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Evidence for Processing of Maize Catalase 2 and Purification of Its Messenger RNA Aided by Translation of Antibody-Bound Polysomes†

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Received September 4, 1985; Revised Manuscript Received November 12, 1985

ABSTRACT: Two-dimensional gel analysis of the in vitro and in vivo labeled catalase 2 (CAT-2) isozyme protein of *Zea mays* L. and western gel analysis of native CAT-2 and in vitro labeled CAT-2 indicated that the protein is processed from a precursor to a lower molecular weight form in the scutellum. The CAT-2 from each source appeared on two-dimensional gels as one major species and two to three subspecies of the same molecular weight. We have also purified the mRNA encoding CAT-2 from scutella of line R6-67 using the procedure of polysome immunoadsorption. As a midcourse check on the progress of purification, we translated a small portion of the purified *Cat2* mRNA-containing polysomes while they were still complexed with CAT-2 antibodies and bound to protein A–Sepharose. This revealed the presence of highly purified *Cat2* polysomes. The final mRNA could not be translated in the wheat germ system but was highly active in the reticulocyte lysate system. The translation product had a molecular weight of 56 000, compared to that of 54 000 for purified CAT-2 protein. We have also enriched for *Cat2* mRNA by size selection on methylmercury–agarose gels. The *Cat2* resided with and slightly above the 18S ribosomal contaminant band of the total poly(A+) mRNA. It is therefore about 1805 bases long, which is 224 bases longer than the calculated coding length of 1581 bases.

The enzyme catalase (CAT; EC 1.11.1.6; H_2O_2:H_2O_2 oxidoreductase) of maize (*Zea mays* L.) exists as a tetramer of 54-kDa[1] subunits held together without disulfide bonds. Three electrophoretically distinct isozymes of maize CAT have been identified and shown to be encoded in three unlinked genes (Scandalios et al., 1980a; Scandalios, 1983). The three structural genes (*Cat1*, *Cat2*, and *Cat3*) have been mapped to different locations within the maize genome (Roupakias et al., 1980). In this study, we have focused on the CAT-2 isozyme as part of an effort to understand the developmental regulation of its synthesis.

In the scutellum of certain genetic lines, during early seedling growth, the product of a trans-acting temporal regulatory gene (*Car1*) acts to maintain an elevated level of CAT-2, which would otherwise decline after 4 days from imbibition (Scandalios et al., 1980b). CAT-2 is also inducible by chemical and environmental stimuli (including light in leaves) (Scandalios, 1983) and displays cell-type specificity (Tsaftaris et al., 1983), tissue specificity (Scandalios, 1979), and organellar targeting (Scandalios, 1974).

In order to further study these phenomena, we have enriched for *Cat2* mRNA sequences by polysome immunoadsorption and by size fractionation of total poly(A+) mRNA. In the course of these studies we have determined the approximate size of the *Cat2* mRNA and its concentration as a percent of total translatable mRNA. Evidence for processing in vivo is presented, as well as observations of maize mRNA translation and polysome immunoadsorption. Of particular usefulness was the finding that the *Cat2*-containing polysomes could be translated in situ while still bound to protein A–Sepharose beads via CAT-2 antibodies. This is a useful technique to employ as a midcourse check for those wishing to purify mRNAs by polysome immunoadsorption.

Materials and Methods

Materials. RNase-free sucrose and reticulocyte lysates were purchased from Bethesda Research Laboratories, protein A–Sepharose was from Pharmacia, low melting point agarose was from Marine Colloids, oligo(dT)–cellulose was from Collaborative Research, and nitrocellulose filters were from Schleicher and Schuell. All other chemicals were reagent-grade, purchased from Sigma Chemical Co.

Polysome Immunoadsorption. Seeds of the high CAT-2 activity line R6-67 (Scandalios et al., 1980b) were surface-

† This work was supported by Research Grants GM-22733 and GM-33817 from the National Institutes of Health to J.G.S. This is paper 9935 of the journal series from the North Carolina Agricultural Research Service, Raleigh, NC.
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[1] Abbreviations: Da, dalton; 2-D, two-dimensional; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PVP-40, polyvinylpyrrolidone-40; DTT, dithiothreitol; PMSF, phenylmethanesulfonyl fluoride; CAT-1, CAT-2, and CAT-3, isozymic forms of maize catalase; *Cat1*, *Cat2*, and *Cat3*, structural genes encoding for the three catalase isozymes, respectively; RNase, ribonuclease; Tris, tris(hydroxymethyl)aminomethane; PPO, diphenyloxazole; IgG, immunoglobulin G; TCA, trichloroacetic acid; EDTA, ethylenediaminetetraacetic acid.

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sterilized with 1% sodium hypochlorite and grown in the dark at 25 °C for 4–6 days. Scutella were excised, frozen immediately in liquid N_2, and stored at −70 °C. Polysomes were prepared by a modification of the procedure of Davies and Knight (1972). Scutella were ground in a mortar in liquid N_2 and transferred to an ice-cold mortar for further grinding in 0.2 M Tris-HCl (pH 8.5 at 22 °C), 30 mM $MgCl_2$, 0.2 M KCl, 2% (w/v) PVP-40, 0.25 M RNase-free sucrose, and 5.8 mM β-mercaptoethanol. The sample was always maintained at 0–4 °C. Six milliliters of grinding buffer per gram of scutella was used. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15000*g* for 15 min in a fixed-angle rotor. The supernatant was stirred for 15 min with Triton X-100 at a final concentration of 1% (v/v) and centrifuged at 25000*g* for 15 min. The supernatant was layered over 8-mL pads of 1.75 M sucrose in 40 mM Tris-HCl (pH 8.5 at 22 °C), 10 mM KCl, and 10 mM $MgCl_2$ in 32-mL polycarbonate bottles and centrifuged in a Ti 70 rotor at 190000*g* (average) for 90 min. After the supernatant above the pads was aspirated off and the sides were washed with sterile distilled H_2O, the pad was removed, and polysome suspension buffer [5 mM Hepes–KOH (pH 7.6), 0.5 mM DTT, 0.625 mM $Mg(OAc)_2$, and 25 mM KOAc] was added. The polysomes were frozen as droplets in liquid N_2 and stored at −70 °C. Antibodies against purified CAT-2 (Chandlee et al., 1983) were prepared from New Zealand white rabbits. The antibodies were bound to protein A–Sepharose (Goudswaard et al., 1978; *Pharmacia Fine Chemicals Handbook*, 1979) and washed with 0.2 N NaSCN to remove possible RNase (Gough & Adams, 1978). Immunoadsorption of polysomes was conducted essentially according to the method of Shapiro and Young (1981). Antibody (1600 μg) was added to 640 A_{260} units of polysomes in 20 mL of immunoadsorption buffer [25 mM Tris-HCl (pH 7.6 at 22 °C), 150 mM NaCl, 5 mM $MgCl_2$, 0.5% (v/v) Nonidet P-40, and 20 units/mL heparin] (Shapiro & Young, 1981). After incubation for 2.5 h at 4 °C, the immunoconjugates were batch-adsorbed for 30 min to 1 mL (packed volume) of protein A–Sepharose, which had been washed and charged (*Pharmacia Fine Chemicals Handbook*, 1979). The mixture was poured through a sialinized Pasteur pipet column and washed overnight with 700 mL of immunoadsorption buffer. CAT-2 antibody (1600 μg) was added to the unbound polysomal fraction, and the immunoadsorption procedure was repeated. This was again repeated an additional 2 times. After washing, approximately 15 μL of packed beads was removed from the column and, later, washed several times with 20 mM Hepes–KOH (pH 7.6), 120 mM KOAc, 5 mM $Mg(OAc)_2$, and 1 mM DTT. The bound polysomes were translated in situ (see below). The *Cat2* mRNA was eluted from the column with 20 mM EDTA, 25 mM Tris-HCl (pH 7.6), and 20 units/mL heparin (Shapiro & Young, 1981) and further purified on oligo(dT)–cellulose (Aviv & Leder, 1972). The mRNA was precipitated overnight at −20 °C after addition of 60 μg of yeast tRNA, KOAc (pH 5.5) to 0.3 M, and 2.5 volumes of 100% ethanol. The mRNA was pelleted by centrifugation in an SW27 rotor at 98000*g* for 2 h and taken up in 40 μL of sterile distilled H_2O.

Purification and Size Fractionation of Total Poly(A+) mRNA. The mRNA was purified from frozen 6- and 7-day-old scutella by homogenization in sodium borate buffer, digestion with proteinase K, and precipitation in LiCl (Hall et al., 1978). The mRNA was purified on oligo(dT)–cellulose and precipitated as above (without carrier tRNA). Ninety-six micrograms of the mRNA was size-fractionated on a 1.2% methylmercury–agarose gel (Bailey & Davidson, 1976); low melting point (Sea Plaque) agarose was used. The sides of the gel were removed, stained in ethidium bromide, and then returned in order to mark the position of the 18S and 26S rRNA contaminants. The gel was sliced horizontally, and the mRNA was extracted with phenol, followed by chloroform extraction of the aqueous phase. The mRNA was precipitated as above and solubilized in distilled H_2O.

Translation of Polysomes and mRNA and Immunoprecipitation of Products. Polysomes were translated in both the wheat germ and reticulocyte lysate systems. Wheat germ was extracted (Marcu & Dudock, 1974) and stored at −70 °C. Reactions were conducted in 50 μL of 20 mM Hepes–KOH (pH 7.6), 2 mM DTT, 1 mM ATP, 20 μM GTP, 2.5 mM $Mg(OAc)_2$, 100 mM KOAc, 50 μM each of 19 unlabeled amino acids, 8 mM creatine phosphate, 6520 units/mL creatine phosphokinase, 55 pmol of 1000 Ci/mmol [^{35}S]-methionine per A_{260} unit of polysomes, and 6–12 μL of wheat germ extract. Reactions were at 25 °C. Reticulocyte lysate reactions (Pelham & Jackson, 1976) in 30-μL volumes consisted of 25 mM Hepes–KOH (pH 7.2), 10 mM creatine phosphate, 50 mM each of 19 unlabeled amino acids, 55 pmol of 1000 Ci/mmol [^{35}S]methionine, 3 mM cyclic AMP, 40 mM KCl, and 10 μL of rabbit reticulocyte lysate. When polysomes were translated, one A_{260} unit, 58 mM KOAc, and 2.5 mM $Mg(OAc)_2$ were used. When mRNA (0.4 μg) was translated, 86.7 mM KOAc and no Mg^{2+} were used. One milliliter of translation immunoadsorption buffer [10 mM Tris–NaOH (pH 8.2), 150 mM NaCl, 10 mM Na_2EDTA, 1% v/v Triton X-100, 0.25% sodium deoxycholate, and 10 mM L-methionine] was added following each reaction. Thirty micrograms of CAT-2 antibody and PMSF (to 2 mM) were then added. The samples were incubated for 1 h at 25 °C, followed by 15 h at 4 °C. The immunoconjugates were batch-adsorbed to protein A–Sepharose beads for 1 h. Products were eluted by boiling the beads for 2 min in Laemmli SDS sample buffer (Laemmli, 1970).

In Vivo Labeling. Two scutella from 7-day-old R6-67 seedlings were isolated and partially submerged in 140 μL of labeling solution: 20 mM sodium phosphate (dibasic, pH 7.2), 25 mM mannitol, and 55 pmol of 1000 Ci/mmol [^{35}S]-methionine. Labeling was conducted for 4.5 h at 24 °C. Each scutellum was rinsed for 15 min at 0 °C in 20 mM sodium phosphate (pH 7.2) and 5 mM unlabeled methionine and then ground in 2 mL of immunoadsorption buffer (above) with a small amount of sand and insoluble PVP-40, 5 mM unlabeled methionine, 0.25% (v/v) β-mercaptoethanol, and 1.5 mM PMSF. The homogenate was centrifuged at 7800*g* for 5 min, after which time 300 μg of CAT-2 antibody was added per scutellum. After incubation at 4 °C for 15 h, the labeled CAT-2–antibody complex was bound to a protein A–Sepharose column, washed with 700 mL of polysome immunoadsorption buffer, and eluted with 0.1 M glycine (pH 2.5). The sample was neutralized with 0.5 M Tris and precipitated with acetone. The precipitate was dissolved in either Laemmli SDS sample buffer for SDS–PAGE or O'Farrell lysis buffer and 9 M urea (1:1 v/v) for 2-D PAGE (O'Farrell, 1975).

Electrophoresis and CAT-2 Detection. SDS–PAGE was conducted according to the method of Laemmli (1970). Eleven percent running gels (9.5 cm) beneath 5% stacking gels (1 cm) were used. Molecular weight standards were phosphorylase *b* (92.5 kDa), bovine serum albumin (67 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa), and α-lactalbumin (14.4 kDa). Two-dimensional PAGE was conducted according to the method of O'Farrell (1975), using a 5% stacking gel and an 11% running gel in

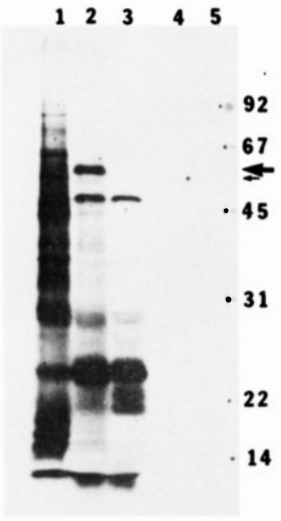

FIGURE 1: Fluorogram of polyacrylamide gel of mRNA translation products from purified mRNA: lane 1, total scutellar mRNA translation products; lane 2, purified mRNA products (same preparation as in Figure 5); lane 3, control translation without exogenous mRNA, showing endogenous reticulocyte lysate mRNA products; lane 4, position of purified unlabeled CAT-2 enzyme (radioactive ink dot); lane 5, positions of molecular weight markers. The large arrow indicates the position of the in vitro labeled CAT-2, while the small arrow indicates the position of the main form of endogenous native CAT-2 seen by Coomassie staining.

the SDS–PAGE dimension, as above. Gels were stained with Coomassie brilliant blue, impregnated with PPO, dried, and fluorographed (Laskey & Mills, 1975). When proteins were to be electrophoretically transferred to nitrocellulose filters (Towbin et al., 1979; Burnette, 1981), the gels were run for 3 h after the bromophenol blue dye front ran off the gel. Native CAT-2 was detected on the nitrocellulose filter immunologically by horseradish peroxidase conjugated goat–anti-rabbit IgG (Towbin et al., 1979; Avrameas & Guilbert, 1971).

Results

Immunoadsorption of CAT-2 from total mRNA translation products revealed that the *Cat2* constituted approximately 0.2% of the total translatable mRNA from 5- to 6-day-old scutella. A comparison of the CAT-2 labeled in vitro and stained purified native CAT-2 revealed that the former is roughly 2000 Da (18 amino acids) larger than the latter (Figure 1). To determine whether this difference may have resulted from possible degradation of the native CAT-2 during enzyme purification, CAT-2 was labeled in vivo, immunoprecipitated, and analyzed together with in vitro labeled CAT-2 on 2-D gels. It can be seen (Figure 2) that the in vitro CAT-2 is again slightly larger than the native CAT-2. It is still possible that the in vivo CAT-2 was degraded during grinding and immunoadsorption. However, when the in vivo labeled native CAT-2 is analyzed alone on 2-D gels, three spots can be seen in positions slightly above the main spots (parts B and A of Figure 3, respectively). It is possible that these represent precursor forms of the CAT-2 that may undergo processing. In order to compare the size of in vitro labeled CAT-2 with undegraded native CAT-2, a homogenate of 5-day-old unlabeled scutella was prepared by grinding directly in SDS sample buffer. In vitro and in vivo immunoprecipitates and the scutellar homogenate were electrophoresed together by PAGE. Western transfers of the gels were subjected to immunological CAT-2 detection. These revealed that the undegraded scutellar CAT-2 is represented by two species, roughly 2000 Da apart. Autoradiography revealed that the

FIGURE 2: In vivo vs. in vitro labeled CAT-2 immunoprecipitates. Polysomes from 4-day-old W64A scutella were translated in the wheat germ system, and CAT-2 products (top arrow) were isolated by immunoprecipitation of the products with CAT-2 antibodies. Scutella from 7-day-old R6-67 were labeled in vivo by partial submersion in buffer containing [^{35}S]methionine. CAT-2 was isolated from scutellar homogenates by immunoprecipitation (bottom arrow). The two immunoprecipitates were electrophoresed together on 2-D gels.

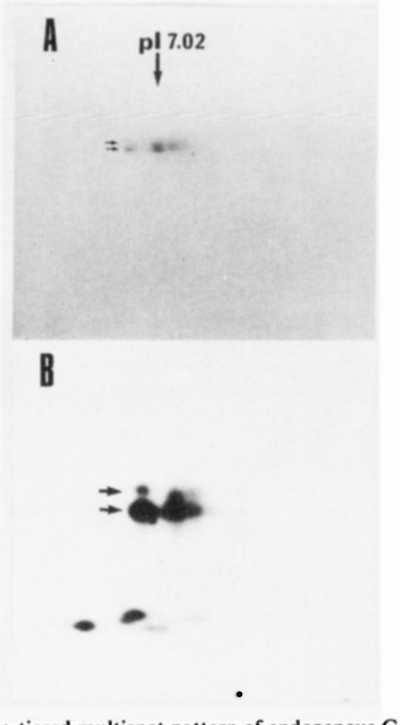

FIGURE 3: Two-tiered multispot pattern of endogenous CAT-2. (A) The native CAT-2 enzyme from 10-day-old R6-67 was purified and presented on a 2-D gel. The upper and lower tiers (arrows) correspond to the presumptive precursor and processed forms of CAT-2, respectively. The major form of CAT-2 (spot in the center) has an isoelectric point of 7.02. (B) Immunoprecipitate of in vivo labeled 7-day-old R6-67 scutella. The upper tier of three spots corresponds to the presumptive precursor form(s) of CAT-2. The more prevalent lower tier of three spots corresponds to the processed form of CAT-2 and matches precisely the lower and most prevalent Coomassie-stained CAT-2 spots (not shown) on the same gel.

in vitro synthesized CAT-2 corresponded to the upper (56-kDa) CAT-2 band (Figure 4). The in vivo immunoprecipitate corresponded to the lower (54-kDa) band. Taken together, these data provide good evidence that CAT-2 undergoes processing.

The use of in situ translation of polysomes bound to protein A–Sepharose provided a useful midcourse check on the progress of *Cat2* mRNA purification (Figure 5A). The CAT-2 products from in situ translation displayed the same multispot two-dimensional gel pattern seen for CAT-2 among total mRNA products (Figure 6A), the immunoprecipitates

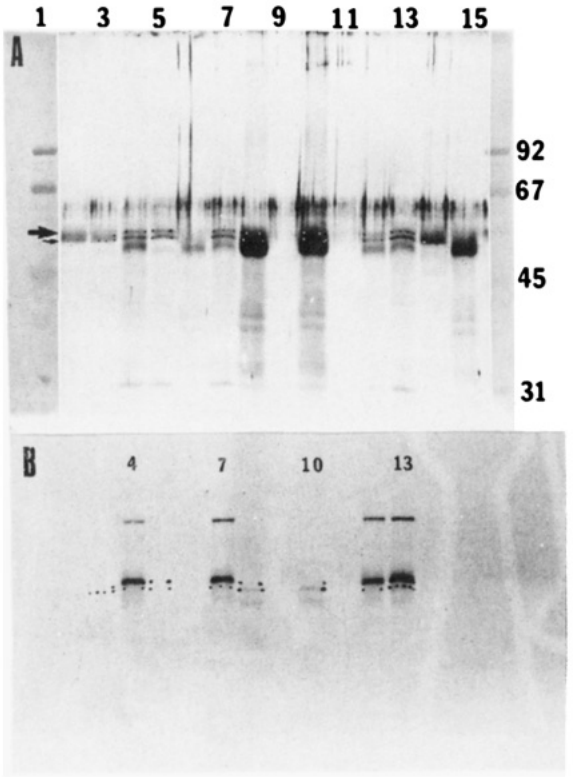

FIGURE 4: In vitro and in vivo labeled CAT-2 in relation to native scutellar CAT-2. (A) Scutella of 5-day-old R6-67 were homogenized in Laemmli SDS sample buffer (see Materials and Methods). One microliter of the homogenate contained 10 μg of TCA-precipitable protein and represented 0.13% of a scutellum. One microliter was loaded in lanes 10 and 12, 2 μL in lanes 4, 5, and 7, and 4 μL in lane 15. A CAT-2 immunoprecipitate from the in vitro translation products of 5-day-old R6-67 scutellar mRNA was loaded in lanes 4, 7, 12, and 13. A CAT-2 immunoprecipitate from in vivo labeled 5-day-old scutella of R6-67 was loaded in lanes 8 and 10. Lanes 1 and 16 contained molecular weight markers. Lanes 2 and 3 contained 0.6 and 0.3 μg of purified CAT-2 enzymes from unlabeled 10-day-old R6-67 scutella. Lane 6 contained 1 μg of CAT-2 antibody alone. Lanes 9 and 11 were not loaded. Electrophoresis on the 11% gel was continued at 150-V constant voltage for 3 h after the dye front ran off in order to further separate the antibody from the CAT-2 bands. The proteins were transferred to a nitrocellulose membrane (western transfer). The membrane was cut so that lanes 1 and 16 could be stained in amido black. The rest of the membrane was incubated in a CAT-2 antibody solution and then in peroxidase-conjugated goat–anti-rabbit antibody. After reaction in the chromogenic solution, the CAT-2 bands (large and small arrows) were marked with ink containing ^{14}C. (B) The membrane in (A) was autoradiographed on X-ray film. The in vitro CAT-2 (lanes 4, 7, 12, and 13) corresponds to the upper native CAT-2 band (large arrow), while the in vivo CAT-2 (lanes 8 and 10) corresponds to the lower native CAT-2 band (small arrow).

of those products (Figures 6B,C and 2), purified native CAT-2 (Figure 3A), and in vivo labeled scutella (Figures 3B and 2). The central species had an isoelectric point of 7.02 (Figure 3A). The in situ translation proved that the *Cat2* polysomes were, in fact, highly purified. The final purified mRNA was translated in the wheat germ system, and the products were analyzed by SDS–PAGE and fluorography. No CAT-2 could be detected. The in situ polysome translation results encouraged us to attempt translation in the reticulocyte system. This was successful and yielded full-length CAT-2 products (Figures 5B and 1). Thus, it appears that *Cat2* mRNA is not efficiently translated in the wheat germ system. The most efficient reticulocyte translation system for this and for total mRNA was one in which no Mg^{2+} (except for the 1.17 mM final concentration from the reticulocyte) or spermidine was used.

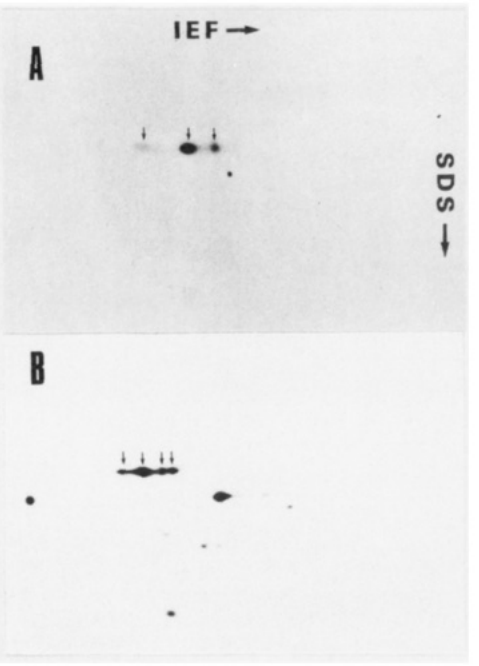

FIGURE 5: Fluorograms of in vitro labeled CAT-2 from *Cat2* polysome purification. (A) In situ translation of purified polysomes. Scutellar polysomes were incubated with CAT-2-specific antibodies, and the immunoconjugates were bound to a protein A–Sepharose column and washed. A small portion of beads was removed and added to a wheat germ translation reaction. The products were electrophoresed on 2-D gels and fluorographed. CAT-2 (arrows) from highly purified polysomes is seen. (B) Translation of purified *Cat2* mRNA. The bound polysomal mRNA was eluted from the column with EDTA and further purified on an oligo(dT)–cellulose column. A portion of the mRNA was translated in the reticulocyte lysate system, and the products were analyzed on 2-D gels. The *Cat2* mRNA products (arrows) produced a four-spot pattern. Other spots resulted from incomplete CAT-2 and endogenous lysate products.

Only 12% of the *Cat2* polysome yield was recovered in the first immunoprecipitation. Almost all was recovered with the second 1600 μg of antibody used for immunoadsorption. None was found in the third and fourth immunoadsorptions. Thus, 5 μg of antibody per A_{260} unit of polysomes was required for titration of *Cat2* polysomes from scutella. The polysome yields were doubled, from 2.5 to 5.0 A_{260} units per gram fresh weight of scutella, by using 6 mL of grinding buffer per gram fresh weight, instead of 2 mL. Translatability was also slightly improved. (For seedling leaf polysomes, it was found that a 6:1 ratio was required for obtaining translatable polysomes.)

Total scutellar mRNA was size-fractionated on methylmercury–agarose gels in order to enrich for *Cat2*, prior to cloning. Translation of the sized mRNAs and immunoadsorption of the products revealed that most of the *Cat2* mRNA is contained with and slightly above the 18S rRNA contaminant (Figure 7). Its length is therefore slightly above 1805 bases. In addition, smaller amounts are contained in slices representing mRNA as large as 26 S.

Discussion

The processing of CAT-2 in vivo is under further study. Previous studies have shown that the maize CAT-2 exists both free in the cytoplasm and within glyoxysomes (Scandalios, 1974). We commonly purify the enzyme from scutella harvested 10 days after germination (Scandalios, 1974; Chandlee et al., 1983; Tsaftaris & Scandalios, 1981), when glyoxysome levels are sharply reduced. Therefore, processing on or in glyoxysomes may not account for all of the CAT-2 that is reduced in size relative to in vitro labeled CAT-2. It is possible that a specific diffusible enzyme exists in the cytoplasm and

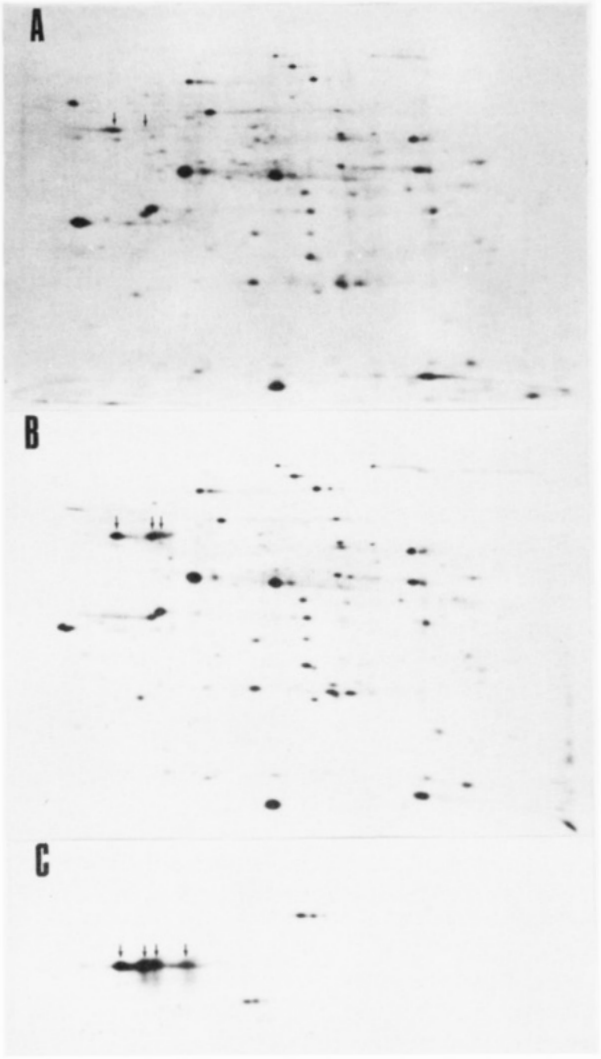

FIGURE 6: Location of in vitro synthesized CAT-2 in relation to total mRNA products. (A) Total poly(A+) mRNA from 6-day-old scutella was translated in the reticulocyte lysate system and electrophoresed on a 2-D gel. (B) A portion of the reaction was immunoprecipitated with CAT-2 antibodies, and the immunoprecipitate (arrows) was electrophoresed together with the total products on a 2-D gel. (C) Immunoprecipitate used in (B) (same amount). (A) and (B) were 1-day fluorogram exposures. (C) was a 6-day exposure.

is capable of processing CAT-2 regardless of the glyoxysome flux. The size reduction of roughly 2000 Da corresponds to a loss of 18 amino acids, which is in the lower end of the range estimated for most signal peptides (Watson, 1984). These data argue strongly in favor of specific proteolytic processing of a higher molecular weight CAT-2 precursor. This is in contrast to rat liver peroxisomal catalase, in which the in vivo and in vitro products are the same molecular weight (Mainferme & Wattiaux, 1982). Our results are very similar to those for cucumber catalase, which was found to be processed from a 55-kDa precursor to a 54-kDa product (Becker et al., 1982). In pumpkin cotlyedons, catalase is processed from an enzymatically inactive 59-kDa precursor to an active 55-kDa product (Yamaguchi et al., 1984). We are currently attempting to determine the mechanism(s) that may be responsible for the processing of CAT-2.

The ability to translate a small fraction of a preparation of immunoadsorbed polysomes in situ, while still attached to protein A–Sepharose beads, offers a helpful tool in the cloning of low-abundance mRNAs. The steps following the elution of the mRNA involve many possibilities for losing submicrogram quantities. A measurement of the final mRNA is

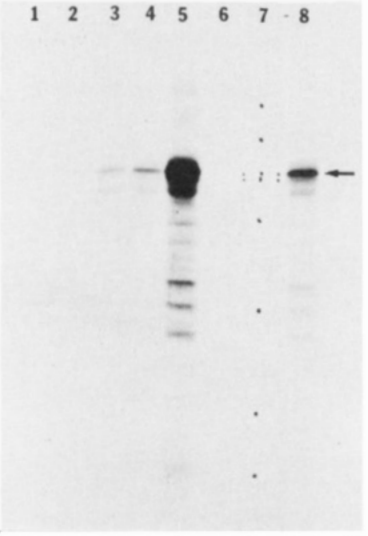

FIGURE 7: Size selection of *Cat2*-enriched mRNA. Total poly(A+) mRNA from 6- to 7-day-old R6-67 scutella was electrophoresed on 1.5% low melting point agarose containing 20 mM methylmercury hydroxide. The gel was sliced horizontally into size fractions. Sizes were determined relative to the ethidium bromide stained 18S and 26S ribosomal RNA contaminants in the mRNA. The mRNA was extracted from the melted gel by sequential phenol and chloroform extraction. A portion (0.13 μg) of the mRNA was translated and immunoprecipitated with CAT-2 antibodies, and the products were electrophoresed on 11.5% polyacrylamide gels. Lanes 1–4 represent products from equal fractions taken from immediately below the 26S band (lane 1) to immediately above the 18S band. Lane 5 represents the 18S band, and lane 6 represents the fraction immediately below the 18S band. Lane 7 shows the positions of molecular weight markers, including native CAT-2 (radioactive ink marks the upper and lower staining bands, the lower of which is predominant). Lane 8 is the same as lane 5, but half as much loaded. The arrow denotes CAT-2. The bands below CAT-2 resulted from incomplete CAT-2 products.

usually not practical, and its presence must be judged by translatability. The in situ translation provided a useful midcourse check, which demonstrated that the *Cat2* mRNA had not been lost or degraded up to and including the protein A–Sepharose column stage. This provided an incentive to look for an alternative translation system after the failure of the final purified mRNA to translate in the wheat germ system. In addition to in situ translation, we have also experimented with the use of prior CAT-2 antibody additions (30 μg per reaction) in the total mRNA translation reactions. We found that CAT-2 synthesis was increased by about 70% (data not shown). The phenomenon of antibody-bound polysome translation may reflect certain translational events that could occur in the cell. Penman (1982) has noted the presence of polysomes bound to internal filaments running throughout the cytoplasm of cells. There is evidence to suggest that this results from mRNA binding. The ribosomes attached to such messages would then translate by free movement along the mRNA. However, it is also possible that nascent polypeptides could target the polysomes to specific sites (e.g., as in signal peptides) where they are bound. In some instances, the mRNA may then be propelled through a growing chain of bound ribosomes. By extrapolation, it is tempting to speculate that highly ordered repetitive protein structures could be synthesized in the cell in this manner, with the mRNA acting in a manner analogous to weaving.

We cannot explain the lack of *Cat2* mRNA translation in the wheat germ system. This is the opposite of the results of Robbi and Lazarow (1978), in which rat liver catalase mRNA could be translated more efficiently with wheat germ than with reticulocytes. This plant–animal system crossover is unexpected. We did not use placental RNase inhibitor, as they

had, because the total mRNA translation products displayed diffuse banding, rather than sharp banding, when this was used. It also caused polysome profile deterioration. The 56-kDa size of the in vitro CAT-2 product is well below the size at which the wheat germ system begins to discriminate against mRNAs for larger proteins, 67 kDa, in our hands. The ability of the reticulocyte system to translate *Cat2* mRNA was therefore not due to more efficient translation of the *Cat2* mRNA size class.

The multispot pattern of CAT-2 seen on 2-D gels may possibly result from artifactual charge heterogeneity (O'-Farrell, 1975), although this is not a general feature of the proteins in this size class (Figure 6). It is also possible that this microheterogeneity is due to multiple species of *Cat2* mRNAs.

The use of a second immunoadsorption of the polysomes was fortuitous, as approximately 88% of the *Cat2* mRNA was in this preparation. Our polysome preparations contain high amounts of active CAT-2 tetramers. It is possible that the antibody first preferentially bound the more antigenic tetramers and, after these were removed, then bound the nascent polypeptide chains on the ribosomes.

ACKNOWLEDGMENTS

We gratefully acknowledge the work of Janet Wilson in the typing of the manuscript.

Registry No. CAT, 9001-05-2.

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