The Sea Urchin Multicatalytic Protease: Purification, Biochemical Analysis, Subcellular Distribution, and Relationship to snRNPs

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Abstract. We have purified and extensively characterized a 19-S particle from sea urchin eggs. This particle is the sea urchin homologue of the "prosome", a particle originally identified in duck erythroblasts. We now show that these sea urchin prosomes contain multiple proteolytic activities. As shown for analogous particles from other cells, these particles hydrolyze synthetic substrates containing neutral hydrophobic or basic amino acids at the carboxy terminus of the synthetic peptides. They contain 16–20 small proteins ranging in molecular weight from 20,000 to 32,000. Peptide mapping shows that most of the polypeptides are unique, however, three exist in two isoelectric forms. We have investigated the possible function of the sea urchin multicatalytic proteases (MCPs) by determining their subcellular distribution, their relationship to egg snRNPs, and their possible role in translational repression. There are almost as many MCPs (2×10^8) as ribosomes (6.6×10^8) or mRNPs (1.8×10^7) per egg. This suggests that like ribosomes, the MCPs are stored in the egg for use during later development. We find that a substantial proportion of egg MCPs move into nuclei by the late blastula stage. Using a specific antibody against one of the sea urchin MCP proteins and antibodies against U1-U6, La, and Ro RNPs, we show that the sea urchin particle is distinct from these RNPs, although the anti-U1-U6 RNP antibody cross-reacts with a single MCP protein. In addition, the sea urchin MCP appears to be associated with a large structure in the cytoplasm of unfertilized eggs and is released under the same conditions that activate egg mRNPs in vitro.

THE sea urchin egg contains large numbers of proteins, RNAs, and ribonucleoprotein complexes that are accumulated during oogenesis for use during the early stages of development (see Davidson, 1986, for review). The large complexes include the U1 containing snRNPs, messenger ribonucleoprotein particles (mRNPs), and ribosomes (Brown et al., 1985; Nash et al., 1987; Grainger and Winkler, 1987). While studying sea urchin egg mRNPs, we discovered a particle which is the sea urchin homologue of a 19-S particle called the "prosome" (Schmid et al., 1984). Subsequently, Akhayat et al. (1987) identified this particle using an antibody against vertebrate prosomes.

Prosomes belong to a class of 19-S particles that have been identified in a number of species of animals and plants (see Kloetzel, 1987, for review). They are composed of a characteristic set of proteins between 21,000 and 35,000 D. Some of the particles contain a variable number of small RNAs between 55 and 200 nucleotides long. The assembled particle's molecular weight is ~700,000. Electron micrographs have shown that the particle has a characteristic raspberry-shaped appearance. These particles are found both in the cytoplasm and the nucleus (Martins de Sa et al., 1986; Kloetzel et al., 1987; Arrigo et al., 1988; Grossi de Sa et al., 1988).

These particles are unusual in that they contain several different enzymatic activities. In yeast, *Drosophila*, amphibian, chicken, and mammalian cells, these 19-S particles have multiple proteolytic activities and in the mammalian cells they have been shown to be identical to the prosome (Hough et al., 1987; Arrigo et al., 1988; Falkenburg et al., 1988; Tanaka et al., 1988). Each particle contains up to three different proteolytic activities, based upon the pH optima, substrate specificity, and the differential effects of inhibitors and activators (Tanaka et al., 1986; Hough et al., 1987). Thus, these particles have been renamed "multicatalytic proteases" (MCPs)[1] or "proteosomes" (Arrigo et al., 1988; Falkenburg et al., 1988). In addition, a similar 19-S particle isolated from *Xenopus* ovaries has a similar protein composition to the multicatalytic protease but contains tRNA-processing activity (Castano et al., 1986).

We have begun an investigation of the role of these 19-S particles in sea urchin development. We have developed an improved isolation procedure to purify the sea urchin egg 19-S particle. We purified the sea urchin particle to homogeneity, characterized its protein components in detail, and showed that the particles contain proteolytic activity characteristic of the MCPs. Using an antibody directed against one of the sea urchin 19-S particle proteins, we have shown that there are large numbers of the particle in the unfertilized egg and that

1. *Abbreviations used in this paper*: HS-EDTA, high salt EDTA buffer; LSB, low salt buffer; MCPs, multicatalytic proteases.

these egg particles redistribute between the cytoplasmic and nuclear compartments during development. We have also shown that they are associated with a macromolecular structure that cofractionates with mRNPs on large pore size gel filtration columns. High salt treatment of these complexes results in the release of the particles and the concurrent translational activation of egg mRNPs.

Materials and Methods

Isolation of Sea Urchin 19-S Particles

Eggs from the sea urchin *Strongylocentrotus purpuratus* were spawned by intracoelomic injection with 0.55 M KCl. The eggs were dejellied with pH 5.0 sea water and washed three times in normal sea water. The temperature of a 1% suspension of eggs was rapidly lowered to 0°C by passing the eggs through a stainless steel coil cooled to −4°C. The eggs were then concentrated by continuous flow centrifugaton, washed three times in homogenization buffer (250 mM NaCl, 25 mM EGTA, 5 mM $MgCl_2$, 110 mM glycine, 250 mM glycerol, 10 mM Pipes, pH 6.8, 1 mM DTT, and 1 mM PMSF), and homogenized in an equal volume of buffer in a stainless steel homogenizer (Dura-Grind; Wheaton Industries, Millville, NJ). The homogenate was centrifuged at 22,000 *g* for 15 min and the supernatant was aliquoted and stored at −70°C.

To separate the 19-S MCPs from free cytoplasmic proteins, 190 ml of egg postmitochondrial supernatant were chromatographed on a Biogel A 1.5-cm column (5 × 71 cm) in low salt buffer (250 mM NaCl, 5 mM EGTA, 5 mM $MgCl_2$, 360 mM glycerol, 10 mM Pipes, pH 6.8, 1 mM DTT, 1 mM PMSF). Under these ionic conditions the MCPs are bound to an intracellular component that is excluded from this column matrix. The excluded peak was concentrated using Aquacide IV (Calbiochem-Behring Corp., La Jolla, CA) and rechromatographed on a second Biogel A 1.5-m column in high salt/EDTA buffer (0.5 M NaCl, 10 mM EDTA, 10 mM Pipes, pH 6.8, 1 mM DTT). The MCPs are released from the excluded peak by this high salt treatment. Each fraction was analyzed on a 12.5% SDS polyacrylamide gel and the fractions containing 19-S particles were pooled and concentrated.

The MCPs were then separated from the remaining cellular contaminants by glycerol gradient centrifugation. 12 OD_{260} units of the high salt EDTA buffer (HS-EDTA) MCP fraction were layered on a 35-ml 15–40% glycerol gradient containing 0.5 M NaCl and 10 mM Hepes, pH 7.4 and centrifuged for 30 h at 28,000 rpm in a SW 28 rotor (Beckman Instruments, Inc., Palo Alto, CA). 15 fractions were collected. The fractions containing MCPs were pooled, dialyzed against 0.5 M NaCl, 10 mM Hepes pH 7.4, and concentrated using Aquacide II (Calbiochem-Behring Corp.).

To determine the stability of the purified MCPs to detergent treatment, MCP-containing fractions from either the high salt/EDTA column or MCPs from the glycerol gradients were layered on 15–40% glycerol gradients containing 0.5 M NaCl, 10 mM Hepes, pH 7.4, and 1% Sarkosyl, and centrifuged for 21 h at 35,000 rpm in a SW 41 rotor (Beckman Instruments, Inc.). 10 fractions were collected. Each fraction was treated with 20 μg/ml of RNase A for 15 min at 25°C, 4 vol of 95% ethanol were added to each fraction and the proteins and MCPs precipitated overnight at −20°C. The fractions were redissolved in SDS gel sample buffer and the proteins separated on one-dimensional SDS gels as described below.

Gel Electrophoresis and Peptide Mapping

One-dimensional SDS gels, Western blots, and silver-staining procedures are described in Grainger et al. (1986). Purified MCPs were run on nonequilibrium two-dimensional gels essentially according to O'Farrell et al. (1977). The first dimension was run for 4 h at 400 V. The proteins were separated in the second dimension on 15% SDS gels and stained with Coomassie Blue.

The spots labeled 1-11b were excised from a two-dimensional SDS gel and partially peptide mapped essentially as described by Cleveland et al. (1977). The spots were equilibrated in 0.125 M Tris, pH 6.8, 0.1% SDS, and 1 mM EDTA, placed in gel sample wells with 0.0125 μg of V8 protease (Miles Laboratories Inc., Naperville, IL) and subjected to electrophoresis into the stacking gel. The power was turned off and proteins digested for 30 min. The power was then turned on and the peptide fragments were separated on a 17.5% SDS gel. The fragments were detected by silver staining.

Assay of Proteolytic Activity

Peptidase activity was assayed essentially as described in Tanaka et al. (1986) using two synthetic peptides, n-Benzoyl-Val-Gly-Arg p-nitrolanilide (Bz-val-gly-arg-pNa) and Succinyl-Leu-Leu-Val-Tyr 4-methyl-7-coumaryl amide (Suc-leu-leu-val-tyr-NMec). 1 μg of purified MCPs was added to 600 μl of reaction buffer (50 mM Tris, pH. 8.0, 1 mM $MgCl_2$, 0.1 mM EDTA, and 1 mM DTT) containing either 0.5 mM Bz-val-gly-arg-pNa or 0.1 mM Suc-leu-leu-val tyr-NMec. The hydrolysis of the pNa derivative was measured directly by monitoring the increase in absorbance at 410 nm. The number of moles of NMec released was determined using a standard curve of 0–0.2 mM NMec. 1 U of activity is defined as the number of nanomoles of pNa or NMec released per minute per milligram protein at 25°C.

Antisera against 19-S Particle Protein P1

Purified MCPs were run on nonequilibrium two-dimensional SDS gels, stained briefly with Coomassie Blue, and the MCP protein designated P1 was excised. Approximately 15 μg of P1 protein was mixed with complete Freunds adjuvant and injected subcutaneously into a rabbit. The rabbit was boosted every 2 wk with 15 μg of P1 protein and high titer antisera was obtained after three boosts.

Quantification of MCP Protein P1

The amount of P1 protein in purified MCPs was determined by quantifying the amount of P1 on Western blots. Several concentrations of MCPs (0.10–0.35 μg) purified on high salt gradients and BSA standards (0.05-1 μg) were run on a one-dimensional, 12.5% SDS polyacrylamide gel. A Western blot of this gel was then stained with 0.1% Amido black and the amount of protein in P1 determined by comparison with the BSA standards. The ratio of the amount of P1 per MCP was constant over this concentration range.

To quantify the amount of P1 protein in fractions from the MCP purification, varying amounts of protein from each of the fractions were separated on a 12.5% SDS gel along with a known amount of P1 protein from purified prosomes. A Western blot of this gel was probed with the anti-P1 antibody and ^{125}I-protein A and exposed to Kodak SB-5 x-ray film. By scanning the autoradiograph, the amount of purified P1 per unit density was determined. The amount of P1 per unit density in each fraction was constant over a fourfold concentration range. By using the P1/unit density of the purified MCPs as a standard, we calculated the amount of P1 protein in each of the other fractions. The total amount of protein in each fraction was determined using the Schaffner-Weissman assay (Schaffner and Weissman, 1973). This procedure allows us to quantify the amount of P1 protein in unpurified cellular fractions.

To determine the number of P1 proteins per purified MCPs, varying concentrations (0.1–0.35 μg) of purified MCPs were run on a one-dimensional SDS gel, stained with Coomassie Blue, and the percentage P1/MCP determined by densitometry. At all concentrations, the P1 protein was 8.8% of the total MCP proteins. If the molecular weight of the protein component of the entire MCP is 500,000–700,000 (Tanaka et al., 1988), then 8.8% of the mass is between 44,000 and 61,000 D. If a single P1 protein is 29,000–30,000 D, then there must be one or two P1 proteins per MCP.

Isolation of Cell Fractions and Nuclei Purification

A 1% suspension of dejellied eggs was fertilized with a 1:20,000 dilution of sperm and cultured for 24 h at 15°C. 2 ml of embryos (hatched blastulae) were washed twice in homogenization buffer and homogenized in an equal volume of buffer. The homogenate was then centrifuged at 800 *g* for 10 min (crude nuclear pellet), 12,000 *g* for 20 min (mitochondrial pellet), and 100,000 *g* for 90 min (microsomal pellet and S100 fraction).

Purified nuclei were isolated from these hatched blastula as described by Hinegardner (1962). The nuclei were collected from the sucrose gradient, diluted 1:1 with distilled water, and pelleted by centrifugation at 8,000 *g* for 10 min. The nuclear pellet was resuspended in homogenization buffer, aliquoted, and stored at −70°C.

A proportion of the isolated nuclei and cell fractions were treated with 100 μg/ml protease-free DNase and 50 μg/ml RNase at 4°C for 10 min. They were then dissolved in one-dimensional SDS gel sample buffer and separated on a 12.5% SDS gel. The amount of MCP protein P1 in each fraction was determined as described above.

Gel Filtration Chromatography

2 ml of a postmitochondrial supernatant from unfertilized eggs was chromatographed on a Biogel A 1.5-m column (1.5 × 50 cm) in low salt buffer (LSB) (0.1 M NaCl, 5 mM $MgCl_2$, 10 mM Pipes, pH 6.8, 1 mM EGTA, 1 mM DTT) or in HS-EDTA (0.5 M NaCl, 10 mM EDTA, 10 mM Pipes, pH 6.8, 1 mM DTT). An aliquot from each fraction of the two columns was

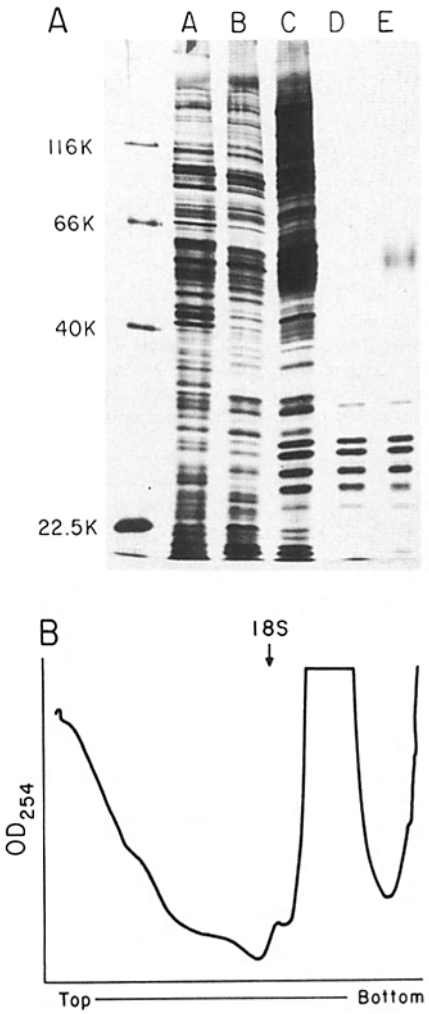

Figure 1. Purification of 19-S particles. (*A*) SDS-PAGE of each stage of purification. Lane *A*, unfertilized egg postmitochondrial supernatant; *B*, excluded peak from low salt Biogel A 1.5-m column; *C*, 19-S particle containing fractions from high salt/EDTA Biogel A 1.5-m column; *D*, 19-S peak from high salt glycerol gradients; *E*, 19-S peak from high salt glycerol gradients containing 1% Sarkosyl. Lanes *A–C* contained 5 μg of total protein, lane *D*, 0.35 μg. Proteins were visualized by silver staining. (*B*) OD_{254} tracing of high salt glycerol gradient. MCPs are contained in the 19-S peak.

run on a 12.5% SDS gel, Western blotted, and incubated with anti-P1 antibody and ^{125}I-protein A. The amount of MCPs in each fraction was determined by scanning each lane of the autoradiograph.

Results

Isolation of Sea Urchin 19-S Particles

A variety of 19-S particles have been isolated from several vertebrate species using a series of centrifugation techniques (Schmid et al., 1984). One possible problem with these isolation procedures is that they include a step in which the particles are pelleted along with free cytoplasmic mRNPs and other cytoplasmic complexes >20 S. This pelleting may result in an artifactual association of 19-S particles with mRNPs or other cytoplasmic particles. In sea urchin eggs, this aggregation may be a particular problem since pelleting of the mRNP fraction results in aggregation of mRNPs and other cellular structures (Moon et al., 1982). Consistent with these results, Akhayat et al. (1987) were unable to separate 19-S prosome particles from other complexes in the unfertilized egg after this pelleting step. To avoid this problem, we purified sea urchin 19-S particles without a pelleting step, using a combination of gel filtration chromatography and glycerol gradient centrifugation. By using this improved procedure, we have purified sea urchin 19-S particles to homogeneity.

The first step in this purification procedure is the fractionation of a postmitochondrial supernatant from unfertilized eggs on a Biogel A 1.5-m gel filtration column. In low salt buffer, the 19-S particles are excluded from the column and remain associated with cellular structures >30 S (see below for discussion). When the excluded peak is rechromatographed over a second Biogel A 1.5-m column in high salt buffer (0.5 M NaCl/10 mM EDTA), a particle containing a characteristic group of small proteins is released from the original excluded fraction (Fig. 1, lane *C*). Centrifugation of this fraction on 15–40% glycerol gradients containing high salt results in the separation of components sedimenting at 19 and 22–24 S (Fig. 1 *B*). Examination of the proteins in these fractions by SDS gel electrophoresis shows that the 19-S peak contains the characteristic cluster of small proteins found in the fraction released by high salt treatment, while the 22-S peak contains a series of high molecular weight proteins (Fig. 1, lane *D*). This 22-S particle is most likely a glycoprotein complex that is stored in the unfertilized egg (Kari and Rottmann, 1985).

We have separated the 19-S particle from the 22-S particle on high salt–Sarkosyl gradients (Fig. 1, lane *E*). A fraction of the high salt/EDTA column was treated with 1% Sarkosyl and 20 μg/ml of RNase A and recentrifuged on Sarkosyl gradients. In this detergent, the 19-S particle is stable while the 22-S particle is converted to an 11-S particle (data not shown). The pattern of proteins from these RNase–Sarkosyl-treated 19-S particles is identical to that from the control particles (Fig. 1, lanes *D* and *E*). The additional higher molecular weight band in the Sarkosyl-treated particles is due to a silver stain artifact and the low molecular weight band is due to the RNase A. Unlike some other small RNPs, RNase treatment does not result in the shift of a protein in the particle to a different molecular weight (Bringmann and Luhrmann, 1986).

Table I. Purification of Sea Urchin 19-S Particles

Stage	Volume	Total protein*	Microgram P1 per milligram‡	Fold	Yield
	ml	*mg*			%
Unfertilized egg homogenate	190	2300	0.90	1.0	100
Peak 1 - LS-Mg column	130	650	3.62	4.0	100
HS-EDTA column	20	27	25.00	27.8	30
HS-glycerol gradients	5	0.65	154.00	171.0	5

* Protein was determined by the Schaffner–Weissman assay (Schaffner and Weissman, 1973) using BSA as a standard.
‡ P1 protein was determined by probing Western blots with an antibody directed against MCP protein P1 (see Materials and Methods for details).

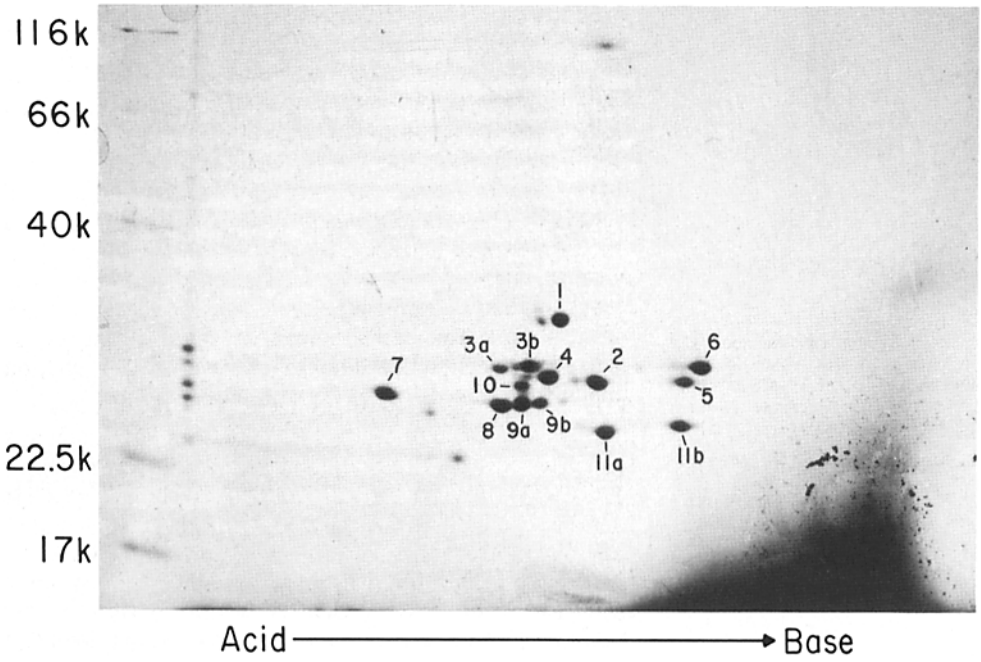

Figure 2. Two-dimensional SDS gel of purified 19-S particles. The proteins from 19-S particles isolated on high salt–Sarkosyl gradients were separated on a nonequilibrium two-dimensional SDS gel as described in Materials and Methods and stained with Coomassie Blue.

We determined the yield and purity of the 19-S particles during the isolation procedure by using an antibody directed against one of the sea urchin 19-S particle proteins (P1) to quantify the amount of particles in each fraction (see below for antibody description). We assume that the amount of P1 per particle remains constant throughout the isolation procedure. As shown in Table I, this isolation procedure results in a 171-fold purification of the particles. To determine if the purified particles were essentially free of egg contaminants, we calculated the expected degree of purification based on the number of particles per egg, the molecular weight of the particles, and the total amount of protein per egg. We calculate that the 19-S particles constitute 0.6% of the total mass of the egg. If the isolated prosomes are completely free from contamination, then the maximal theoretical purification would be 185-fold. This estimate is very close to our experimentally determined value and is consistent with the conclusion that these particles are purified essentially to homogeneity.

Analysis of the 19-S Particle Proteins

The pattern of proteins of the sea urchin 19-S particle is very similar to the protein pattern of prosomes and multicatalytic proteases isolated from yeast, amphibian, *Drosophila*, duck, mouse, and human cells (Schmid et al., 1984; Arrigo et al., 1985; Falkenburg et al., 1988; Arrigo et al., 1988; Tanaka et al., 1988). In addition, all of these 19-S particles are stable to sarkosyl treatment while other RNPs and ribosomes are dissociated in this detergent. It is probably identical to the sea urchin particle identified as a prosome on the basis of immunological cross-reactivity with a monoclonal antibody against duck prosomes (Akhayat et al., 1987). Thus, by these criteria, this 19-S particle appears to be the sea urchin homologue of the multicatalytic protease or prosome isolated from these other systems.

To further substantiate the identity of these particles, we compared the two-dimensional SDS gel pattern of sea urchin particle proteins with those of published protein gels of duck, mouse, and human prosomes (Fig. 2) (Martins de Sa et al., 1986; Arrigo et al., 1987). The purified sea urchin 19-S particles contain 16–20 polypeptides with molecular weights between 22,000 and 30,000 and with isoelectric points between pH 4 and 8 (Fig. 2). The two-dimensional protein pattern of the sea urchin 19-S particle is very similar to the pattern of purified mouse and HeLa cell prosomes. Like the mammalian prosomes, the sea urchin 19-S particles lack the 56,000-D prosome protein found in duck prosomes. No large proteins were observed in the purified sea urchin 19-S particles. The large proteins observed in the previous analysis of sea urchin prosomes are probably due to contamination of the prosome particles with the 22–26-S glycoprotein complex (Akhayat et al., 1987; Kari and Rottman, 1985).

Several of the 19-S particle proteins have identical molecular weights but have different isoelectric points. To determine whether these isoelectric differences are due to posttranslational modifications, we analyzed these proteins by peptide mapping. Protein spots from a two-dimensional gel of purified 19-S particles were excised and digested with V8 protease, as described by Cleveland et al. (1977). Of the 14 proteins examined, 11 are unique (Fig. 3). Protein 8, for example, which appears to migrate very close to proteins 9a and 9b, is clearly distinct from them by peptide mapping. Three of the proteins do exist in two isoelectric forms (see spots labeled 3, 9, and 11) indicating that these proteins are posttranslationally modified.

Sea Urchin 19-S Particles Contain Multicatalytic Protease Activity

Recently, a 19-S particle from *Drosophila* and humans has been shown to contain multicatalytic protease activity (Arrigo et al., 1988; Falkenburg et al., 1988). These particles cleave a variety of synthetic substrates that have two major features in common. They contain either a neutral hydrophobic or basic amino acid in the carboxy-terminal position and

Table II. Multicatalytic Peptidase Activity of the Sea Urchin 19-S Particle

Substrate	Specific activity (nmoles/min/mg protein)
	U
Bz-Val-Gly-Arg-pNa	240
Suc-Leu-Leu-Val-Tyr-Nmec	145
With inhibitors	
0.25 mM leupeptin	2 (0.8%)
0.25 mM pepstatin	336 (140%)

Peptidase activity was determined as described in Materials and Methods. The effect of the inhibitors was determined using the Bz-Val-Gly-Arg-pNa substrate. The inhibitors leupeptin and pepstatin were dissolved in DMSO and added to the MCPs 10 min before the addition of the peptide substrate. Addition of an equivalent amount of DMSO to the control had no effect on proteolytic activity.

they must contain at least three amino acids. We have tested the purified 19-S sea urchin particles for the presence of this activity by using two of these protease substrates, Bz-Val-Gly-Arg-pNa and Suc-Leu-Leu-Val-Tyr-NMec. The results of these assays are shown in Table II. The sea urchin 19-S particles cleave both of these substrates. Their specific activity is relatively high compared with many of the other MCPs isolated from other systems (Tanaka et al., 1986; Hough et al., 1987). This may be due to the removal of an endogenous inhibitor during the gel filtration step or to an unusually high activity in sea urchin eggs. As is found in rabbit reticulocyte and mammalian 19-S MCPs, the proteolytic activity is completely inhibited by the addition of 0.25 mM leupeptin and stimulated 1.5-fold by the addition of 0.25 mM pepstatin A (Hough et al., 1987). Thus, by these criteria, it appears that the sea urchin 19-S particle that we have purified belongs to this class of large, multicatalytic proteases (MCPs) and will from here on be designated as such.

Sea Urchin MCPs Are a Major Constituent of the Unfertilized Egg

From the purification data in Table I, it is clear that there is a large amount of MCP protein in postmitochondrial supernatants of unfertilized eggs. Since knowledge of the relative number of MCPs may help give insights into their function, we have determined the number of MCPs per unfertilized egg. We have used this value to compare their abundance with other cellular structures such as ribosomes and mRNPs. To quantitate the number of MCPs in the egg, we used a rabbit polyclonal antibody which recognizes the sea urchin MCP protein P1 (see 1, Fig. 2). Western blot analysis using this antibody shows that it binds predominantly to this MCP protein (Fig. 4). The two faint spots that react with the P1 antibody may be due to immunological cross-reactivity or to contamination of the original antigen used to prepare the antibody.

To determine the number of MCPs per unfertilized egg, we measured the amount of P1 protein per egg. From the data in Table I, there are 11.7 μg of P1 protein per milliliter of egg homogenate. By using a molecular weight of 29,000 for a single P1 protein and the determination that 1 ml of homogenate equals 1.18×10^6 eggs, we calculate that there are 2×10^8 P1 proteins per egg. By using a stoichiometry of one to two P1 proteins per MCP (see Materials and Methods), we calculate that there are between 2–4 $\times$ 10^8 MCPs per egg. As a comparison, there are $\sim 6.6 \times 10^8$ ribosomes and 1.7×10^7 mRNPs per egg (Davidson, 1986). Thus, there are almost as many MCPs as ribosomes and 10-fold more MCPs than mRNPs. The sea urchin egg contains very large stores of ribosomes and there is little or no ribosome synthesis until later in development (Davidson, 1986). The large number of MCPs in the egg suggests that, like ribosomes, MCPs stored in the unfertilized egg may be used during development without new MCP protein synthesis. This is consistent with the observation that no new synthesis of MCP proteins was detected in the sea urchin embryo (Akhayat et al., 1987; our unpublished results).

MCPs Are Found in Both the Nucleus and Cytoplasm of Sea Urchin Embryos

The majority of MCPs in the unfertilized egg are in the cytoplasm. During development, it appears that they redistribute between the cytoplasm and a crude nuclear fraction (Ahkayat et al., 1987). To determine the relative distribution of MCPs, we prepared a crude nuclear and mitochondrial fraction, a 100,000 *g* pellet that includes free and membrane-bound polysomes, microsomes, and free ribosomes and a 100,000 *g* supernatant (S100), which contains free mRNPs and cytoplasmic proteins from late blastula stage embryos. Analysis of these fractions by SDS gel electrophoresis and Western blotting shows that the majority of the MCPs are found in the S100 fraction, and a small number are found in the microsome fraction (Fig. 5, lanes *A–D*). MCPs were not detected in the crude nuclear fraction because the large amount of yolk in this fraction limited the amount of protein that could be loaded on the gel. The MCP antibody also cross-reacts with a series of high molecular weight proteins in each of these fractions that are not found in purified MCPs. It is not clear if these proteins have similar antigenic sites to the P1 protein or are functionally related.

To rigorously demonstrate that MCPs are found in nuclei, we prepared highly purified nuclei from 24-h embryos (late blastula) and probed Western blots of these nuclei with the sea urchin MCP antibody. As shown in Fig. 5 (lane *E*), the purified nuclei do contain MCPs.

We have compared the number of MCPs in unfertilized eggs to those found in blastula embryos and nuclei. The embryos contain 1.02 μg P1/mg of protein or 2×10^{13} MCPs/mg protein. This is very close to the number obtained from the unfertilized egg, suggesting that there is no change in the overall number of MCPs up to this stage in development. In addition, we cannot detect the synthesis of any new MCP proteins through the blastula stage (data not shown). Thus, the MCPs in the blastula nuclei must be the result of the migration of egg cytoplasmic MCPs.

We have also determined the ratio between the number of MCPs in the blastula nuclei and cytoplasm. The blastula nuclei contain 2.84 μg P1/mg protein or 5.6×10^{13} MCPs/mg protein while the cytoplasm contains 2×10^{13} MCPs per milligram protein. Thus, on a protein basis, MCPs are 2.8-fold more concentrated in the nucleus than the cytoplasm. This suggests that not only do the egg cytoplasmic MCPs migrate to the nucleus, but that there must be some mechanism to concentrate them within the nucleus.

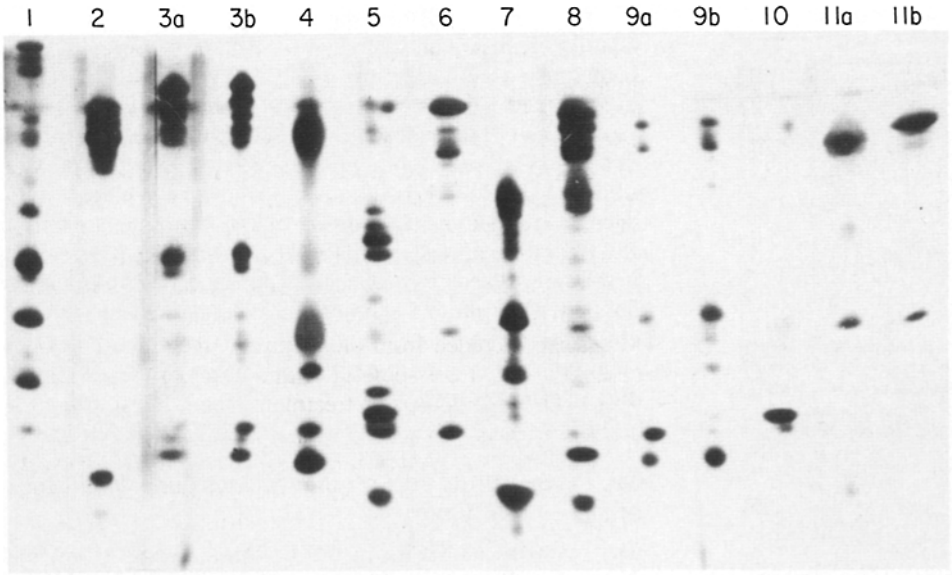

Figure 3. Peptide map of MCP proteins. Spots from a two-dimensional SDS gel of purified MCPs were excised and one-dimensional peptide maps were performed as described in Materials and Methods. The numbers at the top of the gel refer to the numbering scheme presented in Fig. 2. Note that proteins 3, 9, and 11 appear to have very similar peptide maps.

19-S MCPs Are Distinct from Nuclear-splicing RNPs

The unfertilized egg contains large numbers of small RNPs such as U1- and U7-containing RNPs (Brown et al., 1985; Birnstiel et al., 1985; Ruzdijic and Pederson, 1987). To determine if MCPs are related to any of these RNPs, we probed Western blots of one-dimensional SDS gels of unfertilized eggs and purified MCPs with a patient anti-Sm antibody. This antibody recognizes several proteins found in small RNPs containing U1–U6 RNAs (Pettersson et al., 1984). As shown in Fig. 6 (lane *1*), this antibody reacts with three proteins from unfertilized eggs with molecular weights of 33,000, 24,000, and 14,000. Based on these molecular weights, these proteins appear to be the A, C, and D proteins found in U1–U6 RNPs from other species (Pettersson et al., 1984; Bringmann and Luhrmann, 1986). Purified MCPs, however, do not contain cross-reactive proteins of these molecular weights. The antibody binds to a unique MCP protein with a molecular weight of 26,000 (Fig. 6, lane *2*). We have identified this protein as the spot labeled 6 (see Fig. 2) (data not shown). We have also tested antisera against the Ro and La antigens which recognize two other classes of small RNPs (Wolin and Steitz, 1984; Bachmann et al., 1986). The anti-La antibody reacts with several proteins in the unfertilized egg but does not cross-react with any MCP protein (data not shown). No cross-reactivity with any protein in the unfertilized egg was observed with the Ro antisera. Thus, by this antibody analysis, MCPs do not appear to belong to the family of nuclear-splicing RNPs. However, there may be some relationship between these RNP particles since one of the MCP proteins reacts with the anti-Sm antibody; however it should be kept in mind that patient antisera may detect nonrelated proteins in distant species.

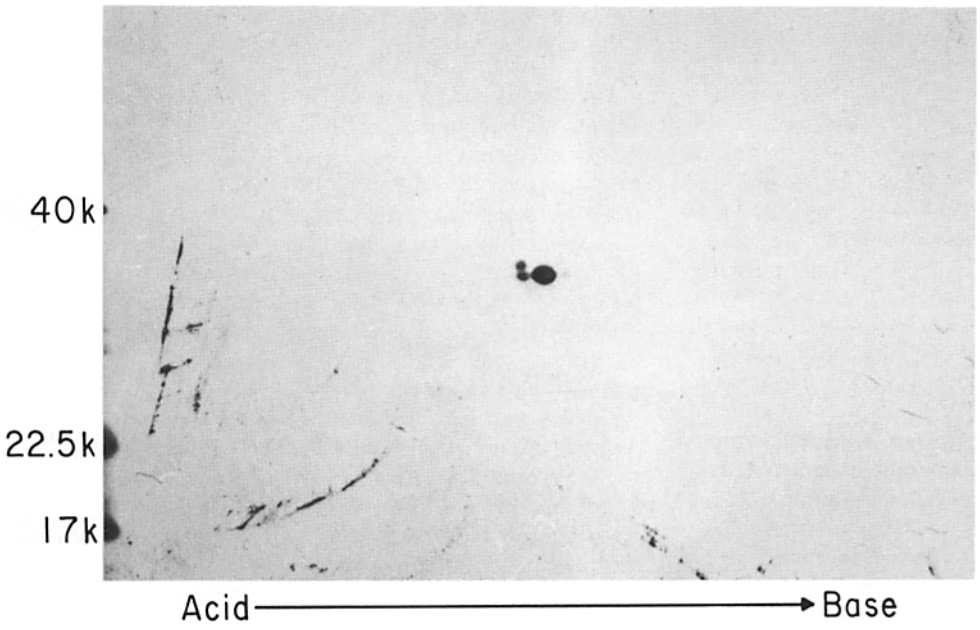

Figure 4. P1 antibody reacts with one set of MCP proteins. Purified MCP proteins were separated on a two-dimensional SDS gel, Western blotted, and probed with the anti-P1 antibody and ^{125}I-protein A.

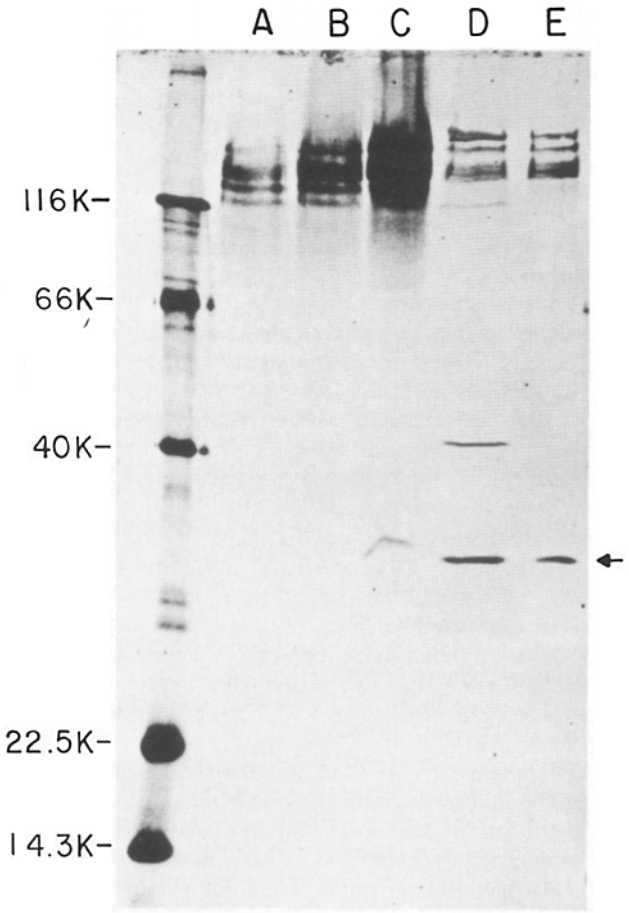

Figure 5. MCPs are present in nuclei of sea urchin blastulae. Equal amounts of protein from the 800 *g* pellet (lane *A*), 12,000 *g* pellet (lane *B*), 100,000 *g* pellet (*C*), 100,000 *g* supernatant (lane *D*), and purified nuclei (lane *E*) were separated on a 12.5% SDS gel, Western blotted, and probed with the anti-P1 antibody. The P1 protein is designated by the arrow. The high molecular weight proteins that react with the P1 antibody are not found in isolated MCPs.

Correlation of MCP Release with mRNP Activation

One possible role of the 19-S particle is the translational repression of cytoplasmic mRNPs (Martins de Sa, 1986). In reticulocytes and tissue culture cells these 19-S particles cofractionate with translationally repressed mRNPs and release of the particles by high salt treatment is correlated with the activation of the mRNPs (Vincent et al., 1983; Schmid et al., 1984). As mentioned previously, one problem with the interpretation of these results is that the 19-S particles and mRNPs were pelleted during these mRNP isolation procedures. This could result in the nonspecific aggregation of mRNPs with the 19-S particles. Thus, the functional inactivity of mRNP-19-S particle complexes may be the result of aggregation of the mRNPs rather than the specific association of these particles with the mRNPs. To avoid this problem, we used gel filtration chromatography instead of sedimentation velocity centrifugation in the initial fractionation or purification steps.

mRNPs fractionated from unfertilized eggs by gel filtration chromatography in low salt buffer are translationally repressed (Grainger and Winkler, 1987). Subsequent treatment of these mRNPs with high salt buffer followed by rechromatography activates these mRNPs. We used the P1 antibody to determine if sea urchin MCP release correlated with mRNP activation (Fig. 7). A postmitochondrial supernatant of unfertilized eggs was chromatographed on a Biogel A 1.5-m column in either low salt or high salt buffer. The proteins in each fraction were separated on an SDS gel, Western blotted, probed with anti-P1 antibody and ^{125}I-protein A. The densitometer tracings corresponding to the location of the MCPs and the OD_{260} of the excluded peak are shown in Fig. 7. Under low salt conditions, all of the MCPs are excluded from the column (Fig. 7 *a*). This indicates that they are associated with a complex larger than 1 $\times$ 10^6 D. After high salt treatment, the MCPs shift to a smaller apparent size, becoming partially included in the column and fractionate at the same place as free MCPs (Fig. 7 *B*). The change in fractionation behavior of the MCPs indicates that in the egg postmitochondrial supernatant, the MCPs are associated with some large cellular structure and that high salt treatment results in the release of the MCPs from this structure. This association is probably not due to an artifactual interaction since no pelleting steps were used.

This column analysis demonstrates that MCPs cofractionate with mRNPs under low salt conditions and are released from the partially purified mRNP fraction after high salt treatment. This release correlates with the translational acti-

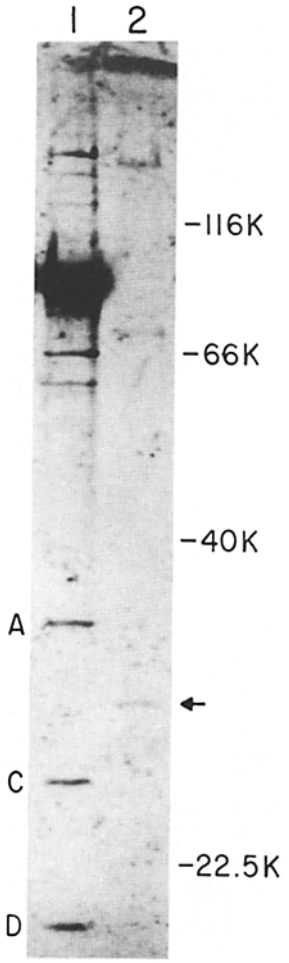

Figure 6. Sea urchin MCPs are unique from U1-6 snRNPs. The proteins from a postmitochondrial supernatant from unfertilized eggs (lane *1*) and from purified egg MCPs (lane *2*) were separated on a 12.5% SDS gel, Western blotted, and probed with the anti-Sm antibody. The bands marked *A*, *C*, and *D* refer to the standardized nomenclature for U1-6 snRNP proteins. The MCP-specific protein is marked by the arrow.

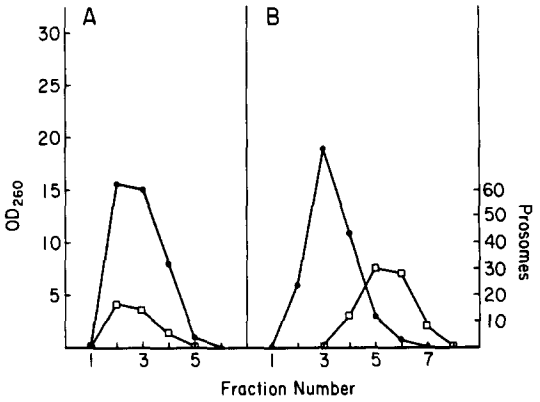

Figure 7. mRNP activation and MCP release. A postmitochondrial supernatant from unfertilized eggs was chromatographed on a Biogel A 1.5-m column in either (*A*) low salt or (*B*) high salt buffer. The fractionation of the MCPs was determined by probing Western blots of each fraction with the P1 antibody. (●) OD_{260}; (□) MCPs. Only the first six fractions are shown.

vation of the mRNPs by high salt treatment. Thus, the MCP could be a "masking" factor that is released upon mRNP activation in vitro. However, we have not demonstrated a direct association of the MCP with egg mRNPs or a role in their translational regulation.

Discussion

We have purified a 19-S particle that is the sea urchin homologue of the 19-S multicatalytic protease. There are large stores of MCPs in the unfertilized egg and a portion of these appear to become associated with nuclei later in development. The sea urchin MCP appears to be unique from other large complexes stored in unfertilized eggs, including particles involved in mRNA processing. We have shown that in the unfertilized egg, MCPs are associated with some large cellular structure and that high salt treatment dissociates the MCPs from this structure. This suggests that the native state of the MCP in the unfertilized egg may contain additional proteins or other macromolecules. A possible candidate are the translationally repressed mRNPs stored in the unfertilized egg.

The U1-containing small RNPs are another class of relatively small particles that have been characterized in detail in sea urchin eggs (Nash et al., 1987). Both the U1 RNPs and MCPs are present in relatively large numbers in the unfertilized egg. There are $\sim 1 \times 10^7$ U1 snRNPs and 2×10^8 MCPs. There are distinct differences between these two particles, both in structure and behavior during development. No new synthesis of MCP proteins or RNAs are detected during development (Ahkayat et al., 1987; our unpublished results). This suggests that the MCPs that are present in nuclei of sea urchin embryos come from cytoplasmic stores in the unfertilized egg. In contrast, there is new synthesis of U1 snRNPs and these newly synthesized snRNPs migrate to the nucleus early in development (Nash et al., 1987). The proportion of egg U1 RNPs present in the nuclei of later developmental stages is not known.

The protein constituents of 19-S particles from several species appear to be highly conserved. Both the molecular weights and isoelectric points of many of the proteins are quite similar. Monoclonal antibodies against duck 19-S particles (called prosomes) cross-react with *Drosophila*, sea urchin, frog, mouse, human, and plant 19-S particle proteins indicating a highly conserved function (Arrigo et al., 1987). We show here that the sea urchin 19-S particle, originally identified as a prosome, contains multicatalytic protease activity as in *Drosophila* and humans. Whether 19-S particles from all of these species contain this activity is not known.

Some of the 19-S particles contain small RNAs (Kloetzel, 1987). Duck erythroblast prosomes contain only two small RNAs, Hela cell prosomes contain at least 12 different RNA species, and *Drosophila* prosomes (MCPs) contain at least four RNAs (Schmid et al., 1984; Arrigo et al., 1985). However, a 16–18-S particle isolated from *Xenopus* ovaries, which has a very similar protein pattern as the sea urchin MCP and the rat MCP, contain no detectable RNA species (Castano et al., 1986; Arrigo et al., 1988). The $OD_{260/280}$ ratio of our highly purified sea urchin MCPs is 0.7, indicating that at most, the MCPs contain 1% RNA (our unpublished results). This would correspond to a single 40-nucleotide RNA per 19-S particle. This result suggests that sea urchin MCPs do not contain RNA. However, it is possible that during the high salt/EDTA step in the isolation procedure, additional proteins or RNAs may have been lost from the sea urchin MCPs.

The function of the 19-S MCP or their role in development has not been clearly established. One major role may be in nonlysosomal protein degradation (Tanaka et al., 1986; Hough et al., 1987). In reticulocytes, there appear to be at least two different large proteolytic complexes (Hough et al., 1987). A 26-S multiproteolytic complex may be involved in the ubiquitin degradation pathway. The 26-S complex has a different subunit composition and is stimulated by ATP. The highly purified 19–20-S or prosome-like MCP activity is independent of ATP and stimulated by the presence of detergents. It is possible that a component of the 19-S MCP that contains ATP sensitivity was lost during the purification. There is evidence that the 19-S MCP isolated from HeLa cells in its native state and rapidly purified 19-S MCPs from skeletal muscle require ATP for maximal activity (Arrigo et al., 1988; Driscoll and Goldberg, 1989). A clarification of these differences will require the purification of the native form of the 19-S MCP.

It is clear that in sea urchins, these particles are associated with some large cellular complex in unfertilized eggs. This complex could contain inactive egg mRNPs or some other cellular structure involved in regulating the subcellular distribution of the MCPs or their proteolytic activity. In addition, it is possible that the egg MCPs lose this attachment when they migrate to the nucleus during development and their primary function may change from a proteolytic to an RNA-processing role. This nuclear migration may involve a change in MCP proteins since there is some evidence in *Drosophila* that several of the MCP proteins become modified during development (Kloetzel, 1987). We are now trying to identify the complexes that associate with MCPs in the egg cytoplasm and determine whether these complexes regulate MCP activities.

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