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Differential Synthesis *in Vitro* of Barley Aleurone and Starchy Endosperm Proteins

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ABSTRACT

To widen the selection of proteins for gene expression studies in barley seeds, experiments were performed to identify proteins whose synthesis is differentially regulated in developing and germinating seed tissues. The *in vitro* synthesis of nine distinct barley proteins was compared using mRNAs from isolated endosperm and aleurone tissues (developing and mature grain) and from cultured (germinating) aleurone layers treated with abscisic acid (ABA) and GA_3. B and C hordein polypeptides and the salt-soluble proteins β-amylase, protein Z, protein C, the chymotrypsin inhibitors (CI-1 and 2), the α-amylase/subtilisin inhibitor (ASI) and the inhibitor of animal cell-free protein synthesis systems (PSI) were synthesized with mRNA from developing starchy endosperm tissue. Of these proteins, β-amylase, protein Z, and CI- 1 and 2 were also synthesized with mRNA from developing aleurone cells, but ASI, PSI, and protein C were not. CI-1 and also a probable amylase/protease inhibitor (PAPI) were synthesized at high levels with mRNAs from late developing and mature aleurone. These results show that mRNAs encoding PAPI and CI-1 survive seed dessication and are long-lived in aleurone cells. Thus, expression of genes encoding ASI, PSI, protein C, and PAPI is tissue and stage-specific during seed development. Only ASI, CI-1, and PAPI were synthesized in significant amounts with mRNA from cultured aleurone layers. The levels of synthesis of PAPI and CI-1 were independent of hormone treatment. In contrast, synthesis of α-amylase (included as control) and of ASI showed antagonistic hormonal control: while GA promotes and ABA reduces accumulation of mRNA for α-amylase, these hormones have the opposite effect on ASI mRNA levels.

One approach to understanding the molecular mechanisms controlling seed maturation and germination is to study organ-specific, developmentally characteristic, and hormonally regulated seed proteins and their mRNAs. Earlier studies have identified such polypeptides in *Gossypium* (7) and *Brassica* (6) and have formed the basis of later work at the molecular level on these crops. We report here the results of similar experiments with barley seeds, a cereal of importance as livestock feed and as malt in the brewing industry.

The barley seed or caryopsis is comprised of several tissues derived from the fertilized ovule (4). The seed proteins of the grain, synthesized during seed development in the endosperm and/or aleurone layer, include the hordeins (30–50% of total N, [17]), and a diverse group of salt-soluble proteins (20–40% total N, [9]). The hordeins, which function as storage polypeptides, are synthesized on RER and accumulate in protein bodies in starchy endosperm cells (17). The salt-soluble proteins have more active physiological functions. β-Amylase is important in starch hydrolysis during germination, while various protease inhibitors, including an α-amylase/subtilisin inhibitor (ASI, [20, 29]), the chymotrypsin inhibitors 1 and 2 (3), and perhaps protein Z (11), and a probable amylase/protease inhibitor (PAPI) (25) may protect the seed against attack by microbial and/or animal hydrolases. It is unclear how the salt-soluble seed proteins are deposited in endosperm cells or to what extent they may act as storage polypeptides (9, 10, 15).

Biochemical data are scarce regarding protein synthesis in developing aleurone cells which differentiate from endosperm cells early in seed development to form a layer surrounding the starchy endosperm tissue. Electron microscopic studies have shown that the ultrastructure of protein bodies found in aleurone cells is different from those of starchy endosperm cells (for reviews see Refs. 1, 28). Such differences imply differential expression during seed development of genes encoding various seed proteins in these two ontogenically closely related tissues.

During germination, proteins and carbohydrates stored in the dead, starchy endosperm cells are mobilized for use by the developing seedling. These storage products are degraded by hydrolases already present in the seed, such as β-amylase, or *de novo* synthesized in the living cells of the aleurone and/or embryonic scutellum (18, 22). The *de novo* synthesis of high levels of these enzymes in germinating aleurone cells has provided a model for studying differential gene expression in plants. Most of the work on this tissue has dealt with the synthesis of α-amylase in cultured aleurone layers from mature, rehydrated seeds, a tissue system developed to simulate events ocurring in aleurone cells during germination. The synthesis of α-amylase and other hydrolases can be stimulated in this system by incubating the layers with GA while their synthesis is blocked by ABA (13, 14, 24). Another protein whose synthesis *in vivo* is specifically regulated in cultured aleurone layers by these hormones has been identified as a seed protein ASI[2] which inhibits both barley α-amylase and the bacterial protease subtilisin (20). Interestingly, ASI synthesis *in vivo* was stimulated in aleurone layers by ABA but reduced by GA, a control antagonistic to that for α-amylase (21). These results have led to speculation on the role of ASI and other seed proteins in aleurone layers, and generally on the effect of ABA on protein synthesis in the

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[2] Abbreviations: ASI, α-amylase/subtilisin inhibitor; CI-1 and 2, chymotrypsin inhibitors 1 and 2; PSI, inhibitor of cell-free protein synthesis; PAPI, probable amylase/protease inhibitor; dpa, days postanthesis.

aleurone tissue system (12, 21).

The present study was carried out to identify other proteins whose synthesis is developmentally and/or hormonally regulated in barley seed tissues. To this end, the synthesis *in vitro* of nine well characterized seed proteins was compared using mRNAs from aleurone and starchy endosperm tissues of developing and mature seeds and from cultured aleurone tissue. Our results document differences in the pattern of expression of genes encoding these proteins.

METHODS

Sources of Seeds and Chemicals. Barley seeds of a proanthocyanidin-free cultivar (*Hordeum vulgare* L. cv Bonus, mutant Ant 13, 1981 crop) were supplied by Dr. John Ingversen, Carlsberg Plant Breeding, Copenhagen, Denmark. The antibiotics used were from Sigma, L-[^{35}S]methionine (1300 Ci mmol^{-1}) and rabbit reticulocyte lysate (N 90) were obtained from Amersham Corp., Amersham, U.K. Poly-U and protein-A sepharose 4B were from Pharmacia, Uppsala, Sweden.

Preparation of Antibodies. Antibodies toward ASI (20), PSI (2, also referred to as protein K, 10), protein Z, homologous with members of the α_1-antitrypsin family of plasma protease inhibitors (11), β-amylase (26), and protein C (10) were raised according to standard procedures. Antibodies towards CI-1 and 2 (3), and towards PAPI (25) were prepared by immunization after gluteraldehyde polymerization of the antigens. PAPI is homologous to an α-amylase inhibitor from Indian finger millet and to the eggplant trypsin inhibitor (25). Antibodies against barley malt α-amylase 2 (EC 3.4.16.1) were used as a control in experiments with hormone-treated aleurone layers (22). IgGs were purified from the crude antisera by chromatography on protein-A sepharose 4B and the protein concentration of the antibody preparations adjusted to 4 mg/ml.

Tissue Preparation and Isolation of Poly A$^+$ RNA. Developing starchy endosperm tissue was squeezed from immature seeds 20 and 30 dpa. After removal of the outer pericarp, the corresponding aleurone layers were halved and washed three times for 15 min in 10 mM Na_3PO_4, 150 mM NaCl (pH 7.4) in a metabolic shaker. Aleurone and starchy endosperm tissues were prepared from mature, dry seeds after removal of the germ with a scalpel, followed by decortication in a rice polisher equipped with a file disc (Pearlest, Kett Electric Laboratory, Tokyo). Fractions decorticated between 3 and 20% and 40 and 100% were used as aleurone and starchy endosperm, respectively. Cultured aleurone layers were prepared by a modification of established procedures (5): embryoless half grains were surfaced sterilized with 0.2% $AgNO_3$ and imbibed 3 d on sterile, wet sand. Samples of 2000 half-seeds were incubated in 500 ml solution containing the hormones (50 μM ABA or 10 μM GA_3). After 16 h the starchy endosperm was squeezed from the half-seeds and the aleurone tissue frozen in liquid N_2. Poly(A$^+$)-RNA was isolated from the tissues by extraction in guanidinium hydrochloride, ethanol precipitation, phenol extraction, and chromatography on poly(U)-Sepharose 4B as previously described (22).

Scanning Electron Microscopy. Dissected aleurone/pericarp and starchy endosperm were prepared by fixation in 1 and 2% gluteraldehyde followed by stepwise dehydration in 20 to 100% ethanol. They were then critical point dried with CO_2 as translation fluid in a CPD 0201 (Balzers Union) and plated with 180 to 200 nm of gold in a SCD 030 sputtering device (Balzers Union) and viewed in a stereoscan 100 scanning electron microscope (Cambridge Instruments) at 5 kV.

***In Vitro* Translation and Analysis of Proteins.** The rabbit reticulocyte lysate system was used as per the manufacturer's instructions. A standard assay consisted of 2 μl mRNA (0.05 OD_{260} units), 7.5 μl lysate, and 1.5 μl [^{35}S]methionine incubated for 1 h at 30°C. TCA precipitable counts were determined and aliquots taken for analysis of total *in vitro* synthesized polypeptides (1×10^5 cpm/lane) and for immunoadsorption of specific proteins (1×10^6 cpm/lane) after (15). Hordein polypeptides were isolated (5×10^5 cpm/lane) by extraction with 55% isopropanol (17). Preliminary experiments using antibody dilution series and repeated precipitations showed that a volume of 20 μl of antibody solution was sufficient in all cases to specifically precipitate more than 90% of a given antigen from the lysate assays.

Samples were electrophoresed in 12.5% SDS-PAGE slab gels and the gels prepared for fluorography as previously described (15). Films were exposed 1 to 5 d for bands of normal and high intensity, up to 2 months for weaker bands. Densitometric scanning of fluorographs in a Shimadzu MPS 5000 spectrophotometer was used to estimate the relative amounts of radioactivity in lanes.

RESULTS

Microscopy of Tissue Preparations. Scanning EM shows that the starchy endosperm preparations consist of homogenous cells rich in starch and protein bodies (Fig. 1A), indicating that our endosperm tissue is not contaminated with other cell types. The outer tissue layers are composed of several cell types which adhere tightly to the aleurone cells when the starchy endosperm is squeezed from the seed (Fig. 1B, note outer and inner pericarp cells). Examination of cultured aleurone layers showed the same

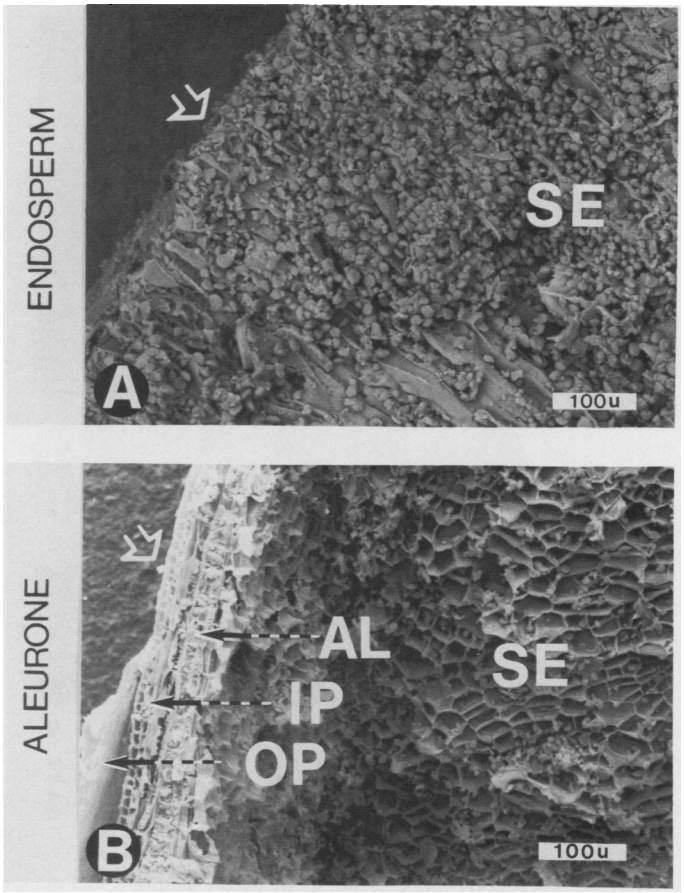

FIG. 1. Starchy endosperm and aleurone/pericarp tissues. Scanning electron micrographs of tissues dissected from 30 dpa developing grains. A, starchy endosperm (SE), showing large homogeneous cells filled with starch granules and protein bodies; B, outer and inner pericarp (OP and IP) and aleurone cells (AL), showing underlying, broken starchy endosperm cells. Arrows approximate the same relative position on each preparation.

tissue structures. The 'aleurone layer' preparations obtained by other workers using both hulled and naked barleys also contain pericarp/testa tissue (4, 5). Similarly, we refer to the aleurone/pericarp tissue used in this study simply as aleurone layers.

Translational Activities of mRNAs from Aleurone and Starchy Endosperm. The profiles of polypeptide products synthesized with mRNAs from different aleurone and starchy endosperm tissues show that our preparations of these tissues contain different mRNA populations (Fig. 2). For example, the dominant B and C hordein bands ($M_r \sim 49–47 \times 10^3$ and $61–63 \times 10^3$, respectively) seen among the 20 dpa starchy endosperm products are missing among the corresponding aleurone products (Fig. 2, lanes 1 *versus* 2). Values for the translational activities (total radioactivity incorporated into protein or TCA precipitable counts) of these mRNA preparations furthermore reflect the relative levels of translatable mRNAs found in the tissues (Table I). These values show that endosperm mRNA levels decline after 20 dpa, becoming negligible by seed maturation and disappearing during imbibition. Aleurone mRNA levels also decline at seed maturity but mRNAs encoding specific polypeptides survive seed desiccation and mRNAs encoding new polypeptides appear during culture (Table I; Fig. 2, lanes 6–9). The effects of ABA and GA on mRNA levels is also clearly illustrated; while GA induces expression of a few genes (lane 7 *versus* 9), ABA-treatment appears to reproduce the polypeptide pattern seen in aleurones of mature seeds (lane 6 *versus* 8).

Table I. *Estimates of Translational Activities (Total TCA Precipitable Radioactivity) of mRNAs Isolated from Different Tissues*

Tissue	Total Activity (10^7 cpm)	
	per g·dry wt of tissue	*per seed*
From developing seed		
20 dpa endosperm	25.10	2.650
20 dpa aleurone	7.20	0.880
30 dpa endosperm	2.45	0.745
30 dpa aleurone	1.70	0.200
Mature endosperm	0.20	0.001
Mature aleurone	1.05	0.009
From organ-culture		
Imbibed endosperm	0.00	0.000
CON aleurone	5.10	0.027
ABA aleurone	6.10	0.032
GA_3 aleurone	4.65	0.025

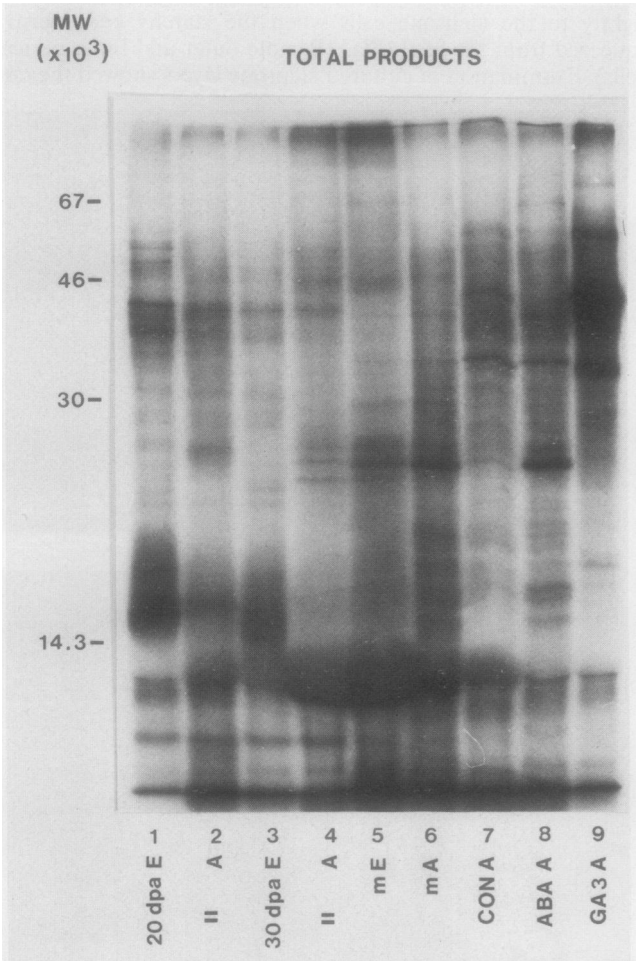

FIG. 2. Total polypeptide synthesis in barley aleurone and starchy endosperm tissues. Fluorogram of SDS-PAGE of [^{35}S]methionine-labeled polypeptides synthesized *in vitro* with mRNAs isolated from: 1, 20 dpa endosperm; 2, 20 dpa aleurone; 3, 30 dpa endosperm; 4, 30 dpa aleurone; 5, mature endosperm fraction; 6, mature aleurone fraction; 7, CON (no hormone control) mature, cultured aleurone; 8, ABA-treated aleurone; 9, GA-treated aleurone. Equal TCA-precipitable counts loaded per lane, film exposed 4 d.

Proteins Synthesized with mRNAs from Developing and Mature Starchy Endosperm. Among the total products synthesized with mRNA isolated from developing endosperm, dominant polypeptides of $M_r \sim 62, 53, 43.8, 43.5, 39, 36, 30.2, 24.3, 20.4, 15.6, 14.3, 12.5$, and 11.5×10^3 can be seen (Fig. 3, lane 4). Hordein polypeptides, extracted from the total products in 55% isopropanol, have $M_r \sim 64, 61, 53.5, 48.5, 45.5, 42.5, 40.3, 38.7, 37 \times 10^3$ (Fig. 3, lane 5). The prominent doublet of $M_r \sim 49–47 \times 10^3$ has the size expected for the precursors of the major B-hordeins of $M_r \sim 46–48 \times 10^3$ (17). The two minor bands of $M_r \sim 64$ and 61×10^3 are presumably C hordeins, while the banding pattern of polypeptides of $M_r \sim 35–45.5 \times 10^3$ is similar to that seen for minor B-hordein polypeptides. Bands below $M_r \sim 30 \times 10^3$ represent A-hordeins (chloroform/methanol-soluble proteins) which are not coded for by hordein genes (23).

Monospecific antibodies raised towards several salt-soluble seed proteins were used to probe these polypeptides among the total products (Fig. 3, lanes 6–12). The relative amounts of the immunoadsorbed polypeptides were estimated by scanning densitometry (Table II). CI-1 was resolved into two major bands (lane 6) which may be precursors of the several isoinhibitor forms described from barley seeds (3). The single form of CI-2 resolved here (lane 7) confirms earlier findings that minor, multiple forms of CI-2 purified from barley seeds are not distinct gene products but represent fragments of a limited number of mature isoinhibitor forms (3). Protein Z was resolved into two major and possibly two minor bands of $M_r \sim 41 \times 10^3$ (lane 8). In contrast, only single forms of β-amylase (lane 9) and ASI (lane 10) were detectable in the gels used here.

Relatively large amounts of protein Z, β-amylase, and CI-1 were synthesized in comparison to ASI and CI-2 (Fig. 3; Table II). These polypeptides are synthesized at the peak of protein deposition about 20 dpa (15). In contrast, only trace amounts of PSI and protein C could be immunoadsorbed from the 20 dpa endosperm preparation. However, in accordance with the late appearance of these basic proteins during grain development (9), strong bands were obtained by immunoadsorption with antibodies towards PSI and protein C from polypeptides synthesized with 30 dpa mRNAs (Fig. 3, lanes 11 and 12). The PSI doublet suggests that the 3 PSI isoinhibitors purified from barley seeds are derived from at least two precursor forms (2). The only seed protein which could not be immunoadsorbed from either the 20 or 30 dpa starchy endosperm products was PAPI (data not presented). This indicates that PAPI is not synthesized in developing starchy endosperm cells before 30 dpa.

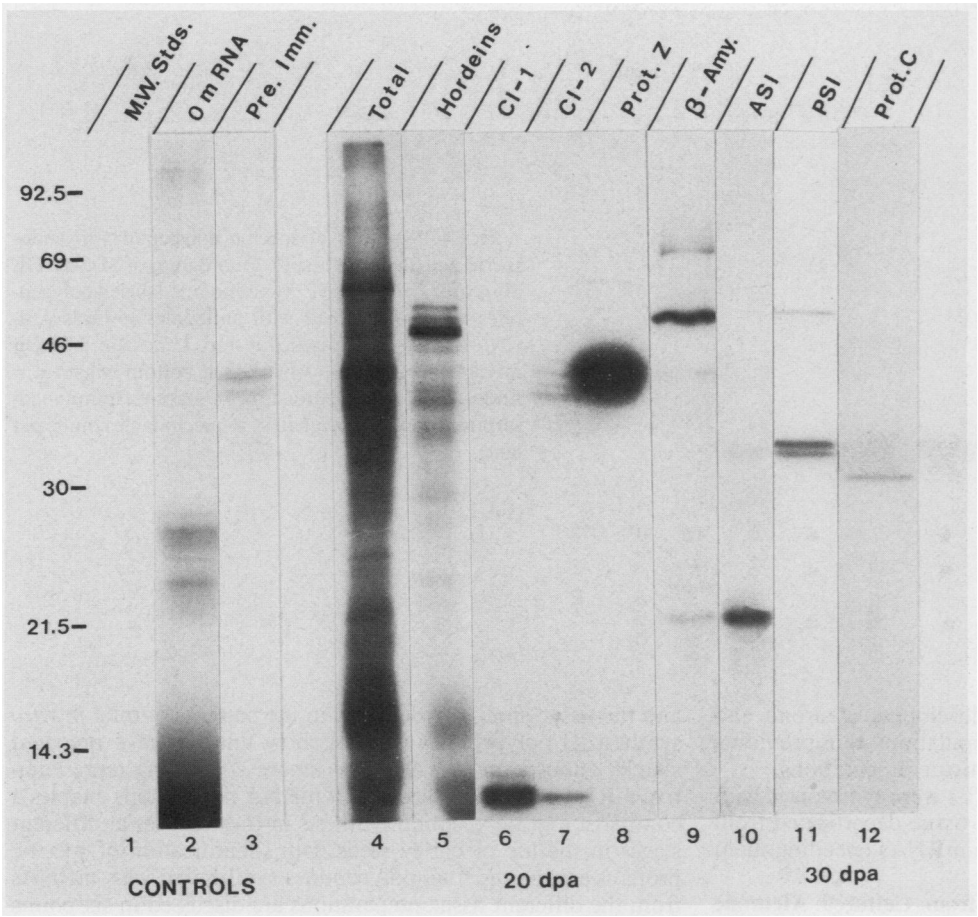

FIG. 3. Synthesis of specific polypeptides in developing (20 and 30 dpa) starchy endosperm. Fluorogram of SDS-PAGE of [^{35}S]methionine-labeled polypeptides synthesized *in vitro* with mRNA isolated from developing starchy endosperm. Lanes 1 to 3 show control experiments: 1, [^{14}C]methylated molecular weight standards (Amersham) from bottom to top, lysozyme, carbonic anhydrase, ovalbumin, BSA, phosphorylase b; 2, control, no mRNA; 3, polypeptides binding nonspecifically to preimmune rabbit serum. Lane 4, total polypeptide products; lane 5, hordein-like polypeptides (55% isopropanol-soluble polypeptides (17). Lanes 6 to 12 show polypeptides immunoadsorbed with antibodies towards salt-soluble proteins: 6, CI-1; 7, CI-2; 8, protein Z; 9, β-amylase; 10, ASI; 11, PSI (30 dpa); 12, protein C (30 dpa). Film exposed 3 to 7 d.

Table II. *Estimates of Relative Levels of Synthesis of Polypeptides Immunoadsorbed from Starchy Endosperm and Aleurone In Vitro Products*

Relative amounts of polypeptides, estimated by scanning densitometry of bands in fluorogram lanes from Figures 3, 4, and 5, are expressed as:

$$\text{relative moles/lane} = \frac{\text{peak area}}{(\text{exp.})(\text{met})(\text{decay})} \times 100$$

where: peak area = density of fluorogram band (520nm), exp. = days exposure, met = number of methionines in mature protein, decay = [^{35}S] methionine decay factor. These values are given for each polypeptide by tissue.

Protein	M_r~ (10^3)	No. of Met.	Ref. No.	Relative Moles/Lane								
				Developing seed				Mature		Hormone treatments		
				20 dpa		30 dpa		Dry seed		Cultured aleurone		
				Endosperm	Aleurone	Endosperm	Aleurone	Endosperm	Aleurone	CON	ABA	GA_3
Protein Z	40	4	11	112	51	70	30	—[a]	—	—	—	—
β-Amylase	57	15	26	21	6	10	—	—	—	—	—	—
CI-1	9	2	3	66	40	75	43	3	1.5	4	4	4
CI-2	9	1	3	18	8	16	4	—	—	—	—	—
PSI	31	4	2	—	—	4	—	—	—	—	—	—
Protein C	28	3	10	—	—	4	—	—	—	—	—	—
PAPI	12	1	25	—	4	—	7	6	19	5	6	5
ASI	21	2	20	7	—	11	—	—	—	6	28	2
α-Amylase 2	41	6	24	—	—	—	—	—	—	16	1	76

[a] No detectable radioactivity.

Mature endosperm tissue yielded relatively little translatable mRNA (Fig. 2, lane 5; Table I). Trace amounts of presumptive B hordein polypeptides, CI-1, and PAPI could be seen after long fluorogram exposure but not bands representing the major salt-soluble proteins protein Z, β-amylase, or CI-2 (data not presented).

Proteins Synthesized with mRNAs from Developing and Mature Aleurone Layers. Traces of B and C hordein polypeptides and much higher levels of β-amylase, protein Z, and CI-1 and 2 were synthesized with the developing aleurone mRNAs (only CI-1 shown, Fig. 4, lanes 5–9). PAPI was synthesized with mRNAs from 20 and 30 dpa aleurones (Fig. 4, lanes 10–12). In contrast, ASI (Fig. 4, lanes 1–4) and PSI (not shown) were not synthesized with mRNAs from developing aleurone tissues. This indicates

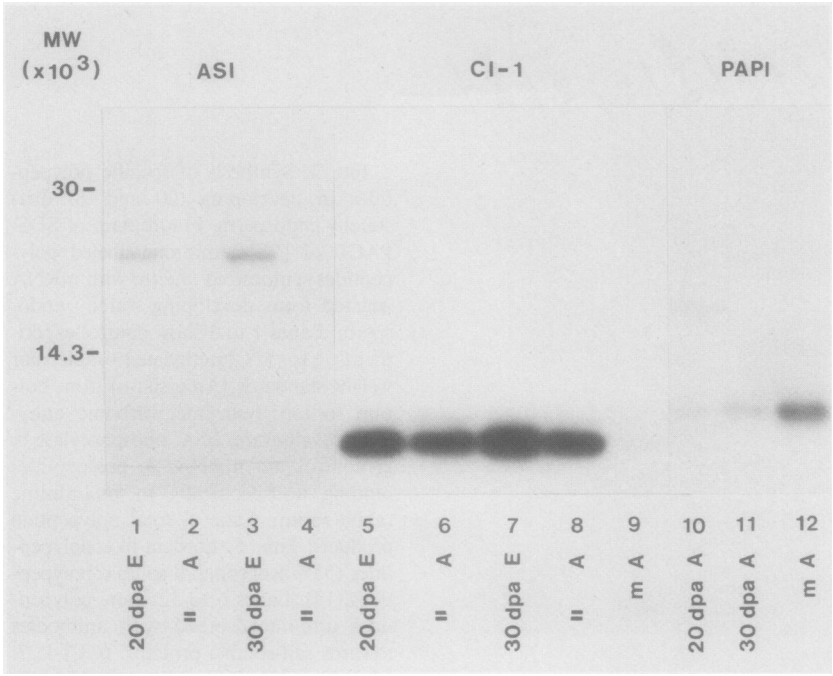

FIG. 4. Synthesis of specific polypeptides in endosperm and aleurone tissues. Fluorogram of SDS-PAGE of *in vitro* synthesized [^{35}S]methionine-labeled polypeptides immunoadsorbed with antibodies towards ASI, CI-1, and PAPI as marked at top. Lanes are different mRNA preparations as marked at bottom where: E = Endosperm, A = aleurone, m = mature. Immunoadsorptions were from equal TCA-precipitable counts per lane.

that the mRNA preparations from developing aleurone and endosperm represent distinct mRNA populations, thus providing another measure of the purity of these tissue preparations.

Only PAPI and smaller amounts of CI-1 were synthesized with mRNA isolated from mature aleurone tissue decorticated from dry seeds (Fig. 4, lanes 9 and 12). The mRNAs encoding them therefore survive seed desiccation.

Proteins Synthesized with mRNA from Cultured Aleurone Layers. Compared to control tissue, dominant ABA-enhanced polypeptides had $M_r \sim 74$, 53 and 25–27 $\times 10^3$ (Fig. 5, lane 1 *versus* 2). The $M_r \sim 25$–27 $\times 10^3$ polypeptide has been observed by other workers and a comparison of published fluorograms of [^{3}H]leucine and [^{35}S]methionine-labeled polypeptides of ABA-treated aleurone layers suggests that this protein is relatively rich in methionine, since it is much less prominent when leucine is used (12, 19). Major GA_3-induced polypeptides include α-amylase ($M_r \sim 44 \times 10^3$) and polypeptides of $M_r \sim 41.5$, 33.5 and 28×10^3. The $M_r \sim 33.5 \times 10^3$ polypeptide has been described previously and is tentatively identified as (1→3, 1→4)β-D-glucanase based on its co-mobility with immunoadsorbed β-glucanase (22).

The pattern of *in vitro* synthesis of α-amylase and of ASI (Fig. 5, lanes 4–6; Table II) is essentially the same as that previously described *in vivo* (21). These two proteins have been precipitated together here to highlight their different patterns of synthesis. The regulation of synthesis of ASI, both *in vivo* (21) and *in vitro* (present study) therefore appears to be the reverse of that for α-amylase; synthesis of the inhibitor is enhanced by ABA while it is inhibited by GA_3 in comparison to untreated aleurones.

Relatively large amounts of CI-1 and PAPI were synthesized independent of hormone treatment (Fig. 5, lanes 7–9). Their synthesis therefore is not regulated by GA_3 or ABA in aleurone layers incubated under standard conditions. In contrast, no synthesis of protein Z, β-amylase, PSI, or CI-2 could be detected. The synthesis of isopropanol-soluble polypeptides could be detected only after prolonged exposure of the fluorograms (data not presented).

DISCUSSION

Taken together, the scanning electron micrographs (Fig. 1), the estimates of levels of translatable tissue mRNAs (Table I), and the tissue-specific differences in the patterns of total *in vitro* synthesized polypeptides (Fig. 2) show that we have prepared starchy endosperm and aleurone tissues containing representative mRNAs. Such a collection of mRNA populations enables a comparison of polypeptide synthesis in these tissues at different stages in the life of barley seeds. Our identification of specific proteins among the total polypeptides synthesized with mRNAs from the different tissue preparations are discussed in six points below. Values for the relative levels of immunoadsorbed proteins from the different tissue preparations are summarized in Table II.

β-Amylase, protein Z, and CI-1 and 2 are synthesized in both developing starchy endosperm and aleurone layer cells (Figs. 3 and 4). Large amounts of B and C hordein polypeptides were also synthesized with developing endosperm mRNA while only traces could be synthesized with mRNA from developing aleurone. In the case of the aleurone preparation, it might be argued that some of the mRNA encoding these polypeptides came from subaleurone, starchy endosperm cells which contaminated the dissected aleurone layers. As mRNAs encoding the hordeins are among the most abundant in starchy endosperm cells, the presence of relatively few such cells could contribute a signifigant amount of these mRNAs to the developing aleurone preparation. However, the absence among the developing aleurone products of ASI and PSI, which are clearly only synthesized in starchy endosperm cells, indicates that contamination of the developing aleurone tissue by starchy endosperm cells is minimal.

ASI, PSI, and protein C are starchy endosperm-specific during seed development while PAPI mRNA is only measurable in aleurone layers up to 30 dpa (Fig. 4, only comparative lanes for ASI shown). In keeping with these results, immunological measurement of PAPI protein in mature seed fractions has shown that PAPI is concentrated in the aleurone fraction and can only be detected in small amounts in fully decorticated endosperm (J Mundy, JC Rogers, unpublished results).

The peak of PSI, protein C, and PAPI synthesis is later than that of the other seed proteins tested (Fig. 3, lanes 11 and 12 and Fig. 4, lanes 10–12). PSI and protein C are members of a small group of basic proteins which have been shown to accumulate after 25 dpa (9). Although the function of protein C is unknown, PAPI and PSI, as enzyme and translational inhibitors respec-

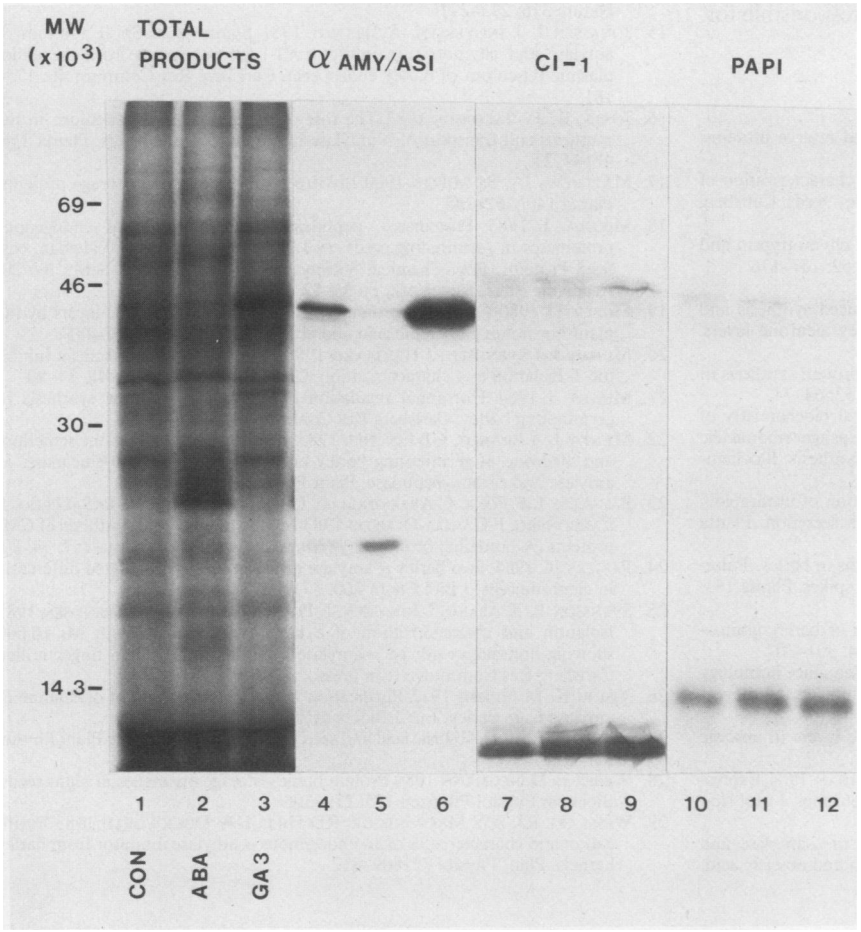

FIG. 5. Synthesis of polypeptides in cultured aleurone layers. Fluorogram of SDS-PAGE of [^{35}S]methionine-labeled polypeptides synthesized *in vitro* with mRNAs isolated from cultured aleurone layers treated with: Lanes 1, CON (no hormone control); 2, ABA; 3, GA_3. The following lanes show polypeptides from the same mRNAs immunoadsorbed with antibodies towards: 4 to 6, α-amylase 2 and ASI (4 d exposure); 7 to 9, CI-1 (28 d exposure); 10 to 12, PAPI (21 d exposure). The faint bands seen at $M_r \sim 45–36 \times 10^3$ are nonspecifically adsorbed polypeptides seen in longer exposures (compare with Fig. 3, lane 3).

tively, presumably guard the seed reserves during dormancy.

mRNAs encoding CI-1 and PAPI are long-lived in aleurone layers and survive seed desiccation (Fig. 4, lanes 9 and 12) Although traces of PAPI and CI-1 could also be measured with the mature endosperm preparation (Table II), the overall data show that mature aleurone cells contain significantly more translatable mRNA than those of the starchy endosperm (Table I). This difference is presumably due to greater degradation of mRNA in mature starchy endosperm cells which are essentially dead at maturity (Table I, mature and imbibed tissue). It is unclear whether the long-lived mRNAs encoding PAPI and CI-1 are translated in aleurone cells at the onset of germination (see below).

Only mRNAs encoding ASI, PAPI, and CI-1 are present in significant amounts in cultured aleurone layers (Fig. 5, lanes 4–12). In general, the results of the immunoadsorption experiments indicate that the levels of mRNAs encoding different salt-soluble seed proteins are differentially regulated in cultured aleurone layers. Some cannot be detected at all (protein Z, β-amylase, and CI-2, PSI, protein C), while those encoding CI-1 and PAPI are present regardless of hormone treatment and those encoding ASI are enhanced by ABA and reduced by GA (see below). In a parallel study we have shown that PAPI is synthesized *in vivo* by cultured aleurone layers although it is not known whether this synthesis is by translation of long-lived mRNA or of newly transcribed message (J Mundy, JC Rogers, unpublished results). Run-off transcription experiments, using a full-length cDNA PAPI probe, are in progress to answer this question. In any event, the different patterns of synthesis of PAPI, CI-1, and ASI make them model candidates for processing studies in aleurone cells. Studies on the intracellular deposition and/or secretion of these proteins might clarify the purported differences between endosperm and aleurone protein bodies (1, 8, 28).

Of the seed proteins tested here, only ASI shows differential hormone regulation by ABA and GA (Fig. 5, lanes 4–7). The enhancement of ASI synthesis by ABA is interesting (a) because ASI mRNA is not detectable in developing aleurone layers and (b) its regulation is anatagonistic to that of α-amylase, an enzyme which ASI specifically inhibits (20, 29). These results indicate that a fine control of the level of ASI is required in aleurone tissue, and suggests that endogenous α-amylase activity may be controlled by ABA and GA at the transcriptional level both directly and via ASI (14, 24). Further work is needed to demonstrate whether ASI polypeptides synthesized in aleurone layers bind to α-amylase molecules during their synthesis and/or transport and secretion in the ER and Golgi apparatus (8, 16).

The ABA-enhanced polypeptides of cultured aleurone layers represent a specific response by that tissue to ABA (Fig. 2, lanes 7 to 9; Fig. 5). Furthermore, the pattern of ABA-enhanced polypeptides, with major *in vitro* products of $M_r \sim 40, 35, 25,$ and $15–13 \times 10^3$, is very similar to that seen from dormant aleurone tissue prepared from mature seeds (Fig. 2, lanes 6 *versus* 8). These results parallel those of other studies showing ABA-stimulation of specific polypeptides in developing seed tissues (6, 7), and are in keeping with theories of the role of ABA as a dormancy/senescence hormone in plants (27).

In conclusion, the present communication identifies proteins whose synthesis is tissue and time-course specific during seed development and which are differently regulated by ABA and GA in cultured aleurone cells. These results widen the selection of known proteins for gene expression studies in barley seed tissues. Genes encoding several of these proteins are now being

studied to identify sequences associated with, or responsible for, their differential expression.

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