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Characterization of toposomes from sea urchin blastula cells: A cell organelle mediating cell adhesion and expressing positional information

(monoclonal antibodies/noncytolytic extraction with butanol/glycoprotein complex/cell membrane/morphogenesis)

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ABSTRACT **Cell adhesion in the sea urchin blastula is mediated by a 22S genus-specific glycoprotein complex consisting initially of six 160-kDa subunits that are processed proteolytically as development proceeds. Noncytolytic removal of the 22S particle from the surface with either 2.5% butanol or trypsin renders dissociated cells reaggregation incompetent, and addition restores reaggregation and development. Polyclonal antibodies against the 22S complex prevent reaggregation in a genus-specific manner while monoclonal antibodies stain cell surface structures in a pattern consistent with a code that specifies the position of a cell in the embryo by a unique combination of subunits in its cell adhesion particles. The existence of similar particles in *Drosophila* and amphibian embryos suggests that these glycoprotein complexes are a general class of organelles, the toposomes, that in the embryo mediate cell adhesion and express positional information.**

Sea urchin embryos are uniquely suited for the study of cell–cell interaction in embryogenesis because removal of Ca^{2+} and Mg^{2+} causes them to dissociate into single cells that, upon restoration of these ions, reaggregate spontaneously to reform a developing embryo (1). Reaggregation is blocked in a strictly genus-specific manner by antibodies (or their Fab fragments) against purified membranes or against butanol extracts from purified membranes (2, 3). Equally inhibitory are antibodies raised against the components extracted noncytolytically from live cells with 2.5% (vol/vol) butanol (2). The proteins solubilized by butanol from purified membranes or from the cell surface neutralize the inhibiting antibodies, stimulate reaggregation, and reconstitute cells that had been rendered reaggregation incompetent by noncytolytic extraction with butanol (2).

Attempts to purify the active component gave results suggesting that the activity was associated with a large glycoprotein complex. Thus, when the butanol extract was chromatographed on phenyl-Sepharose or DEAE-Sepharose, the reaggregation-promoting activity was eluted as a single peak that in $NaDodSO_4$ gels was resolved into a defined set of glycoproteins ranging from about 70 to 180 kDa that was identical for the two purification methods (4). Here we report that all the cell adhesion activity in sea urchin blastula embryos detectable in our bioassays is indeed the property of an oligomeric glycoprotein particle. Similar particles have been isolated previously from a number of other sea urchin genera (cited in ref. 5). While the biological function of this particle remained obscure, it was found to originate in yolk granules and to undergo developmentally regulated processing into a number of fragments held together by S—S bridges and noncovalent bonds (5).

After the studies reported here were completed, strikingly similar glycoprotein complexes were discovered on the surface of imaginal disc cells of *Drosophila* by screening for monoclonal antibodies with positional specificity (6). The similarity of the results in sea urchins and *Drosophila* and the complementarity of the approaches suggest that such complexes, for which the name "toposomes" is proposed, play a general role in position-dependent cell–cell interactions during embryogenesis.

MATERIALS AND METHODS

Preparation of Components. *Yolk granules*: Unfertilized eggs (*Tripneustes gratilla*), homogenized in 0.9 M glycerol/0.1 M Pipes, pH 7.2/5 mM EGTA, were spun at 37,000 rpm and 4°C for 90 min in a Spinco SW 41 rotor. The bright orange middle layer of the sediment, consisting of pure yolk granules, kindly supplied by Robert Kane, was removed by aspiration and frozen at −70°C or extracted immediately. *Membranes*: Preparation for *Paracentrotus lividus* was according to Noll *et al.* (2). For *T. gratilla*, 12- to 15-hr blastulae, washed thrice in Millipore-filtered seawater (MFSW), were suspended in 10 vol of phosphate-buffered saline, pH 7.2 (140 mM NaCl/2.7 mM KCl/8.1 mM Na_2HPO_4/1.5 mM KH_2PO_4), and homogenized on ice with 20 strokes in a Dounce homogenizer with a tight-fitting pestle. The homogenate, underlayered with 7 ml of 60% sucrose in 50-ml centrifuge tubes, was spun in a Sorvall SS-34 rotor at 10,000 rpm for 20 min at 4°C, and the orange material at the interface was collected, resuspended by homogenization in fresh ice-cold phosphate-buffered saline, and spun at 10,000 rpm for 20 min at 4°C. The resuspended membrane pellets were sedimented as before, and the pellets were suspended in 10 vol of 5 mM EDTA, pH 8.0, and either frozen at −70°C or extracted immediately. *Butanol extract*: Membranes or yolk granules from *T. gratilla* suspended in at least 10 vol of 5 mM EDTA were mixed vigorously with ice-cold 1-butanol (about half the volume of the EDTA solution) and kept on ice for 20 min with occasional agitation. The phases were then separated by centrifugation for 10 min at 5000 rpm at 4°C in a Sorvall HB-4 rotor. The interface with the denatured membranes was discarded, and the lower aqueous phase was collected, dialyzed against several changes of cold MFSW, and concentrated as needed.

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Abbreviations: MFSW, Millipore-filtered seawater; mAb, monoclonal antibody.

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RESULTS

Butanol Extract Contains a 22S Glycoprotein Complex Consisting of Six 160-kDa Chains That Are Processed into Smaller Fragments in the Course of Development. The major component of the butanol extract is a 22S glycoprotein complex present in butanol extracts from purified membranes and in the cytoplasm of all sea urchins examined (*T. gratilla, P. lividus, Arbacia lixula, Lytechinus variegatus*, and *Strongylocentrotus purpuratus*). The peak from sucrose gradients is resolved in $NaDodSO_4$ gels under reducing conditions into a number of glycoproteins of unequal molecular mass ranging from 70 to 180 kDa. When the material is isolated from blastulae, this pattern is reproducible and varies according to genus, the degree of similarity reflecting how closely two genera are related in evolution and antigenicity.

Similar particles, though of unknown function, had been isolated from the cytoplasm and were believed to originate from yolk granules (5, 7). Yet, when the same particles were isolated from unfertilized eggs, only one component, about 160 kDa, was observed in reducing $NaDodSO_4$ gels. Processing into multiple smaller fragments was reported to be developmentally regulated (5).

Confirming these reports, we found that both extracts yield 22S particles that are identical by sedimentation (Fig. 1) yet differ in composition as revealed by reducing $NaDodSO_4$ gels; only the particles from the blastulae have been processed proteolytically (Fig. 1*b*).

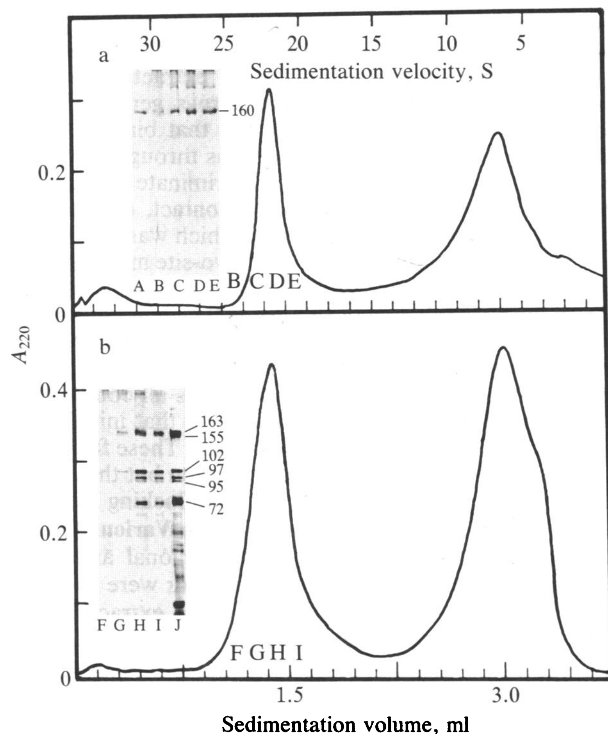

FIG. 1. Analysis of butanol extracts from yolk granules of unfertilized eggs and blastula membranes by sedimentation and polyacrylamide gel electrophoresis. Crude butanol extract (50–100 μl) from yolk granules (*a*) and from membranes (*b*) (A_{280} = 0.5–1.0) were layered over 3.8 ml of an isokinetic (8) sucrose gradient (top concentration = 5%, wt/wt) in MFSW, spun for 90 min at 60,000 rpm and 20°C in a Beckman SW 60 rotor, and scanned spectrophotometrically at 220 nm. (*Insets*) The butanol extracts from yolk granules (3 μg, lane A) or blastula membranes (6 μg, lane J) and gradient fractions B–E and F–I (30 μg) were analyzed on 0.75-mm $NaDodSO_4$/10% polyacrylamide gels under reducing conditions. Silver staining was according to Merril *et al.* (9). Molecular masses are indicated in kDa.

The ratio of the 22S peak to the slower-sedimenting components in butanol extracts is somewhat variable: dialysis against buffers containing Ca^{2+} favors the 22S component at the expense of the slower components, while prolonged dialysis of purified 22S particles against EDTA gives rise to 15S and eventually to a 7S peak with a transient peak at 11S. Since in reducing $NaDodSO_4$ gels the monomer of 22S particles from unfertilized eggs has a molecular mass of about 160 kDa and the particle mass is about 900 kDa (7), the 22S complex appears to be a hexamer that exists in a Ca^{2+}-dependent equilibrium with its subunits: $22S \rightleftharpoons 2(15S) \rightleftharpoons 3(11S) \rightleftharpoons 6(7S)$. In the absence of a reducing agent under denaturing conditions, the breaks remain hidden because of stabilization by intrachain S—S links (5).

Seven monoclonal antibodies (mAbs) reacting with butanol extract were obtained in response to immunization with whole cells or butanol extract from blastula membranes of *T. gratilla*, and all of them immunoprecipitated the 22S particle from total embryo homogenates, as evident from $NaDodSO_4$ gel analysis. The time course of processing followed by this technique shows that the only component detected 3 hr after fertilization (8- to 16-cell stage) is the 160-kDa glycoprotein corresponding to the unprocessed subunit (Fig. 2). At 12 hr (unhatched blastula), the 102- and 72-kDa bands of the processed particle appear, followed by the 97- and 95-kDa bands at 24 hr (early gastrula). All fragments continued to increase at the expense of the 160-kDa parent until reaching a plateau after 36 hr (prism stage). The fact that all seven mAbs recognizing the 22S complex immunoprecipitate no other components of the total cell homogenate makes it unlikely that the 22S complex shares its dominant epitopes with other components of the cell membrane or cytoplasm.

The 22S Complex Neutralizes All Reaggregation-Blocking Antibodies and Reconstitutes Butanol-Extracted Cells. Highly purified 22S complex from *T. gratilla* was tested for neutralization of reaggregation-inhibiting antibodies, using sera or IgG from rabbits immunized with 22S particles, with crude butanol extract, or with purified membranes. The inhibition of reaggregation was reversed by mixing increasing concentrations of 22S particles with a constant antibody concentration before addition of the cells. In the experiment illustrated in Table 1, the titration was against two inhibitory levels (5

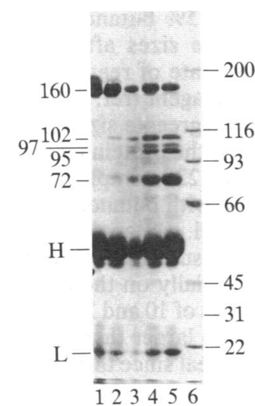

FIG. 2. Processing of 22S complex immunoprecipitated from whole embryos by mAb as a function of development time. Immunoprecipitation with a mAb was performed essentially according to Brugge and Erikson (10), using rabbit anti-mouse IgG absorbed to protein A of Formalin-fixed *Staphylococcus aureus* (for details, see ref. 11). The washed precipitates were dissolved in sample buffer and electrophoresed as described in the legend to Fig. 1. Staining was with Coomassie blue. Lanes 1–5, embryos at 3, 12, 24, 36, and 48 hr of development. Lane 6, molecular mass markers, indicated in kDa. H and L indicate heavy and light chain of the IgG used for immunoprecipitation.

Table 1. Inhibition of reaggregation by anti-membrane serum and reversal by 22S complex

Anti-serum, μl	Extent of reaggregation in presence of 22S complex							
	0 μg	0 μg	0.5 μg	1.0 μg	2.5 μg	5 μg	10 μg	25 μg
5	C*	I	I	CI	C	S	S	S
10	C*	I	I	I	CI	C	S	S

Rabbits were immunized with purified membranes from *T. gratilla* (2, 3). These results were obtained with antiserum 175.1. The assay was in 96-well microtiter plates (Linbrook no. 76-203-05, *not* treated to promote cell adhesion). After the indicated amounts of 22S complex and serum had been mixed in 95 μl of MFSW/10 mM Tris·HCl, pH 8.3, containing gentamycin sulfate at 100 μg/ml, 10^5 cells in 5 μl of MFSW were added quickly with stirring to all wells. Duplicate samples were evaluated by microphotography at 3 and 23 hr. The observations are coded as follows: C*, no-serum control; S, stimulation of reaggregation—all of these wells contained spinning blastoids; C, equal to control containing no 22S complex and no antiserum; CI, partial inhibition; I, inhibition—clumps of rounded cells with no continuous epithelial layer.

and 10 μl) of anti-membrane serum. The endpoint of inhibition of this serum was determined separately (2.5 μl). Inhibition is significant, since after 24 hr the controls developed into spinning blastoids or compact immobile aggregates with smooth epithelium while the inhibited cells remained in a stationary pattern without formation of a continuous epithelial layer.

Clearly, 2.5 μg of 22S complex neutralized all reaggregation-inhibiting antibodies contained in 10 μl of anti-membrane serum. It follows that the 22S complex contains all the epitopes recognized by aggregation-inhibiting antibodies. Similar results were obtained with two other anti-membrane rabbit sera and with purified IgG from rabbits immunized with purified 22S complex. A detailed account of the assay together with a complete microphotographic documentation of the experiment summarized in Table 1 will be published separately.

Reconstitution of butanol-extracted cells with butanol extract and purified 22S particles was compared. In the same experiment, we tested the two preparations also for their ability to stimulate reaggregation of dissociated cells that had not been treated with 2.5% butanol. Stimulation manifests itself in larger aggregate sizes after a given time period, reflecting an increased rate of reaggregation in response to the adhesion-promoting agent (ref. 3 and unpublished data). In Fig. 3, the average aggregate size relative to the controls has been plotted against the protein input. At inputs between 2.5 and 10 μg of purified 22S complex, the rate of reaggregation in cells not treated with butanol increases by a factor of 2.5 to a saturation level. Butanol-treated cells responded considerably slower, presumably because of the much lower ligand concentration initially on the cell surface. Nevertheless, at the highest inputs of 10 and 20 μg of 22S complex, the aggregates were 20–30% larger than the controls after 5 hr. The reconstitution was real since there was the characteristic formation of smooth epithelium after 23 hr.

Trypsin Releases the 22S Complex from the Surface in the Absence But Not in the Presence of Ca^{2+} and Renders the Dissociated Cells Reaggregation Incompetent. Studies with uvomorulin (cadherin) in the mouse embryo have shown that Ca^{2+} is required to maintain these molecules in a trypsin-resistant conformation (12, 13). The same is true for the 22S complex: when dissociated blastula cells of *P. lividus* were incubated with trypsin in the absence, but not in the presence, of Ca^{2+}, the cells lost the ability to reaggregate. At the same time, the 22S complex was released into the supernatant in the absence but not in the presence of Ca^{2+}, as shown

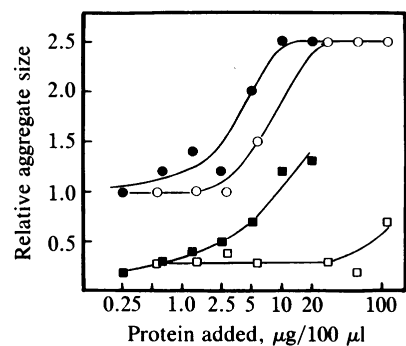

FIG. 3. Dose–response curve of purified 22S complex and butanol extract in stimulation of spontaneous reaggregation and reconstitution of butanol-treated cells. Response after 5 hr to 22S complex (●, ■) or to butanol extract (○, □) of *P. lividus*. Response with dissociated cells that have (lower curves) or have not (upper curves) been treated with butanol. The average aggregate size in each microtiter well after a 5-hr incubation at 15°C, estimated from photographs, was normalized with respect to the control of the untreated cells (= 1.0) and plotted against the protein concentration on logarithmic scale. The butanol-treated cells remained single. All assays were duplicate and more than 80% of the cells were in aggregates at inputs of ≥5 μg protein.

by the time-dependent appearance of the characteristic glycoprotein bands in a $NaDodSO_4$ gel and by a positive reaction with mAbs specific for the 22S complex (Fig. 4).

Two Functionally Distinct Binding Sites on the 22S Complex. Previous experiments have established that reaggregation-inhibiting antibodies are genus-specific, whereas stimulation of reaggregation or reconstitution of extracted cells by butanol extract is equally efficient across genera (3). To resolve this paradox, it was suggested that binding of the active component to the cell surface was through a site that was non-antigenic and failed to discriminate among the surfaces of different genera. Cell–cell contact, on the other hand, was mediated by a second site, which was blocked by the genus-specific antibodies (3). This two-site model (Fig. 5) was confirmed at the molecular level by the demonstration that proteinase K selectively eliminates the ability of butanol extract or 22S complex to promote reaggregation without interfering with its capacity to neutralize reaggregation-blocking antibodies. Proteinase K degrades all components that are larger than 70 kDa into fragments that initially are about 70 kDa and finally 50 kDa or smaller. These fragments are no longer able to stimulate reaggregation but they retain the ability to neutralize the reaggregation-blocking Fab (11).

mAbs Specific for the 22S Complex Stain Various Combinations of Cell Surface Structures. Monoclonal antibodies raised against butanol extract from *P. lividus* were screened for reaction with plastic-absorbed butanol extract by the ELISA assay and for the ability to stain sections of paraffin-embedded embryos by indirect immunofluorescence. Of a total of 19 mAbs, 3 reacted only in the ELISA test, 8 only by immunofluorescence, and 8 in both (14). Seven mAbs reacted with the 22S complex and all of these stained cell surface structures. One (G2) stained cytoplasmic granules as well. Their immunofluorescence staining patterns of embryos are shown in Table 2 and in one photograph (Fig. 6). The other photographs on which this table is based and a detailed account of these results will be published separately.

The most striking feature of the matrix in Table 2 is that nearly all mAbs stain more than one structure in different combinations. This cannot be due to contamination by unrelated mAbs since, according to Poisson statistics, the probability that we obtained in our collection of mAbs one culture containing two or more positive mAbs (in ELISA

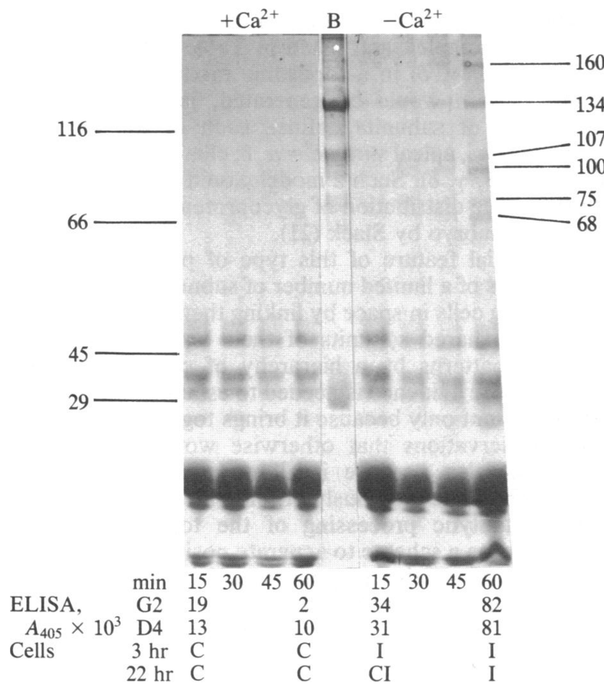

FIG. 4. Release of toposome activity from cell surface by trypsin. A 2.5% cell suspension from *P. lividus* blastulae was incubated in MFSW or Ca^{2+}- and Mg^{2+}-free seawater, each containing 0.01% trypsin, at 20°C for 15, 30, 45, and 60 min; the reaction was stopped with soybean trypsin inhibitor (SBTI); and the dialyzed and lyophilized supernatant was analyzed on a 7.5–12.5% linear gradient $NaDodSO_4$ gel and by ELISA with mAbs D4 and G2 after 15 and 60 min. The lower bands in the gel are from trypsin and SBTI. Positions (in kDa) of typical toposome bands are on the right. Lane B shows a butanol extract of live cells. Staining was with Coomassie blue. ELISA: Binding of toposome-specific mAb to the trypsin-released material immobilized in plastic wells was measured photometrically at 405 nm with anti-mouse Ig-peroxidase. The cells that had been trypsinized for 15 and 60 min were tested for reaggregation after 3 and 22 hr. Aggregation results are coded as follows: C, same as controls; CI, partial inhibition; I, inhibition (mostly single cells).

and/or immunofluorescence) in the particular fusion was less than 0.5%.

We are thus left with the possibility that the 22S complex is heterogeneous with respect to both the composition of the particle and its positional specificity. The other possibility, that the 22S complex shares some of the epitopes recognized by the mAbs with functionally unrelated cell surface components, seems unlikely in view of our earlier observation that all seven mAbs that recognized components of the butanol extract from *T. gratilla* immunoprecipitated exclusively 22S particles (Fig. 2). On the other hand, the idea that the 22S complex is a mixture of position-specific particles is consistent with the observations listed in Table 2 and attractive in view of the discovery of similar glycoprotein complexes in *Drosophila* isolated with the aid of position-specific mAbs (6).

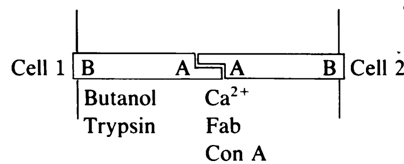

FIG. 5. Two-site model for 22S particle-dependent cell interaction.

Table 2. Positional code deduced from cell surface staining patterns produced by mAbs specific for 22S complex

mAbs	Subunit* specificity	Immunofluorescence staining: Apical surface (α δ —)	Cilia (α β δ)	Cell–cell interface (β γ δ)	ELISA titer × 10^{3}†
D2, G2‡	α	+	+	−	0.5/2.5
B8, B12	β	−	+	+	0.1/12.5
F12	γ	−	−	+	12.5
H10, D4	δ	+	+	+	2.5/2.5

Blastulae of *P. lividus* were embedded in paraffin, sectioned, and stained by immunofluorescence. Mice were immunized by injection of butanol extracts from membranes of *P. lividus* (2). Clones were isolated after fusion by the limiting dilution method (unpublished results). The ELISA tests were carried out in microtiter wells coated with ≈10 μg of 22S complex. The table lists only mAbs positive to butanol extract and 22S complex in an ELISA assay and indicates by a + sign what structures are stained by immunofluorescence.

*The subunits correspond to the 160-kDa monomers after modification by processing (see also *Discussion*).

†Highest dilution of hybridoma culture fluids that produced an absorbance between 0.15 and 0.25 above background. Thus, 1.0 corresponds to 1:1000.

‡G2 also stained cytoplasmic granules.

DISCUSSION

This paper presents evidence that the large oligomeric glycoprotein particle (22S complex) of unknown function isolated previously from yolk granules is the sole structure responsible for the adhesive integrity of the sea urchin blastula.

The molecular properties of the 22S complex summarized in the two-site model in Fig. 5 account for all of the observations on dissociation of sea urchin embryos. The 22S complex seems to be a hexamer of 160-kDa subunits. If this particle is the sole agent linking the cells mechanically, it is likely to be a symmetrical dimer or even-numbered oligomer. The stability of the 22S hexamer in the presence of Ca^{2+} and its tendency to dissociate into two 15S particles upon Ca^{2+} removal suggests the trimer as the functional unit on the cell surface that links two cells by formation of the hexamer. The role of Ca^{2+} as an allosteric stabilizer of the complex is also indicated by its protective effect against trypsin action. It has long been known that dissociation into single cells of the embryos of certain sea urchin species requires only the removal of Ca^{2+}, because even after extensive washing they reaggregate promptly on Ca^{2+} addition. Yet after removal of the complex with butanol, the cells cannot reaggregate despite the presence of Ca^{2+}, unless the complex is added back. It follows that the glycoprotein complex and Ca^{2+} must cooperate. Since the complex is not lost from the cells in the absence of Ca^{2+} or upon dissociation with specific Fab in the

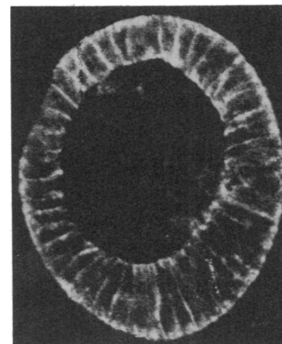

FIG. 6. Immunofluorescence staining of sectioned *P. lividus* blastula with mAb F12. (×300.)

presence of Ca^{2+}, interaction at the cell binding site (B in Fig. 5) is largely Ca^{2+} independent and insensitive to Fab, which leaves only the self-associating site (A) as the target to be modified by Ca^{2+} and Fab binding. On the other hand, cell binding is sensitive to butanol, and proteinase K and trypsin cleave near the cell binding site. The epitopes for blocking antibodies and the fragmentation pattern generated by the developmentally regulated processing are genus-specific in a strictly covariant fashion. For example, the $NaDodSO_4$ gel patterns of the complexes from the immunologically noncross-reacting genera *Arbacia* and *Paracentrotus* or *Arbacia* and *Lytechinus* differ widely, whereas the patterns of the cross-reacting genera *Lytechinus* and *Tripneustes* are very similar (unpublished data). This conforms to the evolutionary relationships derived from morphological criteria and fossil records (15). By contrast, Con A, like blocking Fab, causes live blastulae to dissociate into single cells (16) and also binds strongly to 22S particles, regardless of genus (unpublished results). Clearly, the Fab-sensitive site is more complex than a lectin-specific glycosylation structure.

We do not know how the 22S complex is anchored to the cell surface. According to LeGrue (17), butanol removes external but not integral membrane proteins from live cells. In this respect our complex resembles other external cell surface proteins, and it is probable that, like fibronectin, it is attached through a specific site to an integral membrane protein receptor (18, 19). Our results, when viewed in the context of recent work in other systems, suggest that the very large glycoprotein complexes of the type represented by our 22S particles and the adherons (18, 20) are a novel class of cell organelles specialized in mediating cell–cell and cell-substratum interaction as well as conveying positional information. Schubert and LaCorbiere (20) have shown that adherons, particles of a uniform size that are released into the medium by a variety of higher vertebrate cells, are taken up by cells to promote cell–cell and cell–substratum interaction. Work with position-specific mAbs in *Drosophila* embryos led to the discovery of similar oligomeric glycoprotein complexes by Wilcox *et al.* (6). Although these complexes have not been isolated in their native form, cross-linking experiments indicate a size in the range of 400–500 kDa. This corresponds to half the size (trimer) of our 22S hexamer and to the 12–16S range of adherons described by Schubert and LaCorbiere. Similarly, epimucin from amphibian embryos is most likely a trimer (21). We have recently isolated toposome-like particles from *Drosophila* embryos (unpublished results).

The question arises whether, in view of the general use of detergents in their isolation, all smaller cell adhesion molecules isolated so far might not be fragments of adherons or toposomes. Moreover, purifications relying exclusively on antibody neutralization assays, in contrast to those based on stimulation of reaggregation or reconstitution, do not require functionally and structurally intact molecules.

The most exciting aspect of toposome function is their possible involvement in morphogenesis, suggested by the glycoprotein complexes isolated from *Drosophila* and amphibian embryos (6, 21). That these complexes play a role in cellular adhesion, while so far not supported by a functional test, is strongly implied by their position-specific arrangement on the cell surface. The results suggest a positional code by combining subunits in different ways. If these findings can be generalized and applied to the sea urchin, the 22S particles isolated from whole embryos would be a mixture of subsets characterized by different subunit combinations and positional specificities. The immunofluorescence staining patterns of sectioned embryos generated by monoclonal antibodies recognizing the 22S complex are consistent with such a concept. Thus, if each of the four classes of mAbs positive for the 22S complex and listed in Table 2 recognized one subunit (α, β, γ, δ) in a toposome mixture, the observed staining pattern would be generated, in which a unique combination of subunits defines each of the embryonic structures (e.g., apical surface = α, δ; cilia = α, β, δ; cell–cell interface = β, γ, δ). Such a model would also be consistent with the tissue distribution of glycoproteins observed in the amphibian embryo by Slack (21).

An essential feature of this type of positional code by combinations of a limited number of subunits is the capacity of organizing cells in space by linking them through variable numbers of shared subunits of their toposomes and thus generating patterns by a hierarchy of cell affinities (22). Although more evidence is needed to establish this model, it is attractive not only because it brings together a number of diverse observations that otherwise would remain unexplained but also because it allows predictions that are testable. Thus, the previously obscure developmentally regulated proteolytic processing of the toposome subunits makes sense as a scheme to generate positional diversity. A relatively small number of genes specifying toposome polypeptide chains combined with posttranslational modifications such as proteolytic processing and glycosylation should suffice to determine the growing complexity of morphogenesis of the later stages of development.

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