Cell Differentiation and Development, 26 (1989) 67–76
Elsevier Scientific Publishers Ireland, Ltd.

CDF 00571

Evidence of a precursor–product relationship between vitellogenin and toposome, a glycoprotein complex mediating cell adhesion

Melchiorre Cervello and Valeria Matranga

Istituto di Biologia dello Sviluppo del C.N.R., Palermo, Italy

(Accepted 18 October 1988)

Toposome, a large and oligomeric glycoprotein complex isolated from mesenchyme-blastula embryos, was defined as a cell-adhesion molecule expressing positional information specificities during sea urchin embryogenesis. This report describes the biochemical and functional characterization of the toposome precursor from sea urchin coelomic fluids of both male and female organisms. The molecule is isolated in the form of a 22S particle which has an apparent molecular mass of 200 kDa. An intermediate form is present in yolk granules of unfertilized eggs with a molecular mass of 180 kDa. The 200 kDa and 180 kDa polypeptides are defined as toposome precursors by Western blot and immunoprecipitation analyses using polyclonal and monoclonal toposome-specific antibodies. Comparison of the 200 kDa polypeptide and mesenchyme-blastula toposome by partial-proteolysis peptide-mapping shows that they are related in a precursor–product relationship. A morphogenetic cell-aggregation assay shows that toposome precursors promote cell adhesion of dissociated blastula cells, suggesting that processing is not required for the cell-adhesion function. The studies reported here present the first evidence that cell adhesion molecules first appear in the form of a 200 kDa polypeptide, previously named vitellogenin, and to which only a function as major-yolk-protein precursor has been ascribed.

Cell adhesion molecule; Embryogenesis; Major yolk protein; Toposome; Vitellogenin

Introduction

Cell–cell adhesion is a highly specific interaction that regulates differentiation, morphogenesis and embryonic development. The remarkable ability of sea urchin dissociated cells to reassociate into developing embryos (Giudice, 1962) offers an ideal system for the search for cell-adhesion molecules and for the study of morphogenesis. Recently we have described the characterization of a 22S glycoprotein complex from mesenchyme blastula embryos. Reducing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the molecule showed a reproducible pattern of six major bands ranging from 60 to 160 kDa. For its hypothesized role in position-dependent interactions during embryogenesis we proposed the name of toposome (Noll et al., 1985). The function of toposome in the process of cell adhesion was defined by its ability to: (a) stimulate the rate and the extent of reaggregation of

Correspondence address: Valeria Matranga, Istituto di Biologia dello Sviluppo del C.N.R., Via Archirafi 20, 90123 Palermo, Italy.

dissociated blastula cells; (b) restore reaggregation and embryonic development of cells rendered reaggregation incompetent by butanol extraction; and (c) neutralize antibodies that inhibit cell adhesion (Matranga et al., 1986).

Large glycoprotein complexes with a sedimentation coefficient similar to toposomes were isolated from coelomic fluid of sea urchins (Harrington and Easton, 1982) and referred to as vitellogenin due to their classical role as major yolk protein precursor (Shyu et al., 1986). The isolation of glycoprotein complexes from sea urchin yolk granules has been extensively described (Kane, 1965; Malkin et al., 1965; Stephens, 1967; Borisy and Taylor, 1967; Infante and Nemer, 1968; Kondo, 1972; Kondo and Koshihara, 1972; Il et al., 1978). It has also been documented that the mass of the glycoprotein particle remains constant while a change in the molecular composition appears to occur by limited proteolysis as development proceeds (Kondo, 1972; Kari and Rottmann, 1985). Nevertheless, the biological function of these complexes remains unknown. They are considered to be storage material used as a readily available source of energy for the rapidly dividing fertilized egg (Kavanau, 1954; Williams, 1967).

We have previously reported that particles from yolk-granule butanol-extracts of unfertilized eggs can be purified by sucrose isokinetic gradients showing a single band on reducing SDS-PAGE (Noll et al., 1985). The similarity between the molecular masses of the YGBE-22S particle and the major yolk protein, and the observation that both undergo a similar molecular processing during development support the interpretation that they are in fact the same molecule. In addition, the finding that the major yolk protein has its precursor in the vitellogenin molecule (Shyu et al., 1986) suggests that toposome first appears as vitellogenin, with an intermediate form present in yolk granules.

The studies reported here were undertaken in order to clarify the precursor–product relationships among these molecules and to establish their biological significance. We have been able to demonstrate the biochemical and immunological correlation between toposomes and a 200 kDa polypeptide precursor present in the coelomic fluids of both male and female sea urchins. An intermediate form is also found in yolk granules of unfertilized eggs, which shows an apparent molecular mass of 180 kDa. Furthermore we show that these molecules stimulate adhesion of dissociated blastula cells in a morphogenetic cell aggregation assay, even though in their unprocessed form.

These results strongly support the interpretation that vitellogenin and the major yolk protein, which we found respectively as 200 kDa and 180 kDa polypeptides in the sea urchin *Paracentrotus lividus*, are a storage form of cell-adhesion molecules that will develop into mesenchyme-blastula toposomes.

Materials and Methods

Harvesting of coelomic fluids

Coelomic fluids were collected from single male or female *P. lividus* sea urchin by cutting the peristomial membrane and taking the coelomic fluid with a Pasteur pipette. The material was kept in ice for 15–20 min to allow the formation of a pellet due to the clotting of coelomocytes. The fluid was passed through a 135-μm nylon mesh and centrifuged at $3000 \times g$ for 10 min at 4° C. The supernatant was filtered through a 0.2-μm Millipore filter and concentrated by the Amicon apparatus with an ultrafiltration membrane that has a size cut-off of 30 kDa. To prevent partial proteolysis, 0.1 mM phenylmethylsulphonyl fluoride (PMSF) was added to all solutions.

Isolation of yolk granules

We used the procedure described by Armant et al. (1986) with some minor changes. Briefly, *P. lividus* eggs were collected and washed twice with Millipore filtered sea water (MFSW). Pelleted eggs were resuspended in 10–20 volumes of Ca^{2+} and Mg^{2+} free sea water containing 0.5 mM PMSF (CMFSW/PMSF) and centrifuged at $220 \times g$ for 10 min at 4° C. The pellet was resuspended with 10 volumes of CMFSW/PMSF and homogenized in a Dounce, tight pestle, with 15–20 strokes. The material was diluted five times in the same medium and centrifuged at $27000 \times g$ for 30 min. The pellet was resuspended with 25 ml of CMFSW/

PMSF, 4 ml of this suspension loaded on top of a sucrose step gradient (5 ml 35%, 15 ml 25% and 10 ml 10% (w/v) in CMFSW/PMSF) and centrifuged at 100 000 × *g* for 16 h in a Beckman SW 28 rotor. Fractions of 1 ml each were collected from the bottom and the absorbance measured at 600 nm. Fractions containing the low density granules were pooled, diluted ten fold with CMFSW/PMSF pelleted at 27 000 × *g* for 25 min.

Butanol extraction of yolk granules

Pelleted yolk granules were resuspended in 10 volumes (w/v) of 0.2 M Tris pH 8 and 5 volumes (w/v) of *n*-butanol were added while mixing with a magnetic stirrer. The extraction was performed at 4° C for 15 min with continuous agitation. The phases were then separated by centrifugation for 15 min at 15 800 × *g* at 4° C. The aqueous phase was collected, dialyzed against distilled water containing 0.25 mM PMSF and centrifuged at 12 000 × *g* for 15 min at 4° C to eliminate insoluble material. The butanol extract was concentrated by Aquacide (Calbiochem) and dialyzed against phosphate buffer saline (PBS/PMSF).

Purification of 22S complex from mesenchyme-blastula cells

For large-scale preparations of 22S complexes a butanol extract was prepared by non-cytolytic treatment of dissociated mesenchyme-blastula cells with 10 volumes (w/v) of 2.5% *n*-butanol. The material was then dialyzed and concentrated to about 10 mg/ml by Amicon ultrafiltration, exclusion 30 kDa. The extract was loaded on a S-400 Sephacryl column (80 cm × 2.5 cm) and eluted with Millipore-filtered sea water. Eight-ml fractions were collected. The 22S complex emerged as a sharp peak after two broader peaks and was checked for its purity on a sucrose isokinetic gradient and SDS-PAGE.

Sucrose isokinetic gradients

22S particles were purified from male coelomic fluid (MCF), female coelomic fluid (FCF), yolk-granule butanol-extracts (YGBE) and mesenchyme-blastula toposomes (MBT) by sucrose isokinetic gradient in PBS (top concentration = 5% w/w; Noll, 1967). Material at a concentration of 1 mg/ml in a volume of 100 µl was loaded on top of a 4.2-ml gradient and centrifuged at 50 000 rpm for 3 h at 15° C in a Beckman SW 56 rotor. Ten drop fractions corresponding to about 350 µl were collected from the bottom and the absorbance measured at 220 nm. Fractions to be analyzed by SDS-PAGE were dialyzed against distilled water and lyophilized by speed vacuum centrifugation.

Electrophoretic analyses

Purified male and female coelomic fluids together with butanol extracts from yolk granules and mesenchyme-blastula toposomes were analyzed by SDS-PAGE under reducing conditions according to Laemmli (1970). Protein standards (Pharmacia) were ferritin (220 kDa), phosphorylase *b* (94 kDa), albumin (67 kDa), catalase (60 kDa), ovalbumin (36 kDa). All gels were stained with Coomassie blue unless otherwise specified.

Peptide mapping

Partial proteolysis procedure was carried out according to Cleveland et al. (1977) by using *Staphylococcus aureus* V8 protease from Miles. Products of digestion were resolved in 10% SDS-PAGE and stained with the silver staining method.

Polyclonal and monoclonal antibodies production

Immune serum was obtained by immunizing mice with purified 22S complex at days 1, 13, 16, 20, 24 respectively with 5, 20, 50, 100, 100 µg of antigen, the first injection carried out in Freund's complete adjuvant. Blood was collected from the lateral tail vein, kept overnight in the cold and centrifuged to remove the clot.

For the production of monoclonal antibodies mice were injected i.p. with 100 µg of crude butanol extract from mesenchyme-blastula cells at day 1, 14, 21. Three days after the third boost, the spleen was collected and lymphocytes were fused with myeloma cells (P3 × 63Ag8) according to standard procedures (Kohler and Milstein, 1975). Hybridoma supernatants were screened by ELISA on 22S-coated plates, and positive clones were cloned by limit dilution. Supernatants from these clones were directly used for Western blot and immunoprecipitation analyses.

Immunoblotting

Immunoblot analysis was carried out following the procedure reported by Towbin et al. (1979). Transfer of proteins was performed at 210 mA for 3 h in the cold. After washing and blocking the free sites, the nitrocellulose sheet was incubated overnight at room temperature in 10 ml of 1 : 10 000 diluted mouse anti-MBT. Horseradish peroxidase-conjugated goat anti-mouse IgG (Bionetics) at a dilution of 1 : 500 in PBS/0.05% Tween 20 was used to incubate the nitrocellulose for 2 h at room temperature. Bands were visualized by soaking the blot in a solution of 5 ml 0.3% 4-chloro-1-naphthol in methanol, 20 ml 0.05 M Tris pH 6.8, 7 μl 35% H_2O_2.

Immunoprecipitation

For immunoprecipitations MCF, FCF, YGBE and MBT at a concentration of 160 μg/ml in the immunoprecipitation buffer (0.15 M NaCl, 5 mM EDTA, 10 mM Tris pH 7.5, 2 mM PMSF) were incubated with 50 μl of a 50% suspension of formalin-fixed *Staphylococcus aureus* for 30 min. Samples were centrifuged at 10 000 rpm for 2 min in an Eppendorf microfuge, and the supernatants were incubated overnight with 500 μl of mouse anti-MBT serum diluted 1 : 1 500 in the immunoprecipitation buffer. Samples were incubated for 1 h with 60 μl of *S. aureus*, centrifuged as above and the pellet washed three times in PBS–2 mM PMSF. All incubations were carried out at 4° C with gentle agitation. The immunocomplexes were resuspended in the Laemmli buffer, denatured at 100° C for 5 min and loaded on a 6% SDS-PAGE.

Reaggregation assays

Dissociation of *P. lividus* embryos into single cells was achieved by the technique fully documented by Matranga et al. (1986). Reaggregation of dissociated cells was scored as previously described in microtiter plates by using a cell concentration of 5×10^4 cells per well in a final volume of 100 μl. Aggregation was estimated by measuring the size of 20 reaggregated embryos from photographs taken after 5-h incubation at 15° C. Stimulation of cell aggregation was calculated as follows:

$$\begin{aligned}\%\ \text{aggregation} &= \{\text{average aggregate size(sample)} \\ &\quad - \text{average aggregate size(control)}\} \\ &\quad \times \{\text{average aggregate size(control)}\}^{-1} \\ &\quad \times 100\end{aligned}$$

Results

Identification of a 22S particle in male and female coelomic fluids and yolk-granule butanol-extracts

A large glycoprotein complex present in MCF, FCF and YGBE of the sea urchin *P. lividus* was purified by sedimentation on sucrose isokinetic gradients (Fig. 1). We estimate the size of the particles to be 22S by comparison with the sedimentation profile of mesenchyme-blastula toposome (MBT) used as control (Fig. 1a). When analyzed in SDS-PAGE under reducing conditions the 22S peaks obtained from male and female coelomic fluids show a major 200 kDa glycoprotein band (Fig. 1b, lane 2; Fig. 1c, lane 2). A band with an apparent molecular mass of 180 kDa was found in the 22S peak obtained from yolk-granule butanol-extracts (Fig. 1d, lane 2). The purification of 22S particles from Mediterranean sea urchins is here described for the first time, confirming previous observations reporting the presence of large oligomeric glycoprotein complexes in a variety of other sea-urchin species (Kane, 1965; Malkin et al., 1965; Il et al., 1978; Harrington and Easton, 1982), and sand dollar (Ozaki, 1980; Ozaki et al., 1986).

Biochemical identity between toposome precursor and mesenchyme blastula toposomes

The relationship between the 200 kDa polypeptide and MBT was initially explored by partial-proteolysis peptide-mapping by using staphylococcal protease V8. The polypeptides from

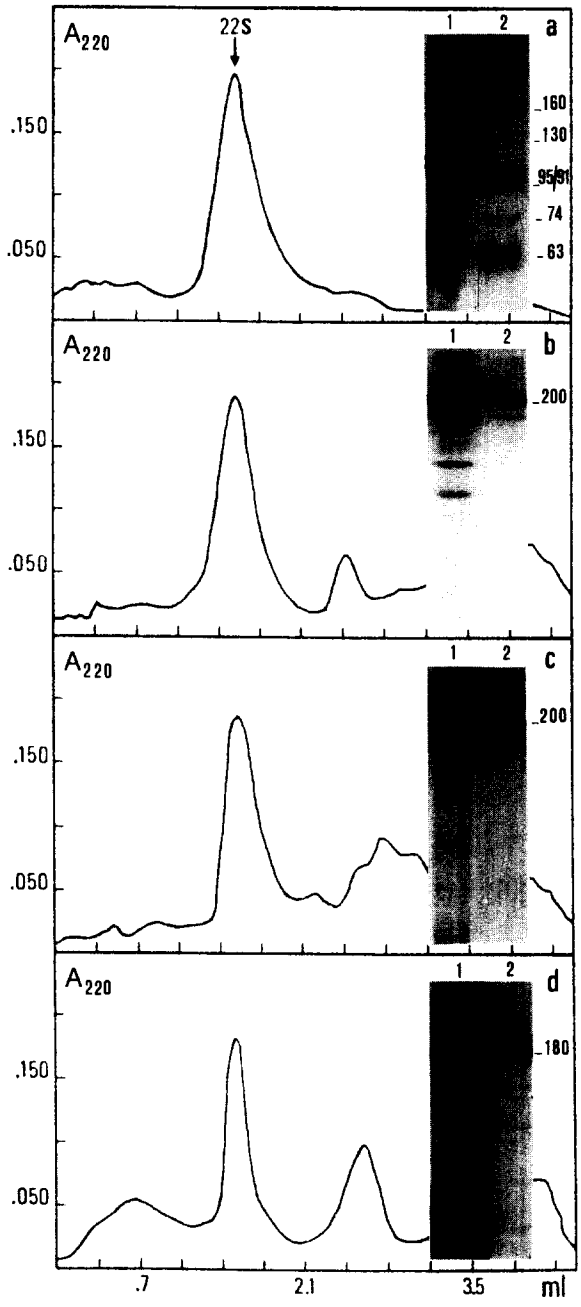

Fig. 1. Analysis of coelomic fluids and yolk-granule butanol-extracts by sedimentation and gel electrophoresis. (a) Mesenchyme-blastula toposomes (MBT), (b) male coelomic fluid (MCF), (c) female coelomic fluid (FCF) and (d) yolk-granule butanol-extracts (YGBE). Sedimentation direction right to left, the arrow is pointing to the 22S peak of MBT used as control. In the right panels 6% SDS-PAGE of crude preparations (1) or purified 22S peaks (2). Sizes are indicated in kDa.

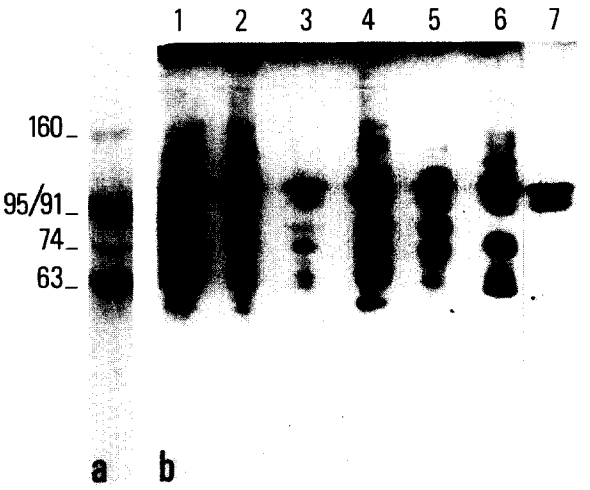

Fig. 2. Toposome precursors and mesenchyme-blastula toposomes generate peptide maps with identical proteolysis fragments. Mesenchyme-blastula toposome major bands (160, 95/91, 74, 63 kDa) were excised from a 6% SDS-PAGE (a) and digested with *S. aureus* V8 protease. Proteolysis fragments were resolved on a 10% SDS-PAGE (b) and shown in the same order in lanes 3–6. MCF and FCF 200 kDa bands obtained from sedimentation on sucrose gradients (see Fig. 1b and 1c lane 2) were digested with V8 protease and run on a 10% gel (b) lanes 1 and 2. Lane 7 contains 5 μg of V8 protease. On the left apparent molecular masses are indicated in kDa. Peptide mapping was stained by the silver-staining method.

male and female coelomic fluids purified by sucrose isokinetic gradients were electrophoresed, excised from the gel and subjected to peptide mapping (Fig. 2). Examination of the patterns reveals that peptide maps from *P. lividus* male and female coelomic fluids are identical (Fig. 2b, lanes 1 and 2). However, when each of the bands obtained from SDS-PAGE of MBT (Fig. 2a) is digested proteolytically it generates peptide fragments that are nearly identical to each other (Fig. 2b, lanes 3–6). The simplest explanation of this result is that each of the bands originates from the 160 kDa component, in agreement with our previous observations (Noll et al., 1985). Minor diversities in the peptide maps may be a reflection of differences in the molecular mass and in the protein content of the bands which have been digested (for example compare lane 3 with 4 and lane 5 with 6 of Fig. 2b).

Comparison of the partial-proteolysis peptide-maps of the 200 kDa band purified from MCF

and FCF with MBT results in the biochemical identity of the examined protein species (Fig. 2b).

Immunological evidence that toposomes originate from a 200 kDa precursor

The immunological correlation among the studied molecular species comes from experiments in which polyclonal and monoclonal antibodies were used to probe male and female coelomic fluids and yolk-granule butanol-extracts in Western blot analyses (Fig. 3). The antibodies recognize a single glycoprotein band with an apparent molecular mass of 200 kDa in both male and female coelomic fluids (Fig. 3b lanes 1, 2 and Fig. 3c lanes 1, 2). In addition, a 180 kDa band is recognized in yolk-granule butanol-extracts from unfertilized eggs (Fig. 3b lane 3 and Fig. 3c lane 3).

Anti-MBT polyclonal antibodies were used to immunoprecipitate proteins from coelomic fluids of both male and female organisms and yolk-granule butanol-extracts from unfertilized eggs. Fig. 4 shows that common epitopes are shared by the 200 kDa band found in the MCF and FCF and the 180 kDa band present in YGBE. No proteins were detected in control experiments with pre-immune serum either in Western blot or immunoprecipitation analyses (not shown). The finding that both polyclonal and monoclonal antibodies recognize only a single band from the total protein content present in MCF, FCF and YGBE suggests that the 200 kDa and the 180 kDa polypeptides are precursors of mesenchyme-blastula cell-adhesion molecules.

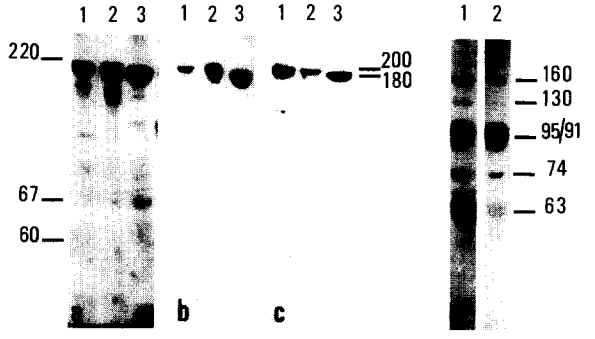

Fig. 3. Male and female coelomic fluids and yolk-granule butanol-extracts are immunologically related to mesenchyme-blastula toposome. (a) 6% SDS-PAGE under reducing conditions of MCF (1), FCF (2), YGBE (3). After transfer to nitrocellulose, samples were probed, in the same order, with anti-MBT polyclonal antibodies (b) and anti-MBT monoclonal antibody D4e11 (c). MBT is revealed in (d) by Coomassie staining (1) or immunoblotting with anti-MBT polyclonal antibodies (2). Apparent molecular masses are indicated in kDa. Size markers on the left.

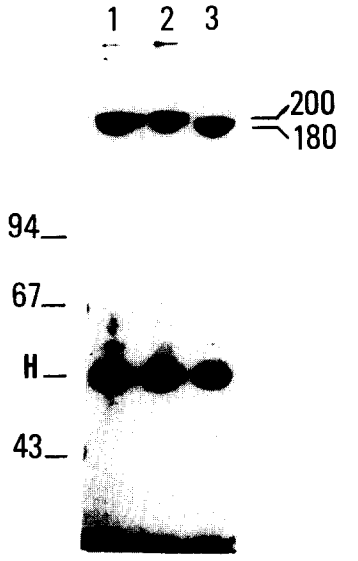

Fig. 4. The 200 kDa toposome precursor present in male and female coelomic fluids shares common epitopes with the 180 kDa band of yolk-granule butanol-extracts. Anti-MBT polyclonal antibodies were used to immunoprecipitate MCF (1), FCF (2) and YGBE (3). Immunocomplexes were resolved on a 6% SDS-PAGE under reducing conditions. Apparent molecular masses are indicated in kDa. Size markers on the left. H indicates the heavy chain of IgGs used for immunoprecipitation.

Functional characterization of toposome precursors

In order to investigate the biological significance of 200 kDa and 180 kDa polypeptides we carried out a morphogenetic cell-aggregation assay on dissociated blastula cells as previously described (Matranga et al., 1986). Fig. 5 shows a dose-response diagram in which crude preparations of MCF, FCF, and YGBE were tested on dissociated blastula cells and inspected after 5 h of incubation. In this experiment the average size of 20 reaggregates was estimated from photographs and the percentage of aggregation relative to controls (i.e. dissociated cells with no additions) is

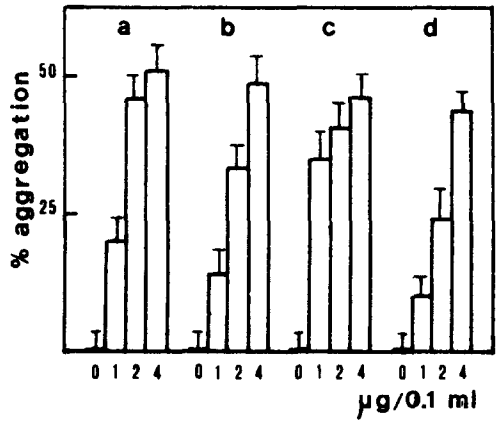

Fig. 5. Stimulation of cell aggregation by increasing concentrations of toposome precursors. MCF (a), FCF (b), YGBE (c) and MBT (d) were tested on dissociated blastula cells. Different concentrations used are expressed by μg per 100 μl of culture. Readings were taken after 5-h incubation. Bars represent the standard deviation of 20 experimental values. % of aggregation is calculated as reported under Materials and Methods.

calculated as reported under Materials and Methods. The lowest amount of protein tested was 0.5 μg/0.1 ml with no observed enhancement of reaggregation in comparison to controls (not shown). At inputs higher than 4 μg per 100 μl culture the size of the reaggregates cannot be measured, since they coalesce into sheets that cover an entire field.

After 24 h of incubation the formation of blastula-like embryoids with a well-defined epithelium was observed with an increase in the number and size of reaggregates (Fig. 6a–e). A dramatic recovery of cells rendered aggregation incompetent by non-cytolytical extraction with 2.5% *n*-butanol is also illustrated (Fig. 6f–j). The formation of a smooth epithelial sheet is our principal criterion in verifying the cell-adhesion activity of the molecules tested, since it has proved successful in the identification of toposomes (Matranga et al., 1986).

Together these results show that although the total protein content of MCF, FCF and YGBE was proved to be active in promoting cell adhesion, it can be easily deduced that the sole

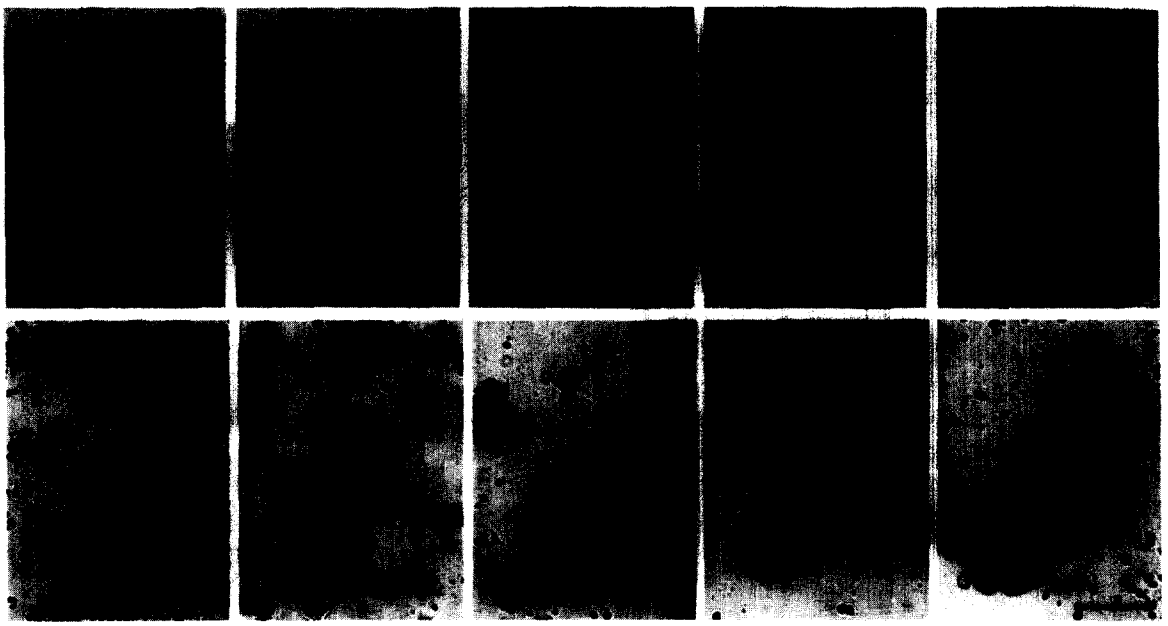

Fig. 6. Morphogenetically significant reaggregation induced by toposome precursors on cell adhesion competent and incompetent cells. Dissociated blastula cells (a–e) and blastula cells rendered incompetent to aggregate by extraction with butanol (f–j) with the addition of 1 μg of MCF (b, g), FCF (c, h), YGBE (d, i) and MBT (e, j). No addition in (a) and (f). Pictures were taken after 24 h of culture. Bar = 100 μm.

molecules involved in the cell adhesion process are the 200 kDa and the 180 kDa polypeptides for the following reasons. First, 22S particles isolated from MCF, FCF and YGBE analyzed by SDS-PAGE reveal the presence of a single band that is the most abundant component in the crude preparations, constituting about 50% of the total protein content (Fig. 1b–d, lanes 1 and 2). On the other hand, it has been documented that mesenchyme-blastula toposomes, isolated in the form of 22S particles, are the sole structures responsible for the adhesive integrity of sea-urchin blastula embryos. This conclusion rests on the demonstration that MBT neutralizes all reaggregation inhibiting antibodies, regardless of whether serum, IgG or monovalent Fabs are used (Matranga et al., 1986). Furthermore, polyclonal and monoclonal anti-22S particle antibodies recognize epitopes that are present only in the 200 kDa and in the 180 kDa polypeptides when MCF, FCF and YGBE were subjected to immunoblotting and immunoprecipitation analyses.

Discussion

In this paper we document the evidence that a toposome precursor is found in the coelomic fluids of both male and female sea urchins with an intermediate form in yolk granules of unfertilized eggs. The immunological evidence that toposomes originate from a 200 kDa precursor, via an 180 kDa intermediate, rests on Western blot and immunoprecipitation analyses with toposome-specific polyclonal and monoclonal antibodies. These findings, together with the demonstration of a biochemical identity between the 200 kDa polypeptides and mesenchyme-blastula toposomes, by means of partial-proteolysis peptide-mapping, strongly argue for the existence of a precursor–product relationship between these molecules.

The biological significance of the 200 kDa and 180 kDa polypeptides was tested using a morphogenetic cell aggregation assay on dissociated blastula cells. Toposome precursors proved to be active in promoting cell adhesion although in their unprocessed form, even when cells rendered aggregation incompetent by non-cytolytic *n*-butanol extraction have been used. It follows that precursors contain all the epitopes of the contact site.

Two processing steps are evident in the formation of mature toposomes: the first is the cleavage of a 200 kDa protein present in MCF, FCF to an intermediate form stored in yolk granules of unfertilized eggs, the second involves the maturation of yolk protein into mesenchyme-blastula toposome. We do not know what the biological significance of this processing is. Since precursors retain a cell-adhesion promoting activity, processing may serve for generating positional diversities among cells during embryogenesis. On the other hand, these new data complement our previous observation that toposome-specific monoclonal antibodies stain cell surface structures in a pattern consistent with a code specifying the position of a cell in the embryo (Matranga and Cervello, 1984; Noll et al., 1985; Matranga et al., 1987).

Here we report that toposome precursors are found in form of a 22S glycoprotein complex, as for toposomes isolated from mesenchyme-blastula embryos. Particles with a similar sedimentation coefficient have been found in coelomic fluids (Harrington and Easton, 1982; Harrington and Ozaki, 1986) and unfertilized egg yolk platelets of a variety of other sea urchin species (Malkin et al., 1965; Infante and Nemer, 1968; Harrington and Easton, 1982; Kari and Rottmann, 1985) and sand dollar (Ozaki, 1980; Ozaki et al., 1986), and referred to respectively as vitellogenin and major yolk protein. The observed similarities in origin and glycoprotein composition of the 200 kDa and the 180 kDa toposome precursors with vitellogenin and major yolk protein strongly argue for their molecular identity.

The accumulation of yolk proteins in oocytes and their formation in various other tissues is known as vitellogenesis and has been extensively studied in vertebrate and invertebrate animals. In invertebrates, vitellogenin is produced in the intestine of hermaphroditic nematodes (Kimble and Sharrock, 1983) and in the fat-body of female insects (Wojchowski et al., 1986). Among vertebrates it is detected in the liver of chickens (Bergink et al., 1974; Tata, 1986) and frogs (Wahli et al., 1981). In the sea urchin it has been shown that vitellogenin is synthesized in the intestine and in

the gonads and can be recovered from the coelomic fluids of both male and female organisms (Shyu et al., 1986). The presence of large quantities of vitellogenin in males seems to exclude its classical role as yolk protein precursor. Furthermore it has been described that this high molecular weight protein is produced by coelomocytes and secreted into the coelomic fluid (Harrington and Ozaki, 1986).

A function as major yolk protein precursor has been reported for vitellogenin (Shyu et al., 1986), whereas 22S complexes packed into yolk granules have been considered the major embryonic source of nutritional material due to their degradation as development proceeds (Kari and Rottmann, 1985). Recently it has been proposed that degradation of yolk proteins occurs by means of a cathepsin-B-like enzyme (Yokota and Kato, 1988). This report documents the first evidence of a direct correlation among the biological function of vitellogenin, major yolk protein and their final form as toposome molecule. Their ability to promote cell aggregation leads us to propose that vitellogenin is the primordial form of cell-adhesion molecule in the sea-urchin embryo. A question arises whether the same mechanisms by which cell-adhesion molecules originate is conserved in other systems. Analysis at the molecular level of the genes coding for vitellogenin in all the systems so far studied and toposome precursors in the sea-urchin embryo will further clarify the relationship between these molecules.

Acknowledgements

The authors wish to thank Prof. G. Giudice, Prof. E. Nakano and Dr. A. Giallongo for helpful discussions and critical reading of the manuscript. Special thanks go to Prof. H. Noll who generously provided the equipment for the maintenance of sea urchins and to Mr. G. Scaturro for their collection.

References

Armant, D.R., D.D. Carson, G.L. Decker, J.K. Welply and W.J. Lennarz: Characterization of yolk platelets isolated from developing embryos of *Arbacia punctulata*. Dev. Biol. 113, 342–355 (1986).

Bergink, E.W., R.A. Wallace, J.A. Van de Berg, E.S. Bos, M. Gruber and G. Ab: Estrogen-induced synthesis of yolk proteins in roosters. Am. Zool. 14, 1177–11933 (1974).

Borisy, G.G. and E.W. Taylor: The mechanism of action of colchicine. J. Cell Biol. 34, 535–548 (1967).

Cleveland, D.W., S.T. Fischer, M.W. Kirschner and U.K. Laemmli: Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. 252, 1102–1106 (1977).

Giudice, G.: Restitution of whole larvae from disaggregated cells of sea urchin embryos. Dev. Biol. 5, 402–411 (1962).

Harrington, F.E. and D.P. Easton: Putative precursor to the major yolk protein of the sea urchin. Dev. Biol. 94, 505–508 (1982).

Harrington, F.E. and H. Ozaki: The major yolk glycoprotein precursor in echinoids is secreted by coelomocytes into the coelomic plasma. Cell Differ. 19, 51–57 (1986).

Il, I., K. Deguchi, S. Kawashima, S. Endo and N. Ueta: Water-soluble lipoproteins from yolk granules in sea urchin eggs. I. Isolation and general properties. J. Biochem. 84, 737–749 (1978).

Infante, A.A. and M. Nemer: Heterogeneous ribonucleoprotein particles in the cytoplasm of sea urchin embryos. J. Mol. Biol. 32, 543–565 (1968).

Kane, R.E.: Identification and isolation of the mitotic apparatus protein. Biol. Bull. 129, 396 (1965).

Kari, B.E. and W.L. Rottmann: Analysis of change in a yolk glycoprotein complex in developing sea urchin embryo. Dev. Biol. 108, 18–25 (1985).

Kavanau, J.L.: Amino acid metabolism in the early development of the sea urchin *Paracentrotus lividus*. Exp. Cell. Res. 7, 530–557 (1954).

Kimble, J. and Sharrock, W.J.: Tissue-specific synthesis of yolk protein in *Caenorhabditis elegans*. Dev. Biol. 96, 189–196 (1983).

Kohler, G. and C. Milstein: Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256, 495–497 (1975).

Kondo, H.: Properties of glycoprotein particles and their changes during development of sea urchin eggs and embryos. Exp. Cell Res. 72, 519–532 (1972).

Kondo, H. and H. Koshihara: Immunological studies on the 26S particles of the sea urchin eggs. Exp. Cell Res. 75, 385–396 (1972).

Laemmli, U.K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685 (1970).

Malkin, L.I., J. Mangan and P.R. Gross: A crystalline protein of high molecular weight from cytoplasmic granules in sea urchin eggs and embryos. Dev. Biol. 12, 520–542 (1965).

Matranga, V. and M. Cervello: Monoclonal antibodies raised against cell surface components of sea urchin embryos show the expression of different antigenic patterns. J. Embryol. Exp. Morphol. 82 (Suppl. 1), 89 (1984).

Matranga, V., B. Kuwasaki and H. Noll: Functional char-

acterization of toposomes from sea urchin blastula embryos by a morphogenetic cell aggregation assay. EMBO J. 5, 3125–3132 (1986).

Matranga, V., F. Zito and M. Cervello: A monoclonal antibody recognizes a subclass of toposomes specifying for mesodermal structures. Eur. J. Cell Biol. 44, Suppl. 21, p. 45 (1987).

Noll, H.: Characterization of macromolecules by constant velocity sedimentation. Nature 215, 360–363 (1967).

Noll, H., V. Matranga, M. Cervello, T. Humphreys, B. Kuwasaki and D. Adelson: Characterization of toposomes from sea urchin blastula cells: A cell organelle mediating cell adhesion and expressing positional information. Proc. Natl. Acad. Sci. USA 82, 8062–8066 (1985).

Ozaki, H.: Yolk proteins of the sand dollar *Dendraster excentricus*. Dev. Growth Differ. 22, 365–372 (1980).

Ozaki, H., O. Moriya and F.E. Harrington: A glycoprotein in the accessory cell of echinoid ovary and its role in the vitellogenesis. Roux's Arch. Dev. Biol. 195, 74–79 (1986).

Shyu, A.B., R.A. Raff and T. Blumenthal: Expression of the vitellogenin gene in female and male sea urchin. Proc. Natl. Acad. Sci. USA 83, 3865–3869 (1986).

Stephens, R.E.: Physical chemical characterization of the 22S protein component and its subunits. J. Cell Biol. 32, 255–275 (1967).

Tata, J.R.: The expression of the vitellogenin gene. Cell 9, 1–14 (1976).

Towbin, H., T. Staehelin and J. Gordon: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. USA 76, 4350–4354 (1979).

Wahli, W., I.B. Dawid, G.U. Ryffel and R. Weber: Vitellogenesis and the vitellogenin gene family. Science 212, 298–304 (1981).

Williams, J.: In: Biochemistry of Animal Development, Vol. 2, ed. R. Weber (Academic Press, New York) pp. 341–382 (1967).

Wojchowski, D.M., P. Parson, J.H. Nordin and J.G. Kunkel: Processing of pro-vitellogenin in insect fat body: A role for high mannose oligosaccharide. Dev. Biol. 116, 422–430 (1986).

Yokota, Y. and K.H. Kato: Degradation of yolk proteins in sea urchin eggs and embryos. Cell Differ. 23, 191–200 (1988).