Carboxyl-Terminal Proteolytic Processing during Biosynthesis of the Lysosomal Enzymes β-Glucuronidase and Cathepsin D†

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ABSTRACT: During pulse–chase experiments in cultured porcine kidney cells, an early 75-kilodalton (kDa) form of β-glucuronidase is converted to a late 72-kDa form. The relative molecular weight difference between the two forms is maintained on removal of high-mannose carbohydrate with endoglycosidase H. Both forms have the same partial NH_2-terminal sequence, and both migrate as single polypeptide chains following reduction, alkylation, and electrophoresis under denaturing conditions. On treatment with carboxypeptidase Y, the early form released [^{35}S]Met faster than the late form. Thus, the late form of β-glucuronidase is generated by COOH-terminal proteolytic processing of the early form. During similar experiments, the mass of the 30-kDa heavy chain of porcine cathepsin D decreased by about 1 kDa. The heavy chain of the two-chain enzyme is derived from the COOH terminus of a 44-kDa single-chain enzyme. On treatment with carboxypeptidase Y, the early single-chain enzyme released COOH-terminal [^{35}S]Met and [^{3}H]Lys faster than the later 29-kDa heavy chain. Like β-glucuronidase, cathepsin D evidently undergoes COOH-terminal proteolytic processing during biosynthesis.

During their synthesis and maturation, lysosomal enzymes undergo a reduction in mass, often in multiple discrete steps, varying from a few to 34 kilodaltons (kDa)[1] (Hasilik & Tanner, 1978; Erickson & Blobel, 1979; Skudlarek & Swank, 1979, 1981; Hasilik & Neufeld, 1980; Brown et al., 1981; Erickson et al., 1981; Müller & Müller, 1981; Myerowitz & Neufeld, 1981). Being glycoproteins, the reduction in mass could affect either the oligosaccharidyl or the peptidyl moiety or both. In fact, both types of processing have been reported. The structural and functional implications of these processing steps remain largely undefined. So far, only two processing events, both proteolytic in nature, have been structurally and functionally defined.

One is the cotranslational cleavage of an NH_2-terminal "prepeptide" which, in the case of porcine spleen cathepsin D, has been shown to be 20 amino acid residues in length (Erickson et al., 1981). This transient peptide functions as a signal peptide, targeting the nascent lysosomal enzyme to the translocation system of the endoplasmic reticulum membrane (Erickson & Blobel, 1979; Erickson et al., 1981). It is likely that all lysosomal enzymes are synthesized with signal peptides (Erickson & Blobel, 1979; Erickson et al., 1981; Müller & Müller, 1981; Proia & Neufeld, 1982; Rosenfeld et al., 1982), although it is possible that the signal peptide is not removed in all cases, in analogy to certain secretory (Lingappa et al., 1978; Palmiter et al., 1978) and membrane proteins (Bonatti & Blobel, 1979; Schechter et al., 1979; Goldman & Blobel, 1981).

The other transient peptide, a "propeptide" cleaved post-translationally, has been detected so far in two lysosomal proteases, carboxypeptidase Y of yeast (Hasilik & Tanner, 1978) and porcine spleen cathepsin D (Erickson et al., 1981). In both cases, the propeptide probably functions as an activation peptide. Support for this role has been obtained in the case of cathepsin D (Erickson et al., 1981; Hasilik et al., 1982). While activation peptides may also occur in other lysosomal proteases, they may not be a common feature of all lysosomal enzymes.

In this paper, we describe a third proteolytic processing event, namely, the cleavage of a 1–3-kDa polypeptide from the COOH terminus of both β-glucuronidase and cathepsin D. The possible functional significance of this processing event is discussed.

Experimental Procedures

Materials. [^{35}S]Met (1000 Ci/mmol) was obtained from New England Nuclear Corp. and [^{3}H]Leu (180 Ci/mmol) from Amersham Corp. Endoglycosidase H was a gift from Dr. Phillips Robbins, Massachusetts Institute of Technology. Carboxypeptidase Y (CpY) from Oriental Yeast Co., Japan, was a gift from Dr. Katsuyoshi Mihara, Osaka University, Japan. The porcine kidney cell line PK(15) was obtained from the American Type Culture Collection (ATCC CCL 33) and maintained as described (Erickson et al., 1981). The buffalo rat liver cell line BRL 3A (ATCC CRL 1442) was maintained similarly, except that calf serum was replaced by fetal calf serum.

Antisera. Cathepsin D antiserum was prepared against the 30-kDa heavy chain of porcine spleen cathepsin D as described (Erickson & Blobel, 1979). The antigen was a gift from Dr. Jordan Tang, Oklahoma Medical Research Foundation. β-Glucuronidase antiserum was prepared similarly by using bovine liver β-glucuronidase obtained from Sigma Chemical Co. as the antigen.

Treatment with Carboxypeptidase Y. Proteins subjected to CpY treatment were radiolabeled during pulse–chase experiments, immunoprecipitated with the appropriate antiserum, purified by PAGE, electrophoretically eluted from the gel, and concentrated by precipitation as previously described (Erickson et al., 1981), except that only 100 μg of ovalbumin was present as unlabeled carrier protein. Each sample (60 μL) was heated to 100 °C in 2.5% SDS for 2 min and adjusted to 0.25% SDS with 0.05 M Tris–acetate buffer at pH 5.8. CpY treatments were conducted under denaturing conditions in the presence of SDS in order to eliminate the effects of chain conformation

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[1] Abbreviations: CpY, carboxypeptidase Y; endoglycosidase H, endo-β-*N*-acetylglucosaminidase (EC 3.2.1.96) from *Streptomyces griseus*; kDa, kilodalton(s); PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

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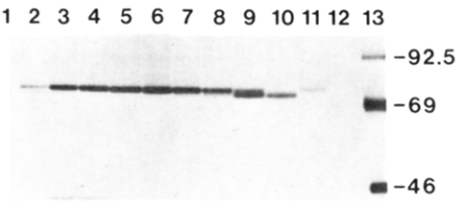

FIGURE 1: Forms of β-glucuronidase synthesized in vivo during a pulse–chase experiment. Porcine kidney cells were pulsed with [^{35}S]Met, and the label was chased with unlabeled Met for the time periods indicated below. Immunoprecipitated proteins, which were analyzed by SDS–PAGE followed by fluorography of the dried slab gel, were obtained from approximately 2×10^6 cells for lane 1, 10^6 cells for lanes 2–10 and 12, and 3×10^6 cells for lane 11. Lane 1 shows proteins immunoprecipitated immediately following a 1-min pulse. Lanes 2 and 3 are like lane 1, except that the pulse period was 5 min for lane 2 and 15 min for lane 3. Lane 4 is like lane 3, except that the 15-min pulse was followed by a 15-min chase period. Lanes 5–10 are like lane 4, except that the chase period was 1 h for lane 5, 2 h for lane 6, 3 h for lane 7, 4 h for lane 8, 8 h for lane 9, and 24 h for lane 10. Lane 11 shows protein labeled in vivo and immunoprecipitated from the cell growth medium after continuous culture of porcine kidney cells for 8 h. Lane 12 is like lane 9, except that nonimmune rabbit serum was substituted for the β-glucuronidase antiserum. Lane 13 shows ^{14}C-labeled phosphorylase *b* (92.5 kDa), bovine serum albumin (69 kDa), and ovalbumin (46 kDa) obtained form New England Nuclear Corp. Similar results were obtained when buffalo rat liver cells were used in place of porcine kideny cells (data not shown).

on the susceptibility to proteolysis. For each time point, CpY (final concentration 250 μg/mL) and a constant amount of radioactivity (approximately 16 000 cpm for [^{35}S]Met and 5000 cpm for [^{3}H]Lys) were incubated at 30 °C for a given time period. Digestion was stopped by transferring the sample to an ice bath followed by precipitation of SDS and polypeptides with KCl (0.4 M final concentration) and trichloroacetic acid (25% final concentration), respectively.

The amount of [^{35}S]Met or [^{3}H]Lys released into the supernant was measured by counting the supernatant in 5 mL of aquasol. The amount released from the zero time point sample, which was mixed with CpY on ice and immediately diluted, was subtracted from the later values. The net amount of radiolabeled amino acid released is expressed as the number of moles of amino acid released per mole of protein digested. The 46-kDa procathepsin D was assumed to have 12 Met and 31 Lys residues (Huang et al., 1979; Erickson et al., 1981), the 44-kDa single-chain form to have 11 Met and 28 Lys residues (Huang et al., 1979), and the 29-kDa heavy chain to have 10 Met and 23 Lys residues (Huang et al., 1979; Takahashi & Tang, 1983). The polypeptide masses of the 75- and 72-kDa forms of β-glucuronidase are 68 and 66 kDa, respectively (see below). Since each of these forms contains about 1.5 mol % of Met (Tulsiani et al., 1978), they were estimated to contain nine Met residues.

Other Methods. Procedures for pulse–chase experiments, treatment with endoglycosidase H, and determination of partial NH_2-terminal sequences have been described (Erickson et al., 1981).

Results

Early and Late Biosynthetic Forms of β-Glucuronidase. During pulse–chase experiments with porcine kidney cells, the earliest form of β-glucuronidase detected was a 75-kDa protein (Figure 1, lane 2). Between 3 and 4 h after translation, this form began to undergo conversion into a 72-kDa protein (Figure 1, lanes 7 and 8). Conversion was completed some time between 8 and 24 h after translation (Figure 1, lanes 9 and 10). During maturation of β-glucuronidase in elicited mouse peritoneal macrophages (Skudlarek & Swank, 1981),

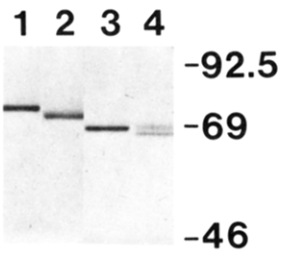

FIGURE 2: Endoglycosidase H treatment of β-glucuronidase. Porcine kidney cells were pulsed for 15 min with [^{35}S]Met, and the label was chased with unlabeled Met for the time periods indicated below. Immunoprecipitated proteins were analyzed by SDS–PAGE followed by fluorography of dried slab gel. Lane 1 shows protein labeled in vivo and immunoprecipitated immediately following a 15-min pulse. Lane 2 is like lane 1, except that the pulse was followed by an 8-h chase period. Lanes 3 and 4 are like lanes 1 and 2, respectively, except that the immunoprecipitated proteins were treated with endoglycosidase H. A blank gel lane (not shown) was obtained when nonimmune serum was added in place of the β-glucuronidase antiserum. Numbers to the right of lane 4 indicate the masses (in kilodaltons) of the ^{14}C-labeled marker proteins (see Figure 1).

a similar decrease in mass was detected by 1 h and completed in 5 h. The slower kinetics of this processing step in porcine kidney cells relative to those in macrophages may not merely reflect a difference between two cell types but rather may be a result of the extensive culturing of the kidney cells. A similar retardation in intracellular processing events in tissue culture cells relative to those in vivo has been observed in the case of human secretory component (Mostov & Blobel, 1982). After porcine kidney cells were continuously radiolabeled for 8 h, the cell culture medium contained an extracellular form of β-glucuronidase that migrated slightly slower than the 75-kDa intracellular form (Figure 1, lane 11 vs. lane 2).

Endoglycosidase H Treatment of Early and Late Forms of β-Glucuronidase. Posttranslational conversion of β-glucuronidase from the 75-kDa form into the 72-kDa form requires the cleavage of a 3-kDa fragment(s) which could be carbohydrate or peptide in nature. In order to determine if the conversion was due to carbohydrate processing, both proteins were treated with endoglycosidase H (Figure 2). This enzyme cleaves the bond between the two *N*-acetylglucosamine residues of Asn-linked high-mannose oligosaccharide chains at a rate that varies with the number of mannose residues (Tarentino et al., 1978). The early 75-kDa form (lane 1) was converted into a 68-kDa form (lane 3) which, except for Asn-linked *N*-acetylglucosamine (Tarentino et al., 1978), was presumably fully deglycosylated because it migrated slightly faster than the unglycosylated primary translation product (data not shown). The late 72-kDa form (lane 2) was converted by endoglycosidase H into two forms (lane 4), a 66-kDa form that was presumably fully deglycosylated and a 68-kDa form that was apparently only partially deglycosylated, perhaps because one of the oligosaccharide moieties became resistant to endoglycosidase H by acquiring complex carbohydrate. These data suggest that the difference in mass between the early 75-kDa and the late 72-kDa forms is largely due to processing of the polypeptide chain rather than the carbohydrate since the difference in mass appears to be maintained after deglycosylation of both forms, yielding 68- and 66-kDa polypeptides, respectively.

Partial NH_2-Terminal Sequence Determination of Early and Late Forms of β-Glucuronidase. The loss of a peptide fragment could occur from the NH_2 terminus, from an internal region, or from the COOH terminus. Since the reduced and alkylated derivatives of the forms of β-glucuronidase were observed by PAGE under denaturing conditions to be single

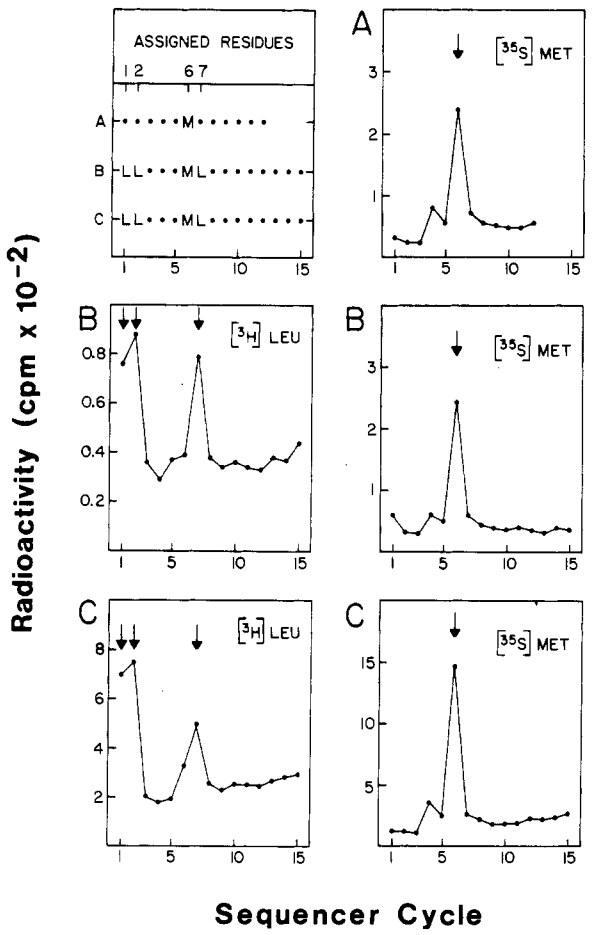

FIGURE 3: Partial NH_2-terminal sequence determination for three forms of β-glucuronidase. The upper left panel summarizes the sequence data presented here, where L is Leu, M is Met, and a dot is an undetermined residue. The other panels show representative results for automated Edman degradation of immunoprecipitated forms of β-glucuronidase synthesized and prepared as described under Experimental Procedures. (Panel A) The intracellular 75-kDa form; (panels B) the extracellular 75-kDa form; (panels C) the intracellular 72-kDa form. Arrows indicate positions where the radiolabeled amino acid is present.

polypeptide chains with masses of 75 and 72 kDa, respectively, loss of an internal fragment is highly unlikely. To determine whether the 3-kDa loss in peptide mass occurred from either or both ends of the early biosynthetic form of β-glucuronidase, we carried out NH_2-terminal sequence analysis of the early and late forms. Porcine kidney cells were labeled with [^{35}S]Met or [^{3}H]Leu, and the immunoprecipitable forms of β-glucuronidase were purified by PAGE. Figure 3 summarizes the partial NH_2-terminal sequences determined and shows the amount of radioactive amino acid released per sequencer cycle. The 75-kDa intracellular form showed a single Met at position 6 (panel A). The 75-kDa extracellular form contained Leu at positions 1, 2, and 7 as well as Met at position 6 (panels B), which suggests that the NH_2-terminal sequences of the intracellular and extracellular 75-kDa forms are identical. The intracellular 72-kDa form also contained Leu at positions 1, 2, and 7 and Met at position 6 (panels C). This identity of 4 of the first 15 NH_2-terminal residues indicates that the 72-kDa protein is not generated from the 75-kDa protein by NH_2-terminal proteolytic processing. Thus, the only remaining possibility is loss of a peptide from the COOH terminus of the 75-kDa form.

Carboxypeptidase Y Treatment of Early and Late Forms of β-Glucuronidase. To obtain direct evidence for COOH-terminal processing of β-glucuronidase, buffalo rat liver cells were labeled with [^{35}S]Met in a pulse–chase experiment. CpY digestion released [^{35}S]Met from the 75-kDa form of β-glucuronidase faster than from the 72-kDa form (Figure 4, panel A). Although the mole fraction of [^{35}S]Met released by CpY treatment was not integral, it is similar to the mole fractions of radiolabled amino acids observed by Kehry et al. (1980), who used CpY digestion to show that the membrane-bound and secreted heavy chains of immunoglobulin M have different COOH-terminal sequences. Taken together, our results strongly suggest that the two forms of β-glucuronidase possess different COOH-terminal sequences and that conversion of the 75-kDa protein into the 72-kDa protein occurs by COOH-terminal proteolytic processing. We cannot rule out the possibility that the late form only has undergone a post-translational modification which retards CpY digestion. However, such a modification would have to alter electrophoretic mobility so that the modified, late form migrates faster on PAGE than the unmodified, early form.

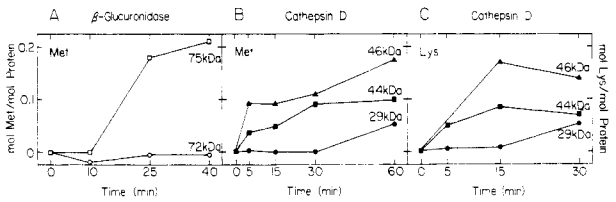

FIGURE 4: Carboxypeptidase Y treatment of β-glucuronidase and cathepsin D. Forms of β-glucuronidase and cathepsin D labeled with [^{35}S]Met or [^{3}H]Lys during pulse–chase experiments were digested with CpY as described under Experimental Procedures for the time periods indicated. Panel A shows release of Met from the 75-kDa early form (□) and the 72-kDa late form (○) of β-glucuronidase; panel B compares release of Met from the early 46-kDa pro (▲) and the 44-kDa single-chain forms (■) of cathepsin D with release of Met from the late 29-kDa heavy chain (●) of the protein; panel C compares release of Lys from the early 46-kDa pro (▲) and the 44-kDa single-chain (■) forms of cathepsin D with release of Lys from the late 29-kDa heavy chain (●) of the protein.

Carboxypeptidase Y Treatment of Early and Late Forms of Cathepsin D. To investigate whether other lysosomal enzymes might undergo similar COOH-terminal processing, we reexamined the various early and late biosynthetic forms of cathepsin D. As mentioned previously (see the introduction), cathepsin D is synthesized as preprocathepsin D (Erickson et al., 1981; Rosenfeld et al., 1982). The pre- and propeptides are removed co- and posttranslationally, respectively (Erickson et al., 1981), yielding an active 44-kDa single-chain enzyme. The latter is then cleaved into a light (15-kDa) and a heavy (30-kDa) chain derived from the NH_2 and COOH terminus, respectively, of the 44-kDa single-chain enzyme (Huang et al., 1979). Close examination of the heavy chain (Figure 5) revealed that between 4 and 8 h after synthesis (compare lanes 1 and 2), the heavy chain begins to undergo a slight reduction in mass (approximately 1 kDa) that is completed 24 h after synthesis (lane 3). A similar reduction in mass has been observed for the cathepsin D heavy chain from human fibroblasts (Hasilik, 1980; Steckel et al., 1982). The kinetics of this conversion step resembled those of the COOH-terminal processing step for β-glucuronidase (see above) and suggested that cathepsin D may also undergo COOH-terminal processing. Because the 30- and 29-kDa forms of the heavy chain are difficult to resolve, we carried out CpY digestion of the 46-kDa procathepsin D, the 44-kDa single-chain form, and the 29-kDa heavy chain, assuming identity in the COOH termini of the 46-kDa proenzyme, the 44-kDa single-chain enzyme, and the 30-kDa heavy chain. The data in Figure 4 (panels B and C) show that CpY digestion released [^{35}S]Met

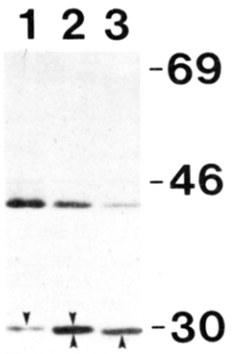

FIGURE 5: Forms of cathepsin D synthesized in vivo during a pulse–chase experiment. Porcine kidney cells were pulsed with [^{35}S]Met, and the label was chased with unlabeled Met for the time periods indicated below. Immunoprecipitated proteins were analyzed by SDS–PAGE followed by fluorography of the dried slab gel. Lane 1 shows protein immunoprecipitated following a 15-min pulse and a 4-h chase period. Lanes 2 and 3 are like lane 1, except that the chase periods were 8 and 24 h, respectively. Numbers to the right of lane 3 indicate the masses (in kilodaltons) of the ^{14}C-labeled marker proteins (see Figure 1; also carbonic anhydrase at 30 kDa). Downward arrows indicate the 30-kDa form, and upward arrows indicate the 29-kDa form of the heavy chain of cathepsin D.

and [^{3}H]Lys faster from the 46-kDa procathespin D and the 44-kDa single-chain enzyme than from the 29-kDa heavy chain. The similarity of the curves for the 46- and 44-kDa forms serves as a demonstration of the reproducibility of the assay. These data suggest, but do not prove, that the 1-kDa loss in mass from the 30-kDa heavy chain may occur by COOH-terminal processing.

Discussion

Our data suggest that the lysosomal enzyme β-glucuronidase undergoes COOH-terminal processing, losing 2–3 kDa in mass. Furthermore, we provide tentative evidence that COOH-terminal processing of a similar magnitude and timing also occurs for the lysosomal protease cathepsin D. Interestingly, three other lysosomal enzymes, glucocerebrosidase (Erickson et al., 1983), α-L-iduronidase (Myerowitz & Neufeld, 1981), and arylsulfatase (Hasilik, 1980) have been shown by pulse–chase experiments to undergo a similar loss of mass at a similar time in their biosynthetic pathway. Although it is not known whether the reduction in mass of these other lysosomal enzymes is due to COOH-terminal processing, the similar timing and magnitude of the loss of mass make it tempting to speculate that it might be analogous.

The role of COOH-terminal processing in lysosomal enzyme maturation is unknown. It is of course possible that the observed processing step is without functional significance and occurs randomly in only a few lysosomal enzymes as a result of collective packaging with and activation of various lysosomal proteases. Another more intriguing possibility is that all lysosomal enzymes might be synthesized with a transient sequence extension (not necessarily always located at the COOH terminus) that might function as a "sorting" sequence (Blobel, 1980) in a receptor-mediated process and that would be cleaved after completion of the sorting. Sorting is an essential step in lysosome biogenesis. Because lysosomal enzymes are initially cosegregated with secretory proteins within the cisternae of the endoplasmic reticulum (Erickson et al., 1981), they need to be separated, as a group, from secretory proteins and to be packaged into primary lysosomes. The recognition marker for sorting has been thought to be phosphomannosyl residues which have been found to be both common and unique to all lysosomal enzymes [see Sly et al. (1981) for a review]. Recent lines of evidence (Miller et al., 1981; Gabel et al., 1982; Owada & Neufeld, 1982; Schwaiger et al., 1982), however, argue against this role for the phosphomannosyl residues in all cell types. Therefore, while it is clear that the phosphomannosyl residues play an important role in receptor-mediated endocytotic uptake of lysosomal enzymes (which may have evolved as a safety mechanism to secure recapture of active lysosomal enzymes which have escaped into the extracellular space), the information for intracellular sorting, at least in some cell types, may reside in another common feature. The transient COOH-terminal segment demonstrated here for β-glucuronidase and tentatively also for cathepsin D could serve such a function and could represent a sorting sequence. Its cleavage in the lysosome would ensure that sorting by a receptor (which would shuttle between primary lysosomes and an upstream cellular compartment) would be a vectoral and irreversible process.

Acknowledgments

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Registry No. Pro-β-glucuronidase, 86921-30-4; procathepsin D, 86921-29-1; β-glucuronidase, 9001-45-0; cathepsin D, 9025-26-7.

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Isolation and Distribution of Elongation Factor 2 in Eggs and Embryos of Sea Urchins†

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ABSTRACT: The subcellular distribution of elongation factor 2 (EF-2) in eggs and early embryos of the sea urchin, *Strongylocentrotus purpuratus*, was studied by employing the diphtheria toxin dependent ADP-ribosylation of EF-2. When egg and embryo homogenates were fractionated by sedimentation, EF-2 was found associated with a low-speed pellet containing yolk, nuclei, and mitochondria. It also sedimented at 80 S and 5 S. No significant amounts of EF-2 were found on polyribosomes. The 5S form of EF-2 probably represents a monomeric unit of the factor as EF-2 had a molecular weight of 95 000 on sodium dodecyl sulfate–polyacrylamide gels. EF-2 could only be isolated intact if soybean trypsin inhibitor or EGTA was present. The total amount of EF-2 was similar in eggs and embryos. However, the distributions of the factor between the various fractions were substantially different for eggs and embryos. Also, a marked difference in the physical association of EF-2 with material in the low-speed pellet occurred after fertilization. Specifically, in eggs, 23% of the EF-2 was associated with the low-speed pellet; in cleavage-stage embryos, only 11% of the EF-2 was associated with the pellet. In eggs, 65% of the EF-2 sedimented as 80 S; by the 16-cell stage, this amount decreased to 44%. Concomitantly, the amount of EF-2 in the 5S fraction increased from about 8% in eggs to 44% in the 16-cell embryos. In addition, Triton X-100 was required for the extraction of EF-2 from the low-speed pellet of eggs, but not of embryos. We suggest that a redistribution of EF-2 after fertilization either may account for the increase in EF-2 activity observed by Felicetti et al. (1972) [Felicetti, L., Metafora, S., Gambino, R., & Di Matteo, G. (1972) *Cell Differ. 1*, 265–277] and, thus, be important in mediating the observed 2.5-fold increase in elongation rates after fertilization or may allow the activity of elongation factors to keep pace with the 50-fold increased rate of translation that occurs by the 2-cell stage.

Following fertilization of sea urchin eggs, there is a rapid and progressive increase of about 50-fold in the rate of protein synthesis (Nakano & Monroy, 1958; Epel, 1967; Gross et al., 1964; Regier & Kafatos, 1977). In the early stages of embryogenesis, this rate increase is independent of the synthesis of new ribosomes and mRNA. Although the major rate-limiting block in translation activity appears to be at the level of mRNA (Jenkins et al., 1978; Ilan & Ilan, 1978) or ribosome availability (Hille et al., 1981; Moon et al., 1982), there is also a 2.5-fold increase in the translational efficiency after fertilization (Hille & Albers, 1979; Brandis & Raff, 1978, 1979). Thus, the increase in translation after fertilization is a multiple of at least two factors. In addition, it is evident that, upon fertilization, numerous different molecular changes in the protein synthesis machinery occur. For instance, after fertilization, (1) the amount of free met-tRNA 40S ribosomal subunits increases (Hille et al., 1980), (2) the translational activity of ribosomes increases (Danilchik & Hille, 1981; Monroy & Tyler, 1967), (3) inhibitors of protein synthesis associated with egg ribosomes are less prevalent (Hille, 1974; Metafora et al., 1971), and (4) the activity of elongation factors increases severalfold (Felicetti et al., 1972). Some of these changes may have no direct effect on the rate-limiting step of protein synthesis. The mechanism of protein synthesis is, however, so complex that the large increase in translational

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