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Evidence That Glyoxysomal Malate Synthase Is Segregated by the Endoplasmic Reticulum[1]

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ABSTRACT

At the onset of castor bean (*Ricinus communis*) germination, 76% of the cellular malate synthase activity of the endosperm tissue was located in the microsomal fraction, with the remainder in the glyoxysomal fraction. During later developmental stages, when rapid malate synthase synthesis was occurring, an increasing proportion of the enzyme was recovered in glyoxysomes. The kinetics of [^{35}S]methionine incorporation into microsomal and glyoxysomal malate synthase in 2-day-old endosperm tissue was followed by employing antiserum raised against glyoxysomal malate synthase to precipitate specifically the enzyme from KCl extracts of these organelle fractions. This experiment showed that microsomal malate synthase was labeled before the glyoxysomal enzyme. When such kinetic experiments were interrupted by the addition of an excess of unlabeled methionine, ^{35}S-labeled malate synthase was rapidly lost from the microsomal fraction and was quantitatively recovered in the glyoxysomal fraction.

Free cytoplasmic ribosomes were separated from bound ribosomes (rough microsomes) using endosperm tissue labeled with [^{35}S]methionine or ^{14}C-amino-acids. Nascent polypeptide chains were released from polysome fractions using a puromycin-high salt treatment, and radioactive malate synthase was shown to be exclusively associated with bound polysomes.

Together these data establish that malate synthase is synthesized on bound ribosomes and vectorially discharged into the endoplasmic reticulum cisternae prior to its ultimate sequestration in glyoxysomes.

In the preceding paper (7) we confirmed the finding of Gonzalez and Beevers (11) that during the early stages of castor bean germination, a significant proportion of the cellular malate synthase activity is recovered in the microsomal fraction. The purified microsomal and glyoxysomal malate synthase proteins are identical in all of the properties we have examined. When isolated glyoxysomes are deliberately broken by osmotic shock, their malate synthase activity is almost completely recovered in the microbody membrane "ghosts," although not an integral membrane protein (2, 14). It therefore seems likely, as suggested previously (11), that any newly synthesized malate synthase temporarily housed in the ER cisternae prior to sequestration in glyoxysomes might likewise remain associated with the membranous components (microsomes) recovered after the inevitable disruption of the ER structure that occurs during tissue homogenization.

In the present paper we provide unequivocal evidence that microsomal malate synthase is ultimately recovered in the glyoxysomal fraction. Since malate synthase activity is largely absent from the soluble fraction recovered during cell fractionation experiments, we are able to present a scheme for the intracellular pathway of malate synthase transport prior to its isolation in glyoxysomes which meets all of the requirements of the accepted model for the segregation of microbody enzymes by the ER.

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MATERIALS AND METHODS

Isolation and Treatment of Cellular Organelles. Growth of seedlings, tissue homogenization, organelle separation, the isolation of malate synthase from glyoxysomes and microsomes, and the preparation of monospecific antiserum to the purified glyoxysomal enzyme were carried out exactly as described previously (7).

[^{35}S]Methionine Pulse-Chase Experiments. Five μCi of [^{35}S]-methionine (465 Ci/mmol; Radiochemical Centre, Amersham, Bucks., U.K.) was added to the inner surface of endosperm halves which were then incubated at 30 C in covered dishes. In the pulse-chase experiments, 5 μl of 2 mM L-methionine were added to each endosperm half 4 hr after the application of radioactivity. At various times, 10 endosperm halves were sampled and the cellular organelles were isolated. KCl extracts (0.2 ml in volume) of osmotically disrupted microsomes and glyoxysomes were prepared as described (7) and malate synthase was specifically precipitated from such extracts by incubating them overnight at 2 C with 0.1 ml of antiglyoxysomal malate synthase serum. This quantity of antiserum gave the maximum yield of radioactivity in the resultant immunoprecipitates. Such immunoprecipitates were washed as described previously (7) then resuspended in 0.1 ml of distilled H_2O and added to 10 ml of Bray's (8) scintillation fluid and their radioactivity content was determined.

Sucrose Gradient Centrifugation of Isolated Microsomal Fraction. The uniformity of the isolated microsomal fraction was examined by recentrifugation on a second sucrose gradient. Microsomes were initially isolated on conventional separation gradients as a discrete protein band at density 1.12 g/ml. After fractionation, gradient fractions containing peak activities of the microsomal marker enzyme cholinephosphotransferase were pooled giving a 3-ml suspension of microsomes in 28% (w/w) sucrose solution. This solution was diluted to approximately 14% (w/w) sucrose concentration by the addition of 3 ml of 1 mM EDTA (pH 7.5). The diluted microsomal suspension was layered onto a sucrose gradient which consisted of 20 ml of sucrose solution increasing linearly in concentration from 20 to 40% (w/w) sucrose over an 8-ml cushion of 40% sucrose. Gradients were contained in 38.5-ml cellulose-nitrate tubes. All sucrose solutions contained 1 mM EDTA (pH 7.5). Gradients were centrifuged at 20,000 rpm (53,000g average) and 2 C for 3 hr in a SW 27 rotor in a Beckman L2 65B ultracentrifuge. After centrifugation, 1-ml fractions were collected using a Beckman gradient fractionator.

Papain Treatment. In order to demonstrate that organelle malate synthase was located inside membrane vesicles, particularly in the case of the microsomal enzyme, its resistance to proteolysis was examined. Following organelle separation, peak gradient

fractions containing microsomes and glyoxysomes were separately pooled and osmotically disrupted by the addition of an equivalent volume of 50 mM Tricine (pH 7.5). The malate synthase-rich membrane fractions were recovered by centrifugation and were gently resuspended in 1 ml of 50 mM Tricine (pH 7.5) containing 20% (w/w) sucrose. An equivalent volume (1 ml) of 5% (w/w) solution of papain (Sigma) in 20% sucrose (to prevent further osmotic disruption of the membrane "ghosts") was added to these suspensions, which were then incubated at 25 C. Samples were withdrawn at intervals, and malate synthase activity was determined. The susceptibility of soluble malate synthase to proteolysis was confirmed by incubating KCl extracts of microsomes and glyoxysomes with papain under the same conditions.

Isolation of Free and Membrane-bound Polysomes. Electron micrographs of cells of germinating castor bean endosperm have shown that the ER membranes are predominantly "rough" surfaced in character, bearing attached ribosomes (18, 20). The gradients routinely used to isolate organelles in the present work contain EDTA and completely strip off membrane-bound ribosomes allowing the microsomal fraction to be isolated as a discrete band at density 1.12 g/ml (17). In order to facilitate the separation of free and bound ribosomes, the tissue was homogenized in grinding medium which should maintain ribosome-membrane attachment (17). Twenty endosperm halves were ground in 7 ml of 150 mM Tricine (pH 7.5) containing 10 mM KCl, 5 mM $MgCl_2$, and 13% (w/w) sucrose, using a chilled mortar and pestle. The slurry was filtered through four layers of cheesecloth and the volume of the filtered homogenate was adjusted to 10 ml with grinding medium. The homogenate was centrifuged at 20,000*g* and 2 C for 10 min. The distribution of cholinephosphotransferase activity between the pellet and supernatant after centrifugation established that rough microsomes were completely sedimented together with the other major organelle fractions. The supernatant (solution A) was used as the source of free ribosomes. The pellet (containing membrane-bound ribosomes) was resuspended in 9 ml of grinding medium and then 1 ml of 10% (w/w) sodium deoxycholate solution was added to release bound ribosomes from the microsomal membranes. After 15-min incubation at 25 C, this suspension was centrifuged at 20,000*g* and 2 C for 10 min to sediment organelles and stripped microsomal membranes. The supernatant (solution B) was used as the source of released bound ribosomes. The suspensions of free and bound ribosomes (solutions A and B, respectively) were each layered over 6 ml of 2 M sucrose solution contained in 38.5-ml cellulose-nitrate tubes. The tubes were then filled (to prevent collapse during centrifugation) by overlayering distilled H_2O and were centrifuged at 24,000 rpm (80,000*g* average) and 2 C for 24 hr in a SW 27 rotor in a Beckman L2 65B ultracentrifuge to pellet the suspended ribosomes. Such pellets contained approximately 600 μg of RNA in the case of free ribosomes and 150 μg of RNA for bound ribosomes.

***In Vivo* Labeling of Nascent Polypeptide Chains.** Three different treatments were employed for the labeling of nascent polypeptide chains. In each case 20 2- to 3-day-old endosperm halves were used and each was supplied with (*a*) 5 μCi of [^{35}S]methionine (465 Ci/mmol); (*b*) 0.5 μCi of [^{35}S]methionine; or (*c*) 0.5 μCi of a ^{14}C-amino-acid mixture (10 Ci/mol; Radiochemical Centre, Amersham, Bucks., U.K.). After 30-min incubation at 30 C, the endosperm halves were homogenized in 7 ml of 150 mM Tricine (pH 7.5) containing 10 mM KCl, 5 mM $MgCl_2$, and 13% (w/w) sucrose. The homogenates were treated exactly as described above in order to isolate free or bound polysomes.

Release of Nascent Labeled Polypeptide Chains from Ribosomes. Pellets of free or bound polysomes radioactively labeled *in vivo* were each suspended in 1 ml of 150 mM Tricine (pH 7.5) containing 500 mM KCl, 5 mM $MgCl_2$, 500 μM puromycin, and 200 μM GTP to release nascent polypeptide chains (5). An aliquot of such suspensions was counted to determine the total ribosome-bound radioactivity. After incubation in an ice bath for 30 min, 1 M $MgCl_2$ was added to give a final concentration of 50 mM (to insure complete recovery of ribosomes at the next centrifugation step) and the suspensions were centrifuged at 37,000 rpm (105,000*g* average) and 2 C for 1 hr in a 40.3 rotor in a Beckman L2 65B ultracentrifuge. The supernatants were decanted and an aliquot was counted to determine the released radioactivity. Seventy to 85% of the labeled polypeptide chains were released from both free and bound ribosomes by the above treatment.

Immunoprecipitation of Nascent Malate Synthase Chains. Solutions containing labeled polypeptide chains released from free or bound ribosomes were incubated with half of their volume of antiglyoxysomal malate synthase serum for 1 hr at 37 C and overnight at 2 C. Immunoprecipitates were recovered by centrifugation, washed, and counted exactly as described (7).

Enzyme Assays. The following enzymes were assayed as described in the accompanying literature references: malate synthase (12), cholinephosphotransferase and NADH-Cyt *c* reductase (17).

Other Methods. RNA was determined by the orcinol method (9) using yeast RNA as the standard. Sucrose concentration was determined refractometrically.

RESULTS AND DISCUSSION

Gonzalez and Beevers (11) have demonstrated that 50% of the cellular malate synthase activity present in homogenates of endosperm tissue removed from 2-day-old castor bean seedlings is recovered on sucrose gradients in a position characteristic of the microsomal fraction, with the remaining activity confined to glyoxysomes. In the case of 4-day-old tissue, the proportion in the microsomal region on gradients has fallen to 10% with a corresponding increase in the proportion of glyoxysomal activity. We have confirmed these developmental changes in cellular location and have further shown that at the onset of germination an even greater proportion of the cellular malate synthase activity (over 70%) is recovered in the microsomal fraction (Fig. 1). During germination, a rapid *de novo* synthesis of malate synthase occurs in the endosperm tissue and the changes in intracellular distribution of the enzyme shown in Fig. 1 are consistent with a route of intracellular transport of this protein via the ER before its ultimate sequestration in its functional cellular compartment, the glyoxysome. This conclusion is based on the assumption that microsomal malate synthase recovered during cell fractionation experiments represents enzyme located in the ER cisternae at the time of

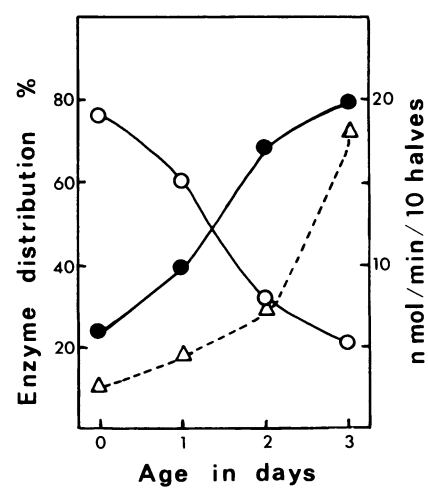

FIG. 1. Effect of seedling age on the total malate synthase activity present in homogenates of 10 endosperm halves (△), and the distribution of this activity between the microsomal (○) and glyoxysomal (●) fractions isolated from such homogenates by sucrose density gradient centrifugation. Day 0 represents imbibed seeds.

homogenization. It seems reasonable to assume that intracisternal malate synthase might avoid solubilization upon disruption of this cellular membrane system since its glyoxysomal counterpart remains almost entirely trapped in the membrane "ghosts" obtained when the isolated organelles are deliberately disrupted (2, 14). This deliberate disruption of the glyoxysomal membrane effectively solubilizes most of the characteristic activities of the organelle matrix, *e.g.* catalase or isocitrate lyase.

Several lines of evidence indicate that microsomal malate synthase is protein trapped inside the membrane vesicles rather than an absorption or other artifact. This activity cannot be attributed to enzyme lost from glyoxysomes, since malate synthase remains associated with glyoxysomal membrane "ghosts" which have a mean buoyant density of 1.21 g/ml (14). More significantly, when microsomal or glyoxysomal "ghosts" (containing malate synthase activity) were incubated at 25 C with papain for up to 1 hr, no loss of enzyme activity was observed, suggesting that the enzyme was isolated in a proteolysis-resistant space. When solubilized malate synthase from either microsomes or glyoxysomes was incubated with papain under identical conditions, 50% of the initial activity was lost after 15 min. Malate synthase activity measured in the microsomal fraction was stimulated at least 2-fold by the inclusion of Triton X-100 in the assay mixture. When solubilized glyoxysomal malate synthase (dialyzed to remove KCl) was incubated with microsomal membranes (devoid of malate synthase after KCl extraction), no association of the enzyme with the membranes was observed.

Although microsomal malate synthase appeared to be membrane-associated, the homogeneity of the microsomal vesicles with respect to malate synthase content was uncertain. It seemed possible that malate synthase activity could be confined to a subfraction of these organelles. More precisely, microsomal vesicles containing this enzyme may themselves have been derived from specialized areas of the ER in which malate synthase activity was temporarily concentrated, perhaps connective elements in the process of vesiculation. When the microsomal fraction recovered from normal organelle separation gradients was recentrifuged on linear gradients constructed to give a narrow range of sucrose concentration, the distribution of malate synthase activity coincided exactly with that of the microsomal membrane enzymes cholinephosphotransferase and NADH-Cyt *c* reductase, suggesting that malate synthase was distributed throughout this fraction (Fig. 2).

The kinetics of [^{35}S]methionine incorporation into KCl extracts of microsomes and glyoxysomes suggested that newly synthesized protein components of these extracts appear first in the ER and subsequently the glyoxysomes. [^{35}S]methionine was applied to excised 2-day-old endosperm tissue. At intervals over a 7-hr period samples were taken, organelles were isolated on sucrose density gradients, and KCl extracts were prepared and counted. During the first 2 hr, the microsomal KCl extract became labeled immediately, whereas a short but significant lag period preceded the labeling of the glyoxysomal fraction (Fig. 3). Similar early labeling kinetic behavior was observed when [^{35}S]methionine incorporation into whole microsomes and glyoxysomes was examined (6). After 2 hr of incubation, further incorporation into the microsomal fraction was not observed, but rather the ^{35}S content of this fraction declined slowly. In contrast, a steady increase in ^{35}S content of the glyoxysomal fraction was observed during this time. More significant changes were observed when the [^{35}S]methionine-labeling kinetic behavior was interrupted after 4 hr by the addition of a large excess of unlabeled methionine. This cold chase resulted in a rapid decrease in ^{35}S content of microsomal KCl extracts with a concomitant increase in the ^{35}S content of glyoxysomal extracts (Fig. 3).

When the labeling kinetics behavior of malate synthase was followed by specifically precipitating this protein from KCl extracts of microsomes and glyoxysomes using antiglyoxysomal malate synthase serum, convincing evidence for the initial segregation of the enzyme by the ER was obtained. These KCl extracts between them contain virtually all of the cellular malate synthase (7). This experiment showed that ^{35}S-labeled malate synthase immediately appeared in the microsomal fraction where it continued to accumulate for 4 hr before leveling off. In the case of the glyoxysomal fraction, little incorporation of [^{35}S]methionine into malate synthase occurred during the first hr of incubation. Beyond this lag period, a steady increase in [^{35}S]malate synthase content of the glyoxysomal fraction was observed (Fig. 4). The effect of the chase of unlabeled methionine demonstrated that the ultimate fate of microsomal malate synthase was its accumulation in glyoxysomes. Apart from an initial increase in microsomal [^{35}S]malate synthase immediately following the chase, possibly due to a stimulated run off of nascent ^{35}S-labeled malate synthase chains from ribosomes, a rapid decrease in microsomal [^{35}S]malate synthase occurred. Simultaneously a stimulated accumulation of [^{35}S] malate synthase in the glyoxysomal fraction was observed (Fig. 4). When the radioactivity in malate synthase lost from the microsomes (estimated by subtracting the values obtained in the chase experiment from the values estimated from the curve drawn in the continuous pulse experiment) was compared with that gained by the glyoxysomes, a quantitative intracellular transport between these organelle fractions was indicated (Fig. 5).

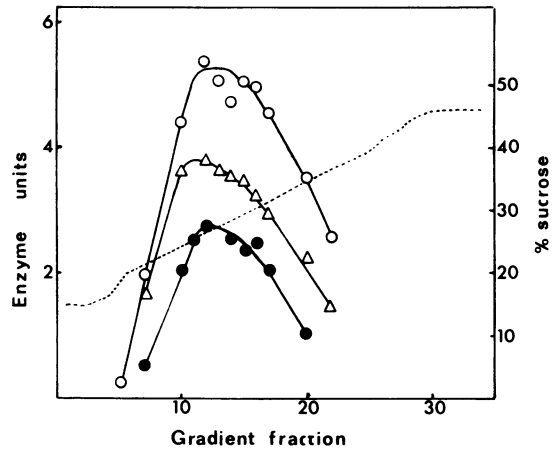

FIG. 2. Sucrose density gradient centrifugation of the isolated microsomal fraction. The microsomal fraction recovered from organelle separation gradients (in 28% [w/w] sucrose) was diluted by the addition of an equivalent volume of 1 mM EDTA (pH 7.5). The diluted microsomal fraction was applied to a second sucrose gradient increasing linearly in concentration from 20 to 40% (w/w) sucrose. After centrifugation at 53,000*g* and 2 C for 3 hr, fractions were collected and the distribution of malate synthase (●, μmol/min [× 100]) was compared with that of the microsomal membrane markers cholinephosphotransferase (○, nmol/hr) and NADH-Cyt *c* reductase (△, μmol/min [× 50]). The broken line shows sucrose concentration.

The data presented in Figure 4 show, at each time point, the total cpm recovered in malate synthase immunoprecipitates from KCl extracts of microsomes and glyoxysomes isolated from homogenates of 10 endosperm halves carefully selected for uniformity. It should be emphasized that the homogenization technique used here is highly reproducible in that good agreement is found between the recovery of total organelle protein, and hence that in KCl extracts, from separate samples of carefully selected seedlings (15). In a series of controls in which malate synthase immunoprecipitates were obtained in identical manner from 10 unlabeled endosperm halves over a 7-hr period, the protein content of KCl extracts and derived immunoprecipitates remained constant within experimental error. The shapes of the curve in Figure 4 would therefore be virtually identical to those shown if expressed on a protein basis. If the experiment had been carried out over a longer period, for example, 24 hr after the chase of unlabeled methionine, the specific radioactivity in glyoxysomal malate synthase expressed on a protein basis would be expected to fall due

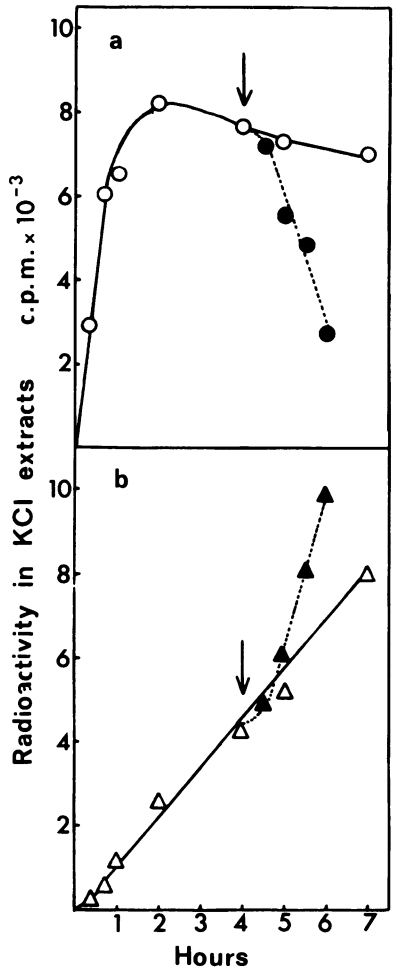

FIG. 3. Progress of [^{35}S]methionine incorporation into KCl extracts of microsomes and glyoxysomes isolated from castor bean endosperm. Endosperm halves removed from 2-day-old seedlings were incubated at 30 C after application of 5 μl of [^{35}S]methionine (containing 5 μCi) to each half. At various times 10 endosperm halves were homogenized and organelles were separated by sucrose density gradient centrifugation. Microsomes and glyoxysomes were collected, osmotically disrupted by adding an equivalent volume of 50 mM Tricine (pH 7.5), and the membranes were recovered by centrifugation. KCl extracts of the membranes were prepared and their radioactivity content was determined. a: Microsomal KCl extracts; b: glyoxysomal KCl extracts. Open symbols: radioactivity present in these extracts during continuous [^{35}S]methionine incorporation; closed symbols: that present after the application of a 1,000-fold excess of unlabeled methionine added after 4 hr as indicated by the arrows.

to a continued synthesis and sequestration of unlabeled enzyme by the tissue (Fig. 1).

This experiment also allowed us to estimate that the minimum time for the synthesis of malate synthase and its segregation and transport by the ER prior to its final sequestration in glyoxysomes is about 1 hr, while the pulse-chase results suggest that a period of approximately 4 hr would be required for the complete transfer of malate synthase located in the ER cisternae to the glyoxysomes.

Since glyoxysomal malate synthase is initially housed in the ER cisternae, it seemed reasonable to expect that this enzyme would be synthesized on membrane-bound ribosomes, the membrane-ribosome junction providing the site for vectorial discharge of the elongating polypeptide chain across the membrane into the intracisternal space. The predominance of rough surfaced ER in this tissue (18, 20) together with its well known capacity for rapid organelle formation in the absence of cell division (and hence in the absence of extensive Golgi apparatus and plasma membrane synthesis or protein secretion) are also consistent with an involvement of bound ribosomes in the synthesis and segregation of organelle membrane and matrix proteins.

The involvement of free or membrane-bound polysomes in the synthesis of malate synthase was investigated by isolating and separating these polysome fractions from endosperm tissue which had been pulsed for 30 min with either [^{35}S]methionine or a ^{14}C-amino-acid mixture. Puromycin treatment in the presence of high

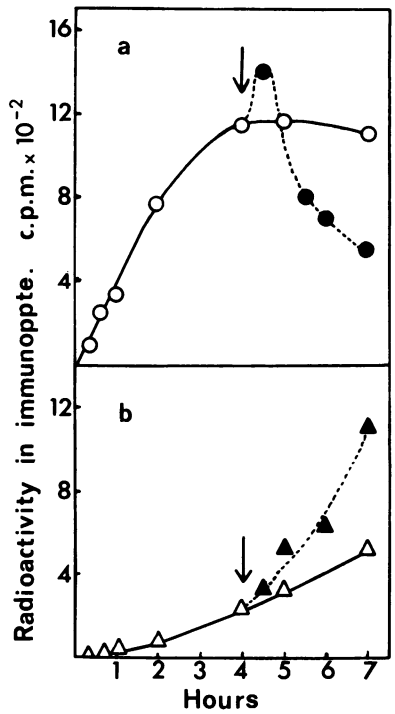

FIG. 4. Progress of [^{35}S]methionine incorporation into immunoprecipitates obtained when KCl extracts of microsomes or glyoxysomes were incubated with antimalate synthase serum. KCl extracts were obtained from microsomes and glyoxysomes isolated from endosperm tissue treated exactly as described in Figure 3. These extracts were incubated with antiglyoxysomal malate synthase serum and the resultant immunoprecipitates were washed and their radioactivity content was determined. a: Microsomal malate synthase immunoprecipitates; b: glyoxysomal malate synthase immunoprecipitates. Open symbols: radioactivity present in immunoprecipitates during continuous [^{35}S]methionine incorporation; closed symbols: that present after the application of a 1,000-fold excess of unlabeled methionine added after 4 hr as indicated by the arrows.

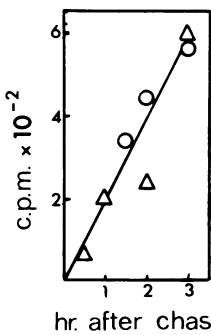

FIG. 5. Quantitative recovery of [^{35}S]malate synthase lost from the microsomal fraction in the glyoxysomal fraction in [^{35}S]methionine pulse-chase experiments. The ^{35}S radioactivity in malate synthase immunoprecipitates lost from the microsomal fraction after the addition of excess unlabeled methionine was estimated (○) and compared with that gained by the glyoxysomal fraction (△). These values were obtained from the differences between the cpm in immunoprecipitates obtained after the chase of unlabeled methionine and the line drawn through the points obtained in the continuous [^{35}S]methionine experiment shown in Figure 4.

Table I The association of nascent malate synthase chains with membrane-bound polysomes.

Twenty 2 to 3 -day -old endosperm halves were each incubated with 5μCi of (^{35}S) methionine (Expt. 1), 0.5μCi of (^{35}S) methionine (Expt. 2) or 0.5μCi of a (^{14}C) amino acid mixture (Expt. 3) at 30°C for 30 min. The tissue was homogenized, free and bound polysomes were separated and nascent radioactive polypeptide chains were released by puromycin treatment as described in the Methods. Malate synthase chains present in released polypeptide preparations were precipitated by incubating with anti-glyoxysomal malate synthase serum and determining the radioactivity present in washed immunoprecipitates. Non-specific precipitation was estimated by substituting null serum for the anti-serum. The values in parentheses indicate the percentage of the total released radioactivity which had been precipitated.

	Ribosomal fraction	Total ribosomal protein	Puromycin released protein	Total precipitated by null serum	Total precipitated by antiserum
		(c.p.m.)	(c.p.m.)	(c.p.m.)	(c.p.m.)
Expt. 1	Free	206750	180100	-	110 (0.06)
	Bound	31800	26150	-	2540 (9.8)
Expt. 2	Free	22100	18000	40 (0.22)	30 (0.16)
	Bound	2540	1740	74 (4.3)	162 (9.3)
Expt. 3	Free	12610	9800	24 (0.24)	30 (0.30)
	Bound	10750	7400	304 (4.1)	770(10.4)

salt concentration effectively released the bulk of the radioactive nascent polypeptide chains from such polysome preparations. When released polypeptides were incubated with antiglyoxysomal malate synthase serum, little radioactivity was precipitated in the case of free polysome-derived polypeptides, whereas a substantial proportion of the released radioactivity was precipitated from the bound polysomes (Table I). One feature of these data is the apparent relatively high degree of nonspecific precipitation obtained from bound radioactive preparations in the presence of null serum. The reason for this is unclear since neither null serum nor antiserum induced significant precipitation when added to the polypeptide chains released from free ribosomes. A considerably higher level of precipitation was obtained from bound ribosome preparations in the presence of antiserum. If it is assumed that a corresponding degree of nonspecific precipitation occurred in the presence of antiserum, then it appears that 5 to 6% of the released labeled polypeptide chains are specifically recognized by antiglyoxysomal malate synthase serum. This corrected figure itself seems relatively high, and the possibility that other radioactive components present in the precipitation mixture are adsorbed onto the immunoprecipitate itself cannot, at present, be ruled out. It should be remembered that these nascent polypeptide preparations were obtained from endosperm tissue harvested at a developmental stage when a rapid and massive net synthesis of malate synthase and other glyoxysomal enzymes is occurring (1). We feel justified in concluding that our results indicate that malate synthase is synthesized on membrane-bound ribosomes.

The data reported in the present paper collectively support a route for the intracellular transport of newly synthesized malate synthase in the castor bean endosperm cell which is in accord with the accepted model for microbody biogenesis. Following transcription from nuclear DNA, the mRNA for malate synthase is translated on ribosomes bound to the ER membrane. This mRNA could be envisaged as interacting with ribosomes already attached to the membrane, but in the light of the recently proposed "signal" hypothesis (3, 4) it is more reasonable to speculate that mRNA for precursor malate synthase undergoes limited translation on a free ribosome before inducing the ribosome to interact with the membrane at a specific receptor site where chain elongation and release occur. The translation of malate synthase mRNA on membrane-bound ribosomes results in the vectorial discharge of the growing polypeptide across the membrane in an irreversible step so that the completed chain, having assumed its characteristic globular conformation, is trapped in the intracisternal space. In the intracisternal space malate synthase possibly associates with other glyoxysomal matrix proteins at specialized sites where vesiculation occurs releasing these enzymes from the endomembrane system in the single membrane-bound glyoxysome. Morphological observations which have shown direct continuity between the ER and microbody membranes may represent the final stages of this process (10, 13, 20).

It is tempting to assume that the intracellular route of malate synthase segregation in castor bean endosperm cells is typical of that undertaken by all microbody enzymes in all cell types. It should be emphasized that similar data have not been reported for other characteristic microbody matrix enzymes. At the developmental stage when considerable microsomal malate synthase is observed in castor bean endosperm, nonglyoxysomal catalase appears in the soluble phase (11; Bowden and Lord, unpublished data). Recent studies of the intracellular pathway of newly synthesized catalase in rat liver have shown that the enzyme appears first in the soluble fraction and then accumulates in the peroxisomes (16, 19). These observations could be explained if catalase is either synthesized on free polysomes or on membrane-bound polysomes which release the nascent catalase into the cytoplasm rather than the ER cisternae. If glyoxysomal catalase of castor bean endosperm was synthesized in this manner, it would mean that different modes of synthesis and segregation would occur for two characteristic glyoxysomal enzymes, catalase and malate synthase. The initial release of catalase into the cytoplasm also presents the problem of transporting this protein (monomer mol wt 60,000) across the microbody membrane, a problem not easily explained in the light of current concepts which hold that a ribosome-membrane junction is a requirement for polypeptide segregation by the membrane (3).

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