Developmental Biochemistry of Cottonseed Embryogenesis and Germination: Changing Messenger Ribonucleic Acid Populations As Shown by in Vitro and in Vivo Protein Synthesis†

Leon Dure III,* Sally C. Greenway,‡ and Glenn A. Galau

ABSTRACT: Changes in messenger ribonucleic acid (mRNA) populations during embryogenesis of cottonseed have been followed by cataloging (a) extant proteins, (b) proteins synthesized in vivo, and (c) proteins synthesized in vitro from extracted RNA, all at specific stages of embryogenesis. Evidence is presented for the existence of five mRNA subsets, all apparently under different regulatory regimes, that produce the abundant proteins of embryogenesis. One of these functions principally during the cell division phase of embryogenesis and encodes among its products the seed storage proteins whose mRNA is superabundant during this period. This subset has disappeared from the abundant group by the mature seed stage. Two other subsets appear in late embryogenesis, one of which may result from the removal of the embryo from the maternal environment, since it is inducible by excision of the young embryo from the seed. The other appears to be induced by the plant growth regulator abscisic acid, whose endogenous concentration increases at this stage. It can be induced by incubating excised young embryos in abscisic acid. The last two subsets exist throughout embryogenesis, but only one of them appears to function in germination.

In order to establish a background for obtaining genomic clones of coordinately expressed genes in a developing system, we have examined the rise/fall of the abundant, functional mRNAs[1] present in cotyledons of cotton embryos during their embryogenesis. This has been done by analyzing two-dimensional (2D)[1] gels of stained proteins (extant proteins), fluorographs of 2D gels of labeled protein from embryos incubated for short periods in radioactive amino acids (measure of in vivo synthesized proteins), and fluorographs of 2D gels of proteins synthesized in vitro in the wheat germ system from isolated RNA. From these three catalogs we have recognized five mRNA subsets that appear to be under different regulatory regimes and that, taken together, give rise to ~90% of the abundant class of proteins. The existence of these subsets has been supported to some extent by an analysis of mRNA populations during embryogenesis by cDNA–RNA hybridization experiments, the results of which are presented in the following paper (Galau & Dure, 1981).

The stages of development used in the construction of the protein–mRNA catalogs were those representing the cell division stage (referred to as the "young embryo" stage hereafter), the maturation stage (referred to as the "older embryo" stage hereafter), and the mature seed (referred to as the "mature embryo" stage hereafter). To distinguish between certain subsets, we followed the presence/disappearance of mRNAs into the first day of germination as well. Immature embryos removed at any point during the cell division or maturation phase will germinate precociously and form viable plants (Ihle & Dure, 1969). This premature germination of excised embryos can be prevented by incubating the embryos in abscisic acid (ABA) at 10^{-6} M (Ihle & Dure, 1970). The concentration of this naturally occurring plant growth regulator increases in cottonseeds in late embryogenesis and is probably the organism's mechanism for preventing "vivipary" (in situ germination of immature embryos inside the cotton boll considered a genetic lethal mutation).

Use of Catalogs To Identify mRNA Subsets. The delineation of the various mRNA subsets is based on what proteins are readily visible as stained or radioactive species on gels at the several developmental stages. Thus, the subsets are comprised only of abundant species. Although there is no obligatory relationship between protein and mRNA abundancy, in most instances proteins that are heavily labeled in in vivo synthesis are recognized as abundant proteins on stained gels. There are, however, exceptions, likely the result of rapid protein turnover. Still other proteins remain abundant in the tissue after their mRNAs are no longer demonstratable by in vivo or in vitro synthesis, the storage proteins being the most notable. The in vivo synthesis catalog serves as the primary basis for subset membership, since it is assumed that proteins whose synthesis is extensive during the incubation in isotopes emanate from abundant mRNAs. The in vitro synthesis catalog, which should reflect the intrinsic concentration of the various mRNAs, has been used to verify this assumption. However, because of the limitations of the wheat germ system which severely underrepresents the population of large mRNAs, this verification is most apparent in the case of proteins of molecular weight below 35 000.

Although subset membership is based on entry into or disappearance from the abundant mRNA class, this does not imply a transcriptional control of these sequences. They most likely exist in the sparse class at developmental stages when their existence is not demonstratable in the catalogs (Galau & Dure, 1981). No attempt has been made to assign mRNAs for all the proteins seen on the gels to specific subsets, but rather to score the proteins (~80 total) that are representative of the most obvious subsets. Neither has there been an attempt to score small quantitative differences in concentration or

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‡ Present address: Plant Sciences Department, Cardiff University, Cardiff, South Wales, Great Britain.

[1] Abbreviations used: 2D, two dimensional; ABA, abscisic acid; NaDodSO$_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; poly(A)+, polyadenylated; RNA, ribonucleic acid; mRNA, messenger RNA; cDNA, complementary deoxyribonucleic acid.

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radioactivity that occasionally are observed between proteins of a given subset at different developmental stages. In fact, most of the members of a given subset seem to bear a constant relationship in amount one to another.

Experimental Procedures

Plant Material and Protein Extraction. Cotton plants (*Gossypium hirustum*, var. Coker 201) were greenhouse grown. To obtain embryonic cotyledons, representing the several developmental stages, we harvested embryos with a wet weight of 50 mg as representing the "cell division" phase of embryogenesis (young embryo stage) and embryos with a wet weight of 110 mg taken to represent the "maturation" phase (older embryo stage). (The mature but undissecated embryo weighs ~125 mg.) Mature embryo cotyledons were taken from dry seeds, and, for germinating cotyledons, embryos were dissected from dry seeds and incubated for varying periods between layers of moist filter paper. The protein content of cotyledons was either extracted in mass with 2% $NaDodSO_4$, 1% mercaptoethanol, and 0.05 M Tris-HCl (pH 8.3) at a concentration of 1 cotyledon pair/2 mL, which yields the "total protein" fraction, or divided into two subfractions, the "soluble" fraction and the "pellet" fraction. The soluble fraction was obtained by homogenizing cotyledons in 0.1 M NaCl, 1% mercaptoethanol, and 0.05 M Tris-HCl (pH 8.3) and taking the supernatant after extracting the tissue for 6 h at room temperature. The pellet fraction was obtained by reextracting the insoluble pelleted material obtained above with 2% $NaDodSO_4$, 1% mercaptoethanol, and 0.05 M Tris-HCl (pH 8.3) for 6 h at room temperature and taking the supernatant after centrifugation. The extracted proteins were precipitated by making the extracts 95% in acetone. These procedures solubilize over 95% of the cotyledon protein (Dure & Chlan, 1981).

In Vivo Protein Synthesis. For determination of the proteins synthesized in intact cotyledons at the various developmental stages, young embryos, older embryos, and mature embryos were harvested, rinsed in distilled water, placed between layers of filter paper moistened with isotope-containing solutions, and incubated in darkness for 6 h. The filter paper was moistened with a solution containing the ^{14}C-labeled algal hydrolysate (Amersham Corp., 50 mCi/mg-atom, 5 μCi/mL), gramacidin D (10 μg/mL), and, where indicated, ABA (5×10^{-6} M). After the incubation period, the embryos were rinsed in distilled water, axes removed and discarded, and the cotyledons extracted for protein as given above. In the pulse–chase experiment, the mature embryos were removed from the filter paper containing the ^{14}C-labeled algal hydrolysate after 6 h of incubation, rinsed well, and incubated an additional 18 h in gramacidin solution. To measure synthesis after several days of incubation with or without ABA, we incubated the dissected, rinsed young embryos on filter paper wetted with the gramacidin solution with or without ABA for 3.5 days in darkness, then transferred them to filter paper wetted with the same medium containing the isotopes, and incubated them for an additional 12 h.

Measurement of Protein Synthesis with the Wheat Germ System (in Vitro Synthesis). Wheat germ supernatant was prepared according to Marcu & Dudock (1974) with potassium acetate replacing potassium chloride. Translation reaction mixtures contained, in a final volume of 50 μL, 35 mM Hepes–KOH (pH 7.5), 1 mM ATP, 0.4 mM GTP, 8 mM creatine phosphate, 100 μg/mL creatine kinase, 2 mM magnesium acetate, 0.15 M potassium acetate, 8.5 mM dithiothreitol, 0.3 mM phenylmethanesulfuryl fluoride, 25 μM each of unlabeled amino acids, 4.5 μM L-[3,4,5-$^3H_3$]leucine (110 Ci/mmol), 18 μL of wheat germ supernatant, and 20–30 μg of total high molecular weight cotton RNA. Incubation was for 90 min at 25 °C after which the mixtures were treated with RNase A (100 μg/mL) for 15 min at 37 °C and precipitated with 10 volumes of acetone. The wheat germ system in our hands (and in the hands of others) has a very slow rate of translation that equates to an average mRNA reading number of only 2–4 per 90-min reaction time (Dure & Galau, 1981). Thus, the 2D fluorographs of in vitro synthesis products show a background of many unfinished polypeptide chains. Limitations of the wheat germ system are discussed in Dure & Galau (1981).

Preparation of Cotton RNA for Translation. Total high molecular weight RNA was prepared from cotyledons by a protocol that involved solubilization by $NaDodSO_4$, deproteinization with phenol and chloroform, and salt precipitations with LiCl and sodium acetate. The details of this procedure have been presented elsewhere (Galau et al., 1981).

Isoelectric Focusing and Electrophoresis. The two-dimensional separation of proteins was performed as described by O'Farrell (1975) with the following modifications. The mixture of ampholines in the isoelectric focusing gel, the overlay solution, and the protein sample was 0.7% each of pH range 3.5–10, 4–6, and 5–8. The sample (in 8 M urea) was applied to the acid end of the gel by underlayering a 7.5 M urea overlay solution. This procedure allows nucleic acids, residual $NaDodSO_4$, and other acidic molecules in the sample to migrate away from the gel rather than transversing it. Samples were prepared for focusing by dissolving acetone precipitates at ~10 μg/μL in the ampholine–urea solution that also contained 2% mercaptoethanol. The second-dimension separation employed the Laemmli system (1970) (10–17% acrylamide gradient) as described by O'Farrell (1975). Gels were stained with Coomassie Brilliant Blue R-250 and fluorographed as described by Lasky & Mills (1975).

Results

In analyzing the composition of the abundant class of proteins existing in the tissue at a given developmental stage and in determining what proteins are being synthesized in abundant amounts at these stages, it has been found beneficial to divide the total tissue protein into the two fractions (soluble and pellet) as outlined under Experimental Procedures. This division of protein puts ~50% of the total embryo protein into each fraction, allows a greater amount of each fraction to be loaded onto the electrophoretic system, and, hence, allows more protein species to be visualized by staining or fluorography. In some cases the total, unfractionated protein complement, solubilized by $NaDodSO_4$, has been visualized by the 2D electrophoresis system.

Embryogenic in Vivo Synthesis. In Figure 1, gels A–C, the stained gel pattern of pellet protein from young embryos (A), older embryos (B), and mature embryos (C) is given. The proteins of this fraction are all contained in purified "protein bodies" (Dure & Chlan, 1981) and, thus, probably represent the nutritional storage proteins that are used by the embryo in germination as a nitrogen–amino acid source. In (C), the principal storage proteins of the mature embryo are seen (upper left quadrant) as two protein sets of about 52 kilodaltons that exhibit p*I* heterogeneity. In (A), two heavier protein sets of about 70 and 67 kilodaltons are observed (indicated by arrows) which are found to decrease relative to the other proteins in the older embryos and to have disappeared by the mature embryo stage. We have found that they are long-lived precursors to the 52- and 48-kilodalton sets (Dure & Galau, 1981). In fact, antibodies formed to either the 52-

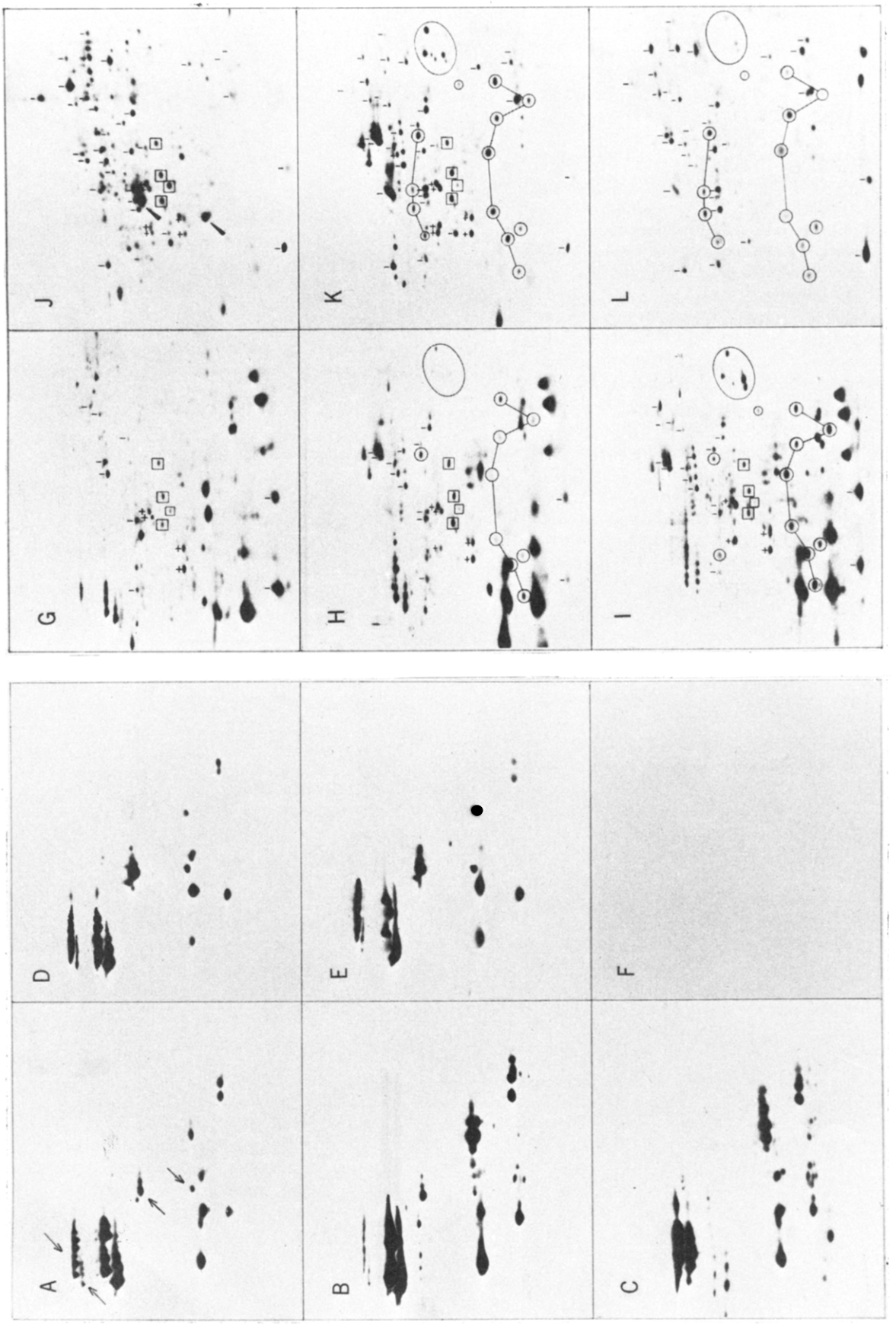
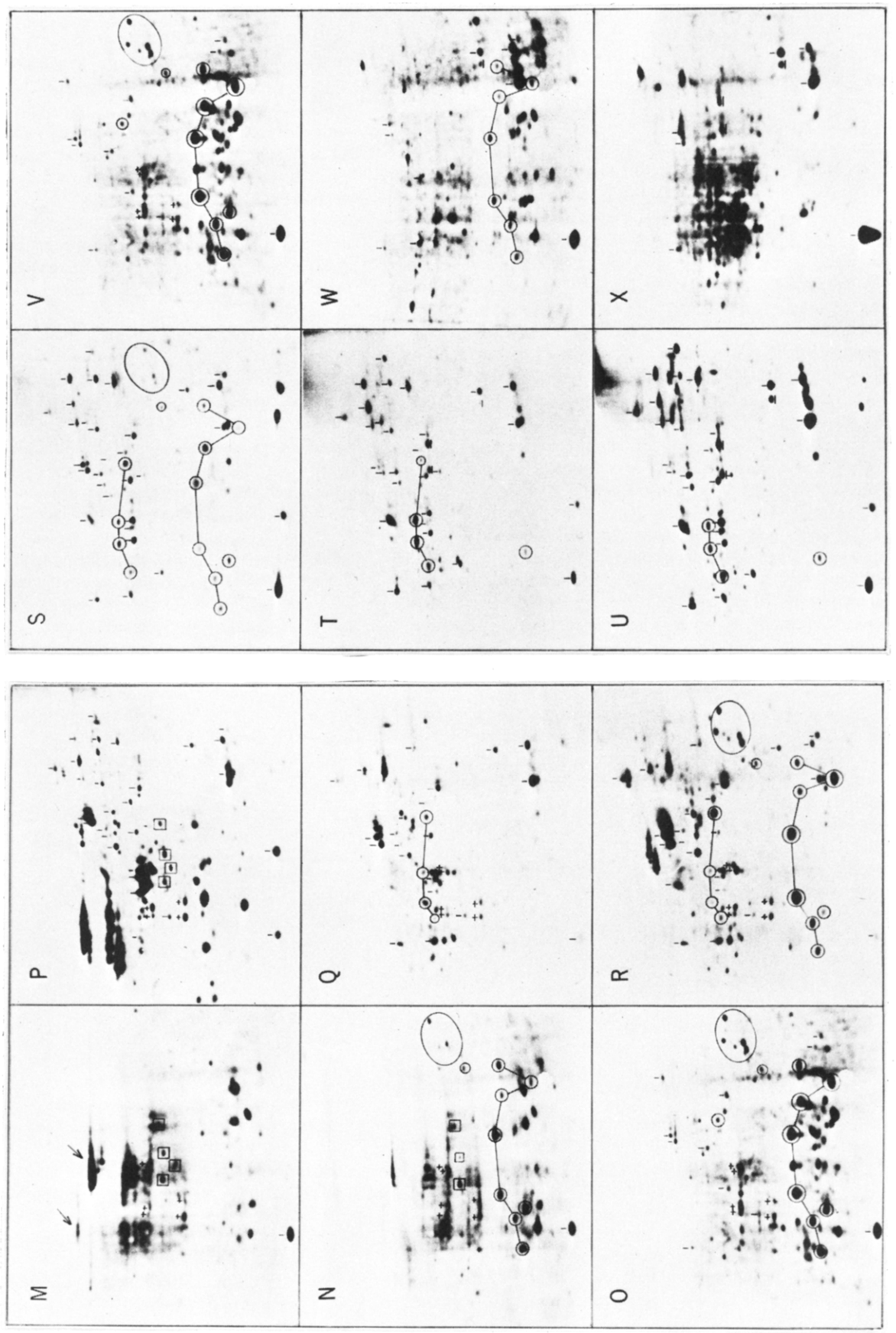


FIGURE 1: Two-dimensional electrophoretic pattern of stained proteins (A–C, G–I); in vivo synthesized proteins (D–F, J–L, P–U); in vitro synthesized proteins (M–O, V–X) from stages of embryogenesis and germination. In the first dimension, isoelectric focusing occurred between pH 4.4 (right side) and 7.4 (left side). Samples were loaded on the acidic end of the cylinders. In gels A–C, pellet protein from 0.05 cotyledon pair of young embryos (A), from 0.02 cotyledon pair of older embryos (B), and from 0.01 cotyledon pair of mature embryos (C) was used. Gels D–F are fluorographs of companion gels to gels A–C. (D) was exposed to X-ray film for 20 days, (E) for 40 days, and (F) for 100 days. In gels G–I, soluble protein from 0.08 cotyledon pair of young embryos (G), from 0.03 cotyledon pair of older embryos (H), and from 0.015 cotyledon pair of mature embryos (I) was used. Gels J–L are fluorographs of companion gels to gels G–I. (J) was exposed for 20 days, (K) for 40 days, and (L) for 60 days. Gels M–O are fluorographs of proteins synthesized in vitro in the wheat germ system from total RNA extracted



from young embryos (M), from older embryos (N), and from mature embryos (O). Gel P is a fluorograph of the total protein synthesized in vivo in young embryo cotyledons. Gels Q and R are fluorographs of the total protein synthesized in vivo in young embryo cotyledons after 4 days of precocious germination (Q) and after 4 days of incubation in ABA at 5×10^{-6} M (R). Protein from 0.05 cotyledon pair was used in each case. Gels S–U are fluorographs of total protein synthesized in vivo by mature embryo cotyledons (S) and in mature embryo cotyledons after 24 h of germination (U) and of the total protein of mature embryo cotyledons that is radioactive after an 18-h chase period following the incubation in isotopes (T). Protein from 0.015 cotyledon pair was used in each case. Exposure times were 60 days (S and T) and 30 days (U). Gels V–X are fluorographs of proteins synthesized in vitro from total RNA extracted from mature embryo cotyledons (V) and from cotyledons of mature embryos germinated 12 h (W) and 24 h (X).

or 48-kilodalton sets from the mature embryo will sequester all four protein sets indicating that they may be members of a multigene family with much sequence homology. The 70- and 52-kilodalton sets are glycosylated, suggesting that the 70-kilodalton set gives rise to the 52-kilodalton set, leaving the 67-kilodalton set to give rise to the 48-kilodalton set. The smaller fragments resulting from the cleavage of the precursors may persist in the protein bodies and constitute some of the smaller proteins seen in gels A–C. Two other abundant proteins [indicated by arrows in (A)] also disappear as embryogenesis progresses. We have some evidence that the heavier of these may be an actin species.

Gels D–F are fluorographs of companion gels of A–C showing the labeling pattern of the proteins of this fraction when the embryos are exposed to radioactive amino acids for 6 h. Most of the proteins of (A) and (B) are radiolabeled (D and E). However, when mature embryos are exposed to the isotopes, essentially no in vivo synthesis of these proteins is detected (F). This suggests that, by maturity, the mRNA for these proteins has vanished from the abundant class.

Gels G–I are of stainable abundant proteins found in the soluble protein fraction from the same three stages, young embryo in (G), older embryo in (H), and mature embryo in (I). Trace contamination of this fraction with the larger storage proteins can be seen at the upper left of these gels. Gels J–L show the in vivo labeling pattern of the proteins of this fraction obtained with the 6-h pulse. In (J) two radioactive proteins that are mostly confined to the pellet fraction are indicated with arrows. A close examination of J–L shows a large number of proteins that are synthesized at all three stages. These are indicated by a perpendicular tick mark above them. Many of this group are abundant enough to be identified in the stained patterns and have been marked in gels G–I as well. This group of proteins–mRNAs is clearly constitutive to the developmental time span studied. There is another group of proteins, indicated by crosses above them, that, although apparent in young and older embryos, are not found among the radioactive abundant proteins of the mature embryo. However, we consider these proteins constitutive to this developmental span also because (a) they can be identified as products of in vitro synthesis with RNA from all three developmental stages and (b) they are identifiable in young embryos that have been excised from the seed and incubated with and without ABA for several days. This procedure brings about the disappearance of the mRNAs that are programmed to disappear in late embryogenesis, as subsequent data will show. The absence of these proteins in gel L must indicate their rapid destruction in the mature embryo during the 6-h incubation, which in reality is the first 6 h of germination. Those constitutive species that are observable in gel L are also recognized in in vivo synthesis after 24 h of germination (data presented later) and may be constitutive to germination as well.

There is a group of proteins synthesized in both sets of immature embryos whose synthesis cannot be demonstrated in mature embryos either in vivo or in vitro. These protein species are enclosed in boxes in all the gels where they are seen. We believe that they represent a subset whose mRNAs leave the abundant class in late embryogenesis just as do those for the storage proteins. They are observable in the stained gels G and H and also in gel I. Their presence in (I) makes their behavior analogous to the storage proteins of the pellet fraction in that the proteins themselves persist, whereas their mRNAs are no longer demonstrable by in vivo or in vitro synthesis.

There is a third group of proteins whose in vivo synthesis is not apparent in young embryos but is readily apparent in older and mature embryos. These species are encircled and some of them linked into constellations for orientation purposes. One part of this group occurs as a cluster, and the entire cluster is encircled. Most of the proteins of this group become abundant enough to be seen in the stained gels. They are faint in older embryos (H) but quite obvious in mature embryos (I).

Embryogenic in Vitro Synthesis. Gels M–O show the products of in vitro translation of mRNA derived from the young embryo (M), the older embryo (N), and the mature embryo (O). The most startling aspect of these gels is the apparent lack of correspondence between them and the gel set portraying in vivo synthesis. As pointed out under Experimental Procedures, this is due in part to the fact that many unfinished polypeptides are present in the in vitro products and most of the high molecular weight products are underrepresented because of the nuclease–protease activity inherent in the wheat germ system. Furthermore, the initial translation products of the principal storage proteins are quite different in size from the long-lived precursors noted in gels A, B, D, and E (Dure & Galau, 1981). These initial products are indicated by arrows in gel M. The sequence of events that transforms the initial products (about 69 and 60 kilodaltons) into the long-lived precursors (70- and 67-kilodalton sets) is thought to involve loss of signal peptides and glycosylation as the initial products are processed into the protein body vesicles. Distinguishable in gels M–O are all of the constitutive species that rapidly vanish from the mature embryo (not found in gel L), and they are denoted again by crosses. This confirms the fact that their mRNAs are present in the mature embryo. Also present in the products from young (M) and older (N) embryos are the disappearing subset members, i.e., the storage protein initial translates and the boxed species. None of these products are obtained with mature embryo RNA (O). Furthermore, some members of the group that appears in late embryogenesis are readily obvious in the older embryo and mature embryo products (encircled). In fact, the mRNAs for these proteins are the most abundant subset in mature embryos.

In Vivo Synthesis in Excised Immature Embryos. In the foregoing, data have been presented that suggest at least three independent mRNA subsets function in embryogenesis, i.e., one that is constitutive, one that disappears, and one that appears. The reality of these putative subsets can be tested by observing the pattern of proteins synthesized by young embryos excised and allowed to precociously germinate for several days. The pattern obtained is presented in gel Q and is compared with the pattern of synthesis found in young embryos pulsed for 6 h directly after excision before precocious germination has begun (gel P). These two gels show the pattern of synthesis obtained with *total* protein. (Thus, gel P is a summation of gels D and J.) In both (P) and (Q) the constitutive subsets can be identified. However, the subset disappearing in late embryogenesis (boxed species and storage proteins) is not evident in Q, whereas *some* of those proteins that appear in late embryogenesis (circled species) have appeared in these young embryos germinating precociously. Other members of this group are not evident in this gel. If the excised young embryos are incubated several days in the presence of ABA, which prevents their precocious germination, the pattern obtained of total, abundant proteins synthesized is given in gel R. Here most of the constitutive species are seen, the disappearing subset has disappeared, and *all* of the late-appearing species are evident. In the case of both (Q) and (R), the embryos have been removed from the maternal environment prematurely and, in the case of (R), have been

exposed to a high level of ABA prematurely. In both cases the synthesis of those proteins destined to cease in late embryogenesis (shown by their absence in gels F and L) has ceased. Those species that arise in late embryogenesis as the ABA level increases seem, from the patterns in Q and R, to represent two distinct subsets. One subset (the heavier constellation) has been induced prematurely in both groups of excised embryos, perhaps in response to the removal from the seed, whereas the other subset appears only in those embryos exposed prematurely to a high level of ABA (lower constellation, circled cluster). This latter subset may be induced by the hormone and play a role in the hormone-mediated prevention of germination, a process known to require RNA synthesis (Ihle & Dure, 1972).

Germination Synthesis. To further delineate these presumptive subsets, we followed the in vivo and in vitro synthesis of proteins into germination, and the synthesis patterns are shown in gels S–X. Gel S is identical with gel L, showing in vivo synthesis in mature embryos labeled 6 h. Gel T gives the pattern obtained when these mature embryos labeled 6 h are removed from the isotope incubation medium and allowed to germinate for an additional 18 h without isotopes (a pulse–chase). Gel U presents the pattern obtained from mature embryos that have germinated normally for 24 h and have been pulsed for the last 6 h of this period, i.e., this pattern shows the proteins synthesized during early germination. Comparing (T) with (S) shows that during the chase period the putative ABA-induced protein subset has been degraded, whereas the other late-embryogenesis subset persists. Further, gel U shows that this latter subset is still being synthesized during the 18–24-h period of germination, whereas there is no sign of the ABA-induced subset. Also notable in gels T and U is the fact that the group of constitutive proteins of embryogenesis that are denoted by the tick marks continue to be synthesized in germination. On the other hand, those embryonic constitutive proteins that seem to vanish rapidly when the mature embryo begins germination (seen in gel O but not L and marked with crosses) are not found in gels T or U. For this reason the mRNAs that appear to be constitutive to embryogenesis have been divided from a regulatory point of view into two distinct subsets, those that are also constitutive to early germination and those that are not.

Gels V–X give the patterns of in vitro synthesis from RNA from mature embryos (V, which is identical with O), from 12-h germinated embryos (W) and from 24-h germinated embryos (X). The putative ABA-induced subset that dominates the mature embryo pattern is found to be much decreased by 12 h of germination and not visible by 24 h. Some members of the subset constitutive to both embryogenesis and early germination are recognizable in these gels of RNA products from germinating embryos (tick marked in gels W and X), but many are not, nor are the members of the subset that become demonstrable in late embryogenesis and continue to be synthesized in germination. The severe limitations of the wheat germ system, we believe, are the cause of this.

It would be expected that during germination new proteins would appear in the abundant class. This is apparent in gel X but is less so in gel U. Some new members of this class that map in areas of the gels that are easily interpreted can be recognized in gels T, U, W, and X and have been designated with a horizontal line beneath them.

Discussion

The five mRNA subsets that have been identified in this period of the organism's ontogeny can be characterized as follows. Subset 1 has 32 members and is constitutive to embryogenesis and early germination (tick marked). Subset 2 has six members and is constitutive to embryogenesis only (cross marked). It is found in mRNA from mature embryos by in vitro translation but is not observed in in vivo synthesis by mature embryos. It is presumably rapidly destroyed in the first hours of germination. Subset 3 has an undetermined number of members, disappears in late embryogenesis, and is not observed in mature embryos. This subset is principally comprised of the storage proteins but has a few readily soluble protein members as well (boxed species). Subset 4 has five members, becomes abundant in late embryogenesis, and is synthesized in abundance in early germination (four members in higher molecular weight circled constellation). It can be induced prematurely in young embryos by excision from the fruit and incubation in water (precocious germination). Subset 5 has 14 members. It becomes abundant in late embryogenesis, but disappears during the first hours of germination (low molecular weight circled constellation, circled cluster). This subset can be induced prematurely in young embryos by excision from the fruit and incubation in ABA (precocious germination arrested).

It is, of course, not surprising that changes in the abundancy of mRNA species have been found to occur during this developmental span since similar changes have been noted in other systems (Alton & Lodish, 1977; Bravo & Knowland, 1979). A case can be made that a tissue's uniqueness is determined by which mRNAs (and their protein products) are in the abundant group. Thus, developmental changes should be reflected in changes in the abundant mRNA–proteins. It is, therefore, not surprising that in the data presented there is an apparent relationship between the flux in the abundant mRNA species and previously observed ontogenic phases of the tissue. When these embryos progress from the cell division phase to the maturation phase, several events occur that are likely to be interrelated from a developmental point of view. First, cell division decreases, and by the older embryo stage no further increase in cell number takes place (Walbot & Dure, 1976). If young embryos are removed from the fruit and allowed to germinate precociously, cell division stops prematurely, giving rise to small, yet viable, seedlings (Ihle & Dure, 1972). The cessation of cell division takes place even in excised embryos whose precocious germination is arrested by ABA. Second, the incipient seed (embryo and nutritive tissues) becomes more of a closed system during this transition as the vascular connection between the seed and the mother plant degenerates. Third, the level of ABA in the seed rises, which prevents the viviparous (premature and lethal) germination of the embryo.

As for mRNA population changes against this background, perhaps the most striking is the disappearance of the storage protein mRNAs from the abundant class by the mature embryo stage. Their disappearance can be brought about prematurely by removing the immature embryo from the fruit and allowing precocious germination to commence (gel Q). Furthermore, they disappear in those excised immature embryos whose precocious germination is prevented by incubation in ABA (gel R). The disappearance of these mRNAs can also be demonstrated by in vitro synthesis programmed with RNA extracted from young embryos that have been incubated with and without ABA for four days. Neither the 69- or 60-kilodalton protein sets are synthesized by these RNAs (data not shown). It is tempting to speculate that the maintenance of these mRNAs as abundant species is dependent upon factors provided by the mother plant.

Some of those mRNAs that become abundant during this developmental transition and persist as abundant mRNAs into germination (subset 4) may also be related to the loss of maternally derived factors, since their premature appearance can be brought about simply by removal from the fruit, regardless of whether they are allowed to precociously germinate (gel Q) or have this germination prevented by ABA (gel R). Those species that arise in late embryogenesis but disappear rapidly in germination (subset 5) may truly constitute a response to ABA. They can be induced prematurely in excised young embryos only when they are incubated in the presence of the hormone (gel R). In general, this plant growth regulator increases in concentration in plant tissues destined to become quiescent or in tissues placed under environmental stress (water stress, chilling stress, etc.) where its increase appears to be a survival adaptation by decreasing metabolic activity. ABA has been shown to prevent certain macromolecular syntheses in germinating barley seeds (Yomo & Varner, 1971) as it prevents germinative syntheses in cotton embryos. In both cases its mode of action requires concomitant RNA synthesis (Ihle & Dure, 1972; Ho & Varner, 1976), and in barley germination, as here, it seems to elicit a new array of proteins (Jacobsen et al., 1979; Mozer, 1980). In this, its action seems analogous to the heat shock phenomenon in *Drosophila* (Ashburner & Bonner, 1979). It is again tempting to speculate that the hormone increases in response to the loss of water pressure brought on by the atrophy of the vascular tissues connecting the seed with the mother plant. The level of ABA declines markedly as the cottonseed desiccates and does not confer dormancy to the seeds of this species. Colaterally, the ABA mRNA vanishes from the abundant class in early germination (gels T, U, W, and X).

The putative ABA-induced proteins are still visible in stained gels after 24 h of germination, although their mRNAs are not demonstrable. These proteins are no longer evident on gels of proteins from cotyledons germinated 48 h. It may be relevant that in cotton and in many other dicotyledoneous seeds that undergo embryogenic development similar to that of cotton, the enzyme activities thought to be unique to germination do not become demonstrable until after the first day of germination (Hock & Beevers, 1966; Radin & Trelease, 1976; Delseny et al., 1976; Becker et al., 1978; Ihle & Dure, 1969; Dilworth & Dure, 1978). Their synthesis may require the degradation of the ABA-induced proteins.

Of the 80-odd abundant proteins that can be identified on these gels with certainty and that have been used to construct the mRNA subsets, about half of them (subsets 1 and 2) are constitutive to embryogenesis and most of these (subset 1) are abundantly synthesized in germination as well. Other than the vesicle-bound nutritive storage proteins (principal members of subset 3), the functions of none of the abundant proteins appearing in this segment of the tissue's ontongeny are known. Of interest will be the function of the putative ABA-induced proteins, which, like the heat shock proteins, appear to act as metabolic repressors.

It is apparent that by utilizing subtractive cDNA–RNA hybridization techniques, cDNA populations highly enriched for subset 3 sequences (storage proteins) and subset 5 sequences (ABA-induced proteins) should be obtainable for cloning, for obtaining genomic clones, etc., experiments which are in progress.

References

Alton, T. H., & Lodish, H. F. (1977) *Dev. Biol. 60*, 180–206.

Ashburner, M., & Bonner, J. J. (1979) *Cell (Cambridge, Mass.) 17*, 241–254.

Becker, W. M., Leaver, C. J., Weir, E. M., & Rieman, H. (1978) *Plant Physiol. 62*, 542–548.

Bravo, R., & Knowland, J. (1979) *Differentiation (Berlin) 13*, 101–108.

Delseny, M., Got, A., & Guitton, Y. (1976) *Physiol. Veg. 14*, 159–178.

Dilworth, M. J., & Dure, L. S., III (1978) *Plant Physiol. 61*, 698–702.

Dure, L., III, & Chlan, C. (1981) *Plant Physiol.* (in press).

Dure, L., III, & Galau, G. A. (1981) *Plant Physiol.* (in press).

Galau, G. A., & Dure, L., III (1981) *Biochemistry* (following paper in this issue).

Galau, G. A., Legocki, A. B., Greenway, S. C., & Dure, L. S., III (1981) *J. Biol. Chem. 256*, 2551–2560.

Ho, D. T., & Varner, J. E. (1976) *Plant Physiol. 57*, 175–178.

Hock, B., & Beevers, H. (1966) *Z. Pflanzenphysiol. 55*, 405–412.

Ihle, J. N., & Dure, L. S., III (1969) *Biochem. Biophys. Res. Commun. 36*, 705–710.

Ihle, J. N., & Dure, L. S., III (1970) *Biochem. Biophys. Res. Commun. 38*, 995–1000.

Ihle, J. N., & Dure, L. S., III (1972) *J. Biol. Chem. 247*, 5048–5055.

Jacobsen, J. V., Higgins, T. J. V., & Zwar, J. A. (1979) in *The Plant Seed* (Rubenstein, I., Phillips, R. L., Green, C. E., & Gengenbach, B. G., Eds.) pp 241–262, Academic Press, New York.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lasky, R. A., & Mills, A. D. (1975) *Eur. J. Biochem. 56*, 335–341.

Marcu, K., & Dudock, B. (1974) *Nucleic Acids Res. 1*, 1385–1397.

Mozer, T. J. (1980) *Cell (Cambridge, Mass.) 20*, 479–485.

O'Farrell, P. H. (1975) *J. Biol. Chem. 250*, 4007–4021.

Radin, J. W., & Trelease, R. N. (1976) *Plant Physiol. 57*, 902–905.

Walbot, V., & Dure, L. S., III (1976) *J. Mol. Biol. 101*, 503–536.

Yomo, H., & Varner, J. E. (1971) *Curr. Top. Dev. Biol. 6*, 111–114.