 3250 rpm. The homogenates were centrifuged at 500 g for 10 min in a Sorvall SS-34 rotor, to remove most of the starch, cell walls and unbroken cells. The supernatants were then centrifuged at 37,000 g for 10 min to pellet the rough endoplasmic reticulum. The pellets also served as a starting material for detached polysome preparation (section 2.4.).

Rough microsomes were prepared from the 37,000 g pellets by resuspension in 10 ml of 2.26 M-sucrose and flotation in a discontinuous gradient consisting of 10 ml portions of 1.75, 1.50 and 0.20 M-sucrose, all solutions made up in HKM buffer B. After centrifugation at 96,000 g_{av} for 16 h on a Beckman SW 27 rotor two bands were obtained, one at the 1.75-2.26 M interface (band I) and one at the 1.50-1.75 M interface (band II). They were both removed with a hypodermic syringe and diluted with an equal volume of HKM buffer B. The isolates were further purified by layering 7 ml of suspension on top of a 1.3 M-sucrose (in HKM buffer B) cushion and centrifuging at 165,000 g_{av} for 1 h in a Beckman 50 Ti rotor. The pellets were suspended in 0.2 M-sucrose HKM buffer B at a

Abbreviations: DOC = deoxycholate; DTT = dithiothreitol; HEPES = N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid; HKM = HEPES-K-Mg-acetate buffer (see 2.1.); Met = methionine; SDS-PAGE = Sodium dodecyl sulfate polyacrylamide gel electrophoresis; TCA = trichloroacetic acid.

concentration of 1 A_{260} unit/10 μ l and stored in liquid nitrogen.

2.4. Polysome isolation

Detached polysomes were prepared from 20 day old Bomi barley endosperm as described previously (4).

2.5. Electron microscopy of the material contained in density gradient bands I and II

The pellet obtained after the sucrose wash of bands I and II (see section 2.3.) was fixed in HKM buffer B containing 2% glutaraldehyde at 0°C and pelleted by centrifugation at 105,000 g_{av} in a Beckman 50 Ti rotor for 30 min. The pellet was resuspended in 2-3 drops of 45°C HKM buffer B containing 2% agar and then immediately applied to a precooled microscope slide. The solidified agar was then cut into 1 mm³ cubes and the embedded material post-fixed with 2% OsO₄ in HKM buffer B for 1 h. The fixed cubes were then washed in water and stained with 0.5% uranylacetate for 2 h at 20°C. Dehydration, final embedding in epoxyresin and sectioning were performed as described earlier (11).

2.6. Cell-free protein synthesizing system

An S-30 wheat-germ extract was prepared as described by MARCU and DUDOCK (15) and used for cell-free protein synthesis.

The standard reaction mixture for incorporation of amino acids into proteins contained 5 μ l S-30 wheat-germ extract, 1-2 A_{260} unit of rough microsomes or polysomes, 1 mM-ATP, 200 μ M-GTP, 25 mM-HEPES pH 7.6, 2.0 mM-Mg acetate, 100 mM-K acetate, 10 μ Ci ³⁵S-Met (specific activity approx. 900 mCi/mmol) and 20 μ M of each of the other 19 amino acids in a final volume of 50 μ l.

The reaction mixture was incubated at 27°C for 60 min, and to measure the incorporation of ³⁵S-Met into TCA precipitable proteins 5 μ l aliquots were pipetted onto Whatman 3MM filter paper discs impregnated with 10% TCA. The discs were heated to 100°C for 10 min in 10% TCA containing 10 mM-Met, washed in 5%

TCA and dried by successive dipping in ethanol (96%), ethanol:diethylether 1:1, and diethylether. The radioactivity precipitated on the discs was counted in a toluene based scintillator, containing 0.4% omnifluor (New England Nuclear), in a Beckman LS 3155T liquid scintillation spectrometer.

2.7. SDS-PAGE and fluorography of polypeptides synthesized in the cell-free system

Cell-free protein synthesis was terminated by the addition of 1 ml 10% TCA, 10 mM-Met and the hordein translation products were extracted into 55% isopropanol containing 2% mercaptoethanol, 10 mM-Met, 10 mM-EDTA and 100 μ g/ml carrier hordein. After removal of insoluble proteins by centrifugation, the isopropanol extracts containing the hordeins were dried down and prepared for electrophoresis. The extracts were suspended in 20 μ l 0.1 M-Tris base, 1 μ l 1% bromophenol blue, 20 μ l 30% sucrose: 5% SDS and 5 μ l 0.1 M-DTT, and boiled for 1 min. 10 μ l of 0.2 M-iodoacetamide were added to each sample 2 h prior to electrophoresis. Electrophoresis was performed according to the procedure of CHUA and BENNOUN (8). Native ¹⁴C-labelled hordeins were co-electrophoresed as markers.

The gels were then prepared for fluorography as described by BONNER and LASKEY (3), and exposed to RP Royal X-omat film (Kodak) at -70° for 4-6 days, and then developed.

2.8. Preparation of in vivo ¹⁴C-labelled hordeins

Five barley spikes were cut 10 cm below the ear 14 days after anthesis. The spikes were placed in a solution of 200 μ Ci [U-¹⁴C]-sodium acetate in 1 ml water. After the acetate had been taken up, the spikes were left for 14 days in a nutrient solution. The seeds were then collected and homogenized in 50 ml 55% isopropanol with 2% mercaptoethanol. After stirring for 1 h, the hordein extract was recovered by centrifugation at 10,000 g for 10 min. The extract was then dialyzed overnight against water and freeze dried. Carrier hordeins were isolated in a similar way from mature non-radioactive seeds.

Figure 1. Rough microsomes from Bomi barley endosperm. Rough microsomes were prepared from 20 day old Bomi barley endosperm by homogenizing tissue in HKM buffer A. The homogenate was centrifuged at 500 g for 10 min in a Sorvall SS-34 rotor. The supernatant was then centrifuged at 37,000 g for 10 min in the same rotor to pellet the endosperm membrane fraction. The pellet was resuspended in 10 ml of 2.26 M -sucrose and overlaid with 10 ml portions of 1.75, 1.5, and 0.2 M -sucrose, all containing HKM buffer B, and centrifuged at 96,000 g_{av} for 16 h in a Beckman SW 27 rotor. The material banding at the 1.75-2.26 M -sucrose interface (band I) and at the 1.5-1.75 M -sucrose interface (band II) was isolated, fixed, stained, embedded and sectioned for electron microscopy. A, B, C are from band I and D from band II. Magnification: A, $\times 43,500$; B, $\times 106,000$; C, $\times 129,000$; D, $\times 43,500$.

3. RESULTS

3.1. Isolation and characterization of barley endosperm rough microsomes

Rough membrane fractions were obtained from Bomi endosperm, banding at two positions on a discontinuous sucrose gradient, namely at the interfaces of 1.5-1.75 M (band II) and 1.75-2.26 M (band I). The yields are shown in Table I.

Electron microscopy of sections through a suspension of the material in band I revealed a high content of microsomes i.e. endoplasmic reticulum derived vesicles with polysomes attached to their outer surface (Figure 1A). Some electron dense material was present inside the microsomes close to the membrane (Figures 1B and C).

Electron microscopy of the material in band II showed a mixed population of microsomes, smooth membrane vesicles and membrane vesicles containing ribosomes (Figure 1D). Since

Table I

Yield of rough microsomes from Bomi barley endosperm. Rough microsomes from 20 day old Bomi barley endosperm were isolated as described under Materials and methods (2.3.). The ratio of the absorption at 260 nm to that at 280 nm is lower than the value of 1.8 found with purified polysomes (4). This is expected because of the protein absorption contributed by the membranes.

	Yield	Ratio
	A_{260} units/10 spikes	A_{260}/A_{280}
Band I	43.5	1.24
Band II	36.0	1.22

Table II

Cell-free protein synthesis directed by rough microsomes from Bomi barley endosperm. Rough microsomes from 20 day old Bomi barley endosperm were isolated and assayed in the standard reaction mixture as described under Materials and methods (2.6.).

Microsome Fraction	S-30 Wheat-germ	cpm/A ₂₆₀ unit	Stimulation over wheat-germ control
Band I	+	602,900	5.9
Band II	+	605,000	5.9
0	+	102,000	
Band I	0	50,900	
Band II	0	35,800	

microsomes were present in band II, it was also studied as a template in the in vitro incubations.

3.2. Cell-free protein synthesis with barley endosperm rough microsomes

The rough microsomes isolated from Bomi endosperm were incubated in the standard reaction mixture at 27°C for 40 min. The in-

corporation of ³⁵S-Met into hot 10% TCA insoluble material in the presence or absence of S-30 wheat-germ extract was determined (Table II).

The addition of membrane-bound polysomes, associated with the two rough microsome fractions of Band I and II, to the wheat-germ cell-free system caused a 6 fold stimulation of protein synthesis. Translation on the membrane-bound polysomes was largely dependent on the wheat-germ extract.

The in vitro translation products were analysed by SDS-PAGE and fluorography. In order to exclude all but the hordein mRNA translation products, the 10% TCA precipitates of the reaction mixtures were extracted with 55% isopropanol containing 10 mM-EDTA and 100 µg/ml carrier hordeins. Trial extractions of in vivo ¹⁴C-labelled hordeins revealed that over 90% of the hordeins could be recovered in an isopropanol extract after TCA precipitation. The fluorogram seen in Figure 2 shows the separation of native hordein in tracks 1 and 2.

Figure 2. SDS-PAGE of 55% isopropanol soluble translation products of rough microsomes from Bomi barley endosperm. Cell-free protein synthesis with rough microsomes from 20 day old Bomi barley endosperm and electrophoresis and fluorography of the products were as described under Materials and methods (2.6., 2.7.). The fluorogram shows in vivo ¹⁴C-labelled hordein marker, track 1; 55% isopropanol soluble hordein marker extracted from TCA precipitate, track 2; translation products of rough microsomes from 2 A₂₆₀ units, track 3 and 1 A₂₆₀ unit, track 4.

The 55% isopropanol solubilized hordeins in track 2 were derived from a TCA precipitate, thereby confirming the reliability of this isolation procedure. Tracks 3 and 4 contain the translation products from band I rough microsomes at two concentrations. Most of the native hordein polypeptides are represented among the in vitro translation products. The preponderance of low molecular weight polypeptides is probably due to premature chain termination and the extraction of low molecular weight wheat-germ translation products.

3.3. Optimization of cell-free protein synthesis with barley endosperm rough microsomes

In order to maximise the efficiency of in vitro protein synthesis we assessed certain parameters in the procedure by measuring the incorporation of ^{35}S -Met into hot 10% TCA insoluble material in the presence of band I rough microsomes. The time-course of in vitro protein synthesis performed at 27°C in the standard reaction mixture (Figure 3A), shows a significant decline in the rate of synthesis after 20 min and by 80 min a slight loss of product is observed. It is probable that the decline in synthesis rate is partially due to a depletion of reaction mixture components, in particular the energy generating system, and partially to proteolysis of the translation products. Incubations of 60 min were considered optimal for subsequent assays.

Figure 3B shows a rise in protein synthesis with increasing concentrations of rough microsomes per assay. A concentration of 1 A_{260} unit/assay was selected for subsequent experiments.

In vitro protein synthesis using the wheat-germ cell-free system has been found to show a sharp magnesium response (15, 17). However, unlike the translation of mRNA associated with free or detached polysomes, the rough microsomes in barley (Figure 3C) appear to be little sensitive to magnesium concentration. It is possible that the ion-binding capacity of membranes enables them to perform a magnesium buffering role.

Figure 3. Optimization of cell-free protein synthesis directed by rough microsomes from Bomi barley endosperm. Rough microsomes (band I) from 20 day old Bomi barley endosperm were isolated and assayed in the standard reaction mixture as described under Materials and methods.

3.4. Membrane transport of hordeins synthesized on barley endosperm rough microsomes

According to the signal hypothesis (1) for the synthesis of vectorially discharged proteins, the translation of their mRNA on membrane-bound polysomes yields nascent chains which are transported through the membrane as an integral part of their synthesis. Thus, when cell-free protein synthesis is directed by rough microsomes, the products are transferred into the microsomal cisternae, a phenomenon best demonstrated by their protection from proteolytic attack (7, 9).

Conditions suitable for the proteolysis of native hordeins were first investigated using *in vivo* ^{14}C -labelled hordeins. 100 μg each of chymotrypsin and trypsin were added to 50 μl of a solution containing 100 μg ^{14}C -hordeins and 5

μl S-30 wheat-germ extract, and incubated at 2°C for 40 or 180 min. Chymotrypsin treatment of the cell-free system is performed at 2°C to prevent further protein synthesis. The reaction was terminated with 10% TCA and the products analysed by SDS-PAGE and fluorography. The fluorogram (Figure 4) shows a partial degradation of hordeins after 40 and 80 min (tracks 2 and 3 respectively), but it was subsequently observed that chymotrypsin alone, at concentrations as low as 10 $\mu\text{g}/\text{assay}$, hydrolysed the hordeins after 120 min incubation. It should be noted that three groups of large peptide fragments are prominent among the cleavage products of hordein proteolysis. One may infer a degree of uniformity of composition among the hordein polypeptides from the limited points of proteolytic cleavage.

The accessibility of hordeins, synthesized *in vitro* on detached polysomes and rough microsomes, to proteolytic attack could then be investigated, measuring the TCA precipitable translation products remaining after chymotrypsin treatment. Since the background count in the precipitates, contributed by the wheat-germ translation products, is lost after chymotrypsin treatment (not shown) it has been subtracted from the untreated assays. A greater extent of proteolysis was found for proteins synthesized on detached polysomes as compared to those made on rough microsomes from band I or band II (Table III). While at first sight the polysomes may appear to afford some protection to their nascent polypeptides, it should be appreciated that a significant proportion of the larger cleavage products may be precipitated by TCA. This conclusion is fully supported by analysis of the chymotrypsin-treated translation products by SDS-PAGE and fluorography. The fluorogram (Figure 5) shows the hordeins synthesized by rough microsomes (band II, track 1; band I, track 3) and by detached polysomes (track 9), and after their respective treatment with 5 or 10 μg chymotrypsin/assay (tracks 2, 4, 5, 10 and 11). Here there is no evidence of protection of the hordeins synthesized on detached polysomes (tracks 10, 11), and release of larger cleavage products can be seen close to the tracking dye. However, the hordeins synthesized on rough microsomes from both bands

Figure 4. SDS-PAGE of native hordeins after proteolytic treatment. *In vivo* ^{14}C -labelled hordein (100 μg) was treated with 100 μg each of chymotrypsin and trypsin at 2°C , in the presence or absence of S-30 wheat-germ extract. The products were analysed by electrophoresis and fluorography as described under Materials and methods (2.7.). The fluorogram shows ^{14}C -hordein marker, tracks 1,5; ^{14}C -hordein with S-30 wheat-germ after 40 and 180 min proteolysis, tracks 2,3; ^{14}C -hordein after 180 min proteolysis, track 4.

I and II (tracks 2, 4, 5), were afforded significant protection from proteolysis. The vectorial discharge of hordeins synthesized on rough microsomes into their lumen is further indicated by the ability of membrane-solubilizing detergents to expose the hordeins to proteolytic attack. Triton X-100 (1%) and DOC (0.05-0.5%), detergents, which did not impair the proteolysis of polysomal translation products (tracks 12, 13), destroyed the microsomal protection of transported hordeins (tracks 6, 7, 8).

The absence of wheat-germ translation products in track 14 (Figure 5) confirms the hordein selectivity of 55% isopropanol extraction for hordeins.

4. DISCUSSION

4.1. Isolation of rough microsomes

The electron micrographs of the microsomal fraction, designated as band I, contained microsomes and what could be interpreted as free polysomes (Figures 1A-C). The method applied for the preparation of the microsomal fraction makes the presence of free polysomes in this fraction highly unlikely, since only membrane-bound polysomes are isolated by the initial 37,000 g spin. If polysomes are detached subsequently they would be pelleted during the

flotation step involving centrifugation at 96,000 g_{av} . The ribosomes not visibly attached to microsomal membranes in Figures 1A-C are therefore interpreted as being members of microsomal polysomes which are not yet in contact with the microsomal membrane. In some cases electron dense material is observed adhering to the inner surface of the microsomal membranes (Figures 1B, C). It is tempting to suggest that this material represents vectorially discharged polypeptides.

4.2. Cell-free synthesis of barley endosperm proteins

Hordeins are hydrophobic proteins, deposited as protein bodies bounded by a tonoplast in the developing barley endosperm (11, 16), and whose synthesis is primarily directed by membrane-bound polysomes (4). In many animal cells, particularly those from secretory tissues, the synthesis of exported proteins on membrane-bound polysomes is closely associated with their subsequent excretion. Techniques developed to demonstrate the concomitant synthesis and vectorial discharge of animal proteins *in vitro*, have been applied in the study of hordein synthesis on barley endosperm rough microsomes. These include the use of proteolytic enzymes to detect the distribution

Table III

Cell-free synthesis of proteins on rough microsomes and detached polysomes from Bomi barley endosperm and their accessibility to proteolytic attack. Rough microsomes and polysomes from 20 day old Bomi barley endosperm were isolated and assayed in the standard reaction mixture as described under Materials and methods (2.6.). TCA precipitable translation products remaining after 2 h chymotrypsin treatment (10 μ g/assay) were measured.

	Chymotrypsin treated	Detergent treated	cpm/assay ⁺	per cent proteolysis
Detached polysomes	0	0	708,600	
-	+	0	451,200	36
-	+	1% Triton X-100	428,800	40
-	+	0.50% DOC	426,400	40
Rough microsomes				
band I	0	0	126,800	
-	+	0	102,700	19
-	+	1% Triton X-100	79,500	37
-	+	0.05% DOC	65,900	48
-	+	0.50% DOC	83,370	34
band II	0	0	166,400	
-	+	0	118,700	29

+ A wheat-germ background count of 80,000 cpm was subtracted from all untreated samples.

of translation products of membrane-bound polysomes between the medium and the microsomal lumen. When cell-free protein synthesis was followed by a two hour chymotrypsin treatment at 2°C, the hordeins synthesized by detached polysomes were extensively degraded, while the hordeins synthesized by rough microsomes were largely preserved. Further confirmation of the vectorial discharge of hordeins synthesized on rough microsomes was gained from the results of chymotrypsin treatment in the presence of membrane-solubilizing detergents.

Concentrations of DOC (0.05%) sufficient only to cause membrane leakiness, as against complete disassembly, have been determined for rat liver microsomes (12). The pores thus created in the microsomes gave access to

proteolytic enzymes which degraded polypeptides released into the microsomal cavity. The location of in vitro synthesized hordeins within the microsomal cavity and not in the membrane, was similarly indicated in the present work by their exposure to proteolytic attack by 0.05% DOC.

Other examples of protein membrane transport in plant cells are limited, but the synthesis of the chloroplast protein ribulose-1,5-bisphosphate carboxylase, whose small subunit is encoded in the nuclear genome, has received particular attention. In both *Pisum sativum* (10) and *Chlamydomonas reinhardtii* (9) the small subunit is synthesized on cytoplasmic polysomes and subsequently transported into the chloroplast. The primary translation product has a higher molecular weight indicating a

Figure 5. SDS-PAGE of 55% isopropanol soluble translation products of rough microsomes and polysomes from Bomi barley endosperm after proteolytic treatment. Cell-free protein synthesis was performed with rough microsomes and polysomes prepared from 20 day old Bomi barley endosperm. The 55% isopropanol soluble translation products remaining after 2 h chymotrypsin treatment at 2°C were analysed by electrophoresis and fluorography as described under Materials and methods (2.7.). The fluorogram shows translation products after the following treatments.

	Template	Chymotrypsin	Detergent
Track 1	rough microsomes (band II)	0	0
Track 2	-	10 µg	0
Track 3	rough microsomes (band I)	0	0
Track 4	-	5 µg	0
Track 5	-	10 µg	0
Track 6	-	10 µg	1% Triton X-100
Track 7	-	10 µg	0.05% DOC
Track 8	-	10 µg	0.50% DOC
Track 9	detached polysomes	0	0
Track 10	-	5 µg	0
Track 11	-	10 µg	0
Track 12	-	10 µg	1% Triton X-100
Track 13	-	10 µg	0.50% DOC
Track 14	wheat-germ S-30	0	0
Track 15	Marker consisting of native ¹⁴ C-hordein		

signal sequence for membrane transport. The extra amino acid sequence is absent from the small subunits isolated from the chloroplasts (9, 10). However, the mechanism of polypeptide transport involved is likely to be different from that envisaged for polypeptides transferred into the lumen of the endoplasmic reticulum, owing to the independence of the synthesis of the small subunit and its subsequent transport.

In the present paper it has been shown that nascent hordein polypeptides are transferred into the lumen of the endoplasmic reticulum of barley endosperm. The next task is to determine whether the newly synthesized hordein chains contain a signal sequence of amino acids.

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