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Isolation of Cleavage Furrows from Eggs of Regular Sea Urchins and Identification of Furrow-Specific Proteins[1]

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We have developed a method for the isolation of cleavage furrows from dividing sea urchin eggs, which is applicable to various sea urchin species. The new method differs from that used for isolating cleavage furrows from sand dollar *Clypeaster japonicus* eggs [Yonemura, S., Mabuchi, I., and Tsukita, S. (1991) *J. Cell Sci.* 100, 73-84] in the type and concentration of detergent included in the isolation medium, the temperature during the treatment of dividing eggs with the isolation medium, and the centrifugation conditions. The contractile ring was included in the isolated cleavage furrows, as seen on rhodamine-phalloidin staining of actin filaments. When the furrows were isolated with the isolation medium containing both NaF and β-glycerophosphate, which are potent protein phosphatase inhibitors, the isolated furrows were found to be accompanied by the mitotic apparatus. When the isolation was carried out in the absence of both NaF and β-glycerophosphate, cleavage furrows without the mitotic apparatus were obtained. The development of a method of isolation of cleavage furrows from regular sea urchin eggs enabled us to compare protein constituents among furrows from different sea urchin and sand dollar species. We found that 32, 36, and 51 kDa proteins were concentrated in common in the cleavage furrows isolated from eggs of the sand dollars, *C. japonicus* and *Scaphechinus mirabilis*, and the sea urchins, *Hemicentrotus pulcherrimus* and *Strongylocentrotus nudus*, on two-dimensional gel electrophoreses.

Key words: actin filament, cleavage furrow, contractile ring, cytokinesis, mitotic apparatus.

It is widely accepted that animal cells divide through contraction of the contractile ring, which is formed in the cleavage furrow cortex. It has been established that its major constituents are actin filaments and these filaments have been shown to be necessary in cytokinesis in various cells (*1-3*). It has also been established that myosin is necessary for the process. Starfish blastomeres that are microinjected with an anti-myosin antibody are not able to undergo cytokinesis (*4, 5*). In *Dictyostelium discoideum* amoebae, expression of antisense RNA of the myosin heavy chain (*6*) or targeting of the myosin heavy chain gene (*7, 8*) results in failure of cytokinesis. These facts indicate that the interaction between myosin and actin filaments causes contraction of the contractile ring. In addition, some actin-modulating proteins have been shown to be involved in cytokinesis in different manners (*2, 3*). The molecular mechanism underlying cytokinesis, however, is not well understood.

The isolation of cleavage furrows from dividing cells is one of the most important methods for elucidating the molecular mechanism underlying cytokinesis. We hand-isolated furrows from newt eggs and found some unique proteins in the furrow preparation (*9*). However, this method has the disadvantage that it is not easy to obtain a sufficient amount of the furrows for analysis of the protein constituents. We have also reported (*10*) a mass isolation method for cleavage furrows from eggs of the sand dollar, *Clypeaster japonicus*. However, this method has not been applicable to the dividing eggs of regular sea urchins.

We believe that comparison of the protein constituents of cleavage furrows isolated from eggs of various sea urchins and identification of the proteins present in common in these preparations would lead to an elucidation of the molecular mechanisms underlying both the formation and contraction of the contractile ring. In this study, we developed an isolation method for cleavage furrows by which we were able to isolate furrows from various sea

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Abbreviations: CBB, Coomassie Brilliant Blue R-250; CL-A, calyculin A; CR-like apparatus, contractile ring-like apparatus; DAPI, 4,6-diamidino-2-phenylindole; DMSO, dimethyl sulfoxide; DOC, sodium deoxycholate; IEF, isoelectric focusing; IM-A, isolation medium-A; IM-B, isolation medium-B; IM-C, isolation medium-C; MOPS, 3-(*N*-morpholino)propanesulfonic acid; NEpHGE, non-equilibrium pH gradient gel electrophoresis; NP-40, Nonidet P-40; pI, isoelectric point; Rh-ph, rhodamine-phalloidin; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

urchin eggs. On analyzing the cleavage furrow fractions by two-dimensional gel electrophoreses, we found that some proteins are unique to the cleavage furrow and are common in the furrow preparations from the eggs of various species. A part of this study has been published in an abstract form (*11*).

MATERIALS AND METHODS

Cell Culture—The sea urchins, *Hemicentrotus pulcherrimus*, *Strongylocentrotus nudus*, and *Pseudocentrotus depressus*, and the sand dollars, *C. japonicus* and *Scaphechinus mirabilis*, were used. All the eggs and sperm were obtained by intracoelomic injection of 0.1 M acetylcholine chloride dissolved in sea water. The eggs of *H. pulcherrimus*, *P. depressus*, *S. nudus*, and *S. mirabilis* were washed twice with ordinary sea water and then dejellied by passage through a 70 μm nylon mesh. The eggs were further washed three times with ordinary sea water. After insemination in ordinary sea water, the fertilization membranes were removed as described previously (*10, 12*). The eggs were washed three times with and then cultured in Ca-free sea water. *C. japonicus* eggs were inseminated in Ca-free sea water, and the fertilization membranes were removed by passage through the nylon mesh (*10, 12*).

Isolation of Cleavage Furrows—We used three media for isolation of the cleavage furrows. Isolation medium A (IM-A) consisted of 0.1% Nonidet P-40 (NP-40), 0.16 M sucrose, 0.1 M KCl, 2 mM $MgCl_2$, 5 mM EGTA, 0.5 mM DTT, 5 μg/ml leupeptin, 0.5 mM PMSF, and 10 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer, pH 7.3. This medium was the same as that previously used for isolation of the cleavage furrows from sand dollar *C. japonicus* eggs (*10*). The following two media were newly devised. Isolation medium B (IM-B) consisted of 0.2% (w/v) sodium deoxycholate (DOC), 0.1 M KCl, 2 mM $MgCl_2$, 5 mM EGTA, 0.5 mM DTT, 5 μg/ml leupeptin, 0.5 mM PMSF, 10% (v/v) dimethyl sulfoxide (DMSO), and 10 mM MOPS buffer, pH 7.3. Isolation medium C (IM-C) consisted of IM-B in which the KCl concentration was reduced to 50 mM, and both 50 mM NaF and 50 mM β-glycerophosphate were added.

The cleavage furrows of *H. pulcherrimus* eggs were isolated with IM-B or IM-C, and those of *S. nudus, P. depressus*, and *S. mirabilis* eggs were isolated with IM-C as follows. When eggs were at the first cleavage stage, they were pelleted by hand-centrifugation, and then mixed with 50 volumes of one of the isolation media. Then they were allowed to settle down for 30 min on ice, followed by mixing and gentle shearing by pipetting. Most of the eggs were disrupted by this procedure. The mixture was further incubated for 30 min on ice. Eggs that did not lyse were removed by passage through two layers of 50 μm nylon mesh. The suspension was then layered onto a discontinuous density gradient of 0.3 and 1.5 M sucrose dissolved in the isolation medium devoid of DOC, and centrifuged at 40 $\times g$ for 1 h at 4°C. A fraction rich in cleavage furrows was recovered at the 0.3 M/1.5 M interface. This fraction was mixed with an equal volume of the isolation medium devoid of DOC and then centrifuged at 40 $\times g$ for 30 min at 4°C. The loose pellet was transferred to an Eppendorf tube, and then 1 ml of the isolation medium devoid of DOC was added. It was then mixed and centrifuged at 15,000 $\times g$ for several seconds to recover the furrows.

The cleavage furrows of *C. japonicus* eggs were isolated with IM-A according to the method previously described (*10*). The cortices of eggs at the late prophase (*13*) and calyculin-A (CL-A)-induced contractile ring-like apparatuses (CR-like apparatuses) (*14*) were isolated as previously described.

Staining with Rhodamine-Phalloidin—Actin filaments in the cleavage furrows were visualized by rhodamine-phalloidin (Rh-ph) staining as previously described (*10*). An about 5 μl sample at each isolation step was stained without fixation for 5 min by adding 1 μl of 3.3 μM Rh-ph in an isolation medium devoid of the detergent. Samples were examined under an Optiphot microscope equipped with an epifluorescence apparatus (Nikon, Tokyo).

Immunofluorescence Microscopy—The cleavage furrows isolated with IM-C were transferred to a test tube, and then an equal volume of glycerol-F-buffer (1 M glycerol, 0.1 M KCl, 2 mM $MgCl_2$, 1 mM EGTA, and 10 mM MOPS buffer, pH 7.3) containing 10% (v/v) formalin was added. They were fixed for 1 h at room temperature. The fixed cleavage furrows were attached to a protamine (10 mg/ml)-coated glass slide, followed by washing with glycerol-F-buffer.

For indirect immunofluorescence microscopy, a monoclonal antibody against α-tubulin (Amersham International plc, Amersham, England) was used as the first antibody. Fluorescein isothiocyanate-labeled goat anti-mouse IgG (Tago, Burlingame, CA, USA) was used as the second antibody. Chromosomes were visualized with 0.5 μg/ml 4,6-diamidino-2-phenylindole (DAPI). Actin filaments were visualized with 0.3 μM Rh-ph.

Observations were carried out under a Carl Zeiss Axio-Scope microscope.

Electrophoreses—Two-dimensional gel electrophoreses were carried out essentially according to O'Farrell (*15, 16*) with slight modifications (*10, 17*). The first gel containing 2% Ampholine (pH 5-7 : pH 3.5-10 = 4 : 1), 3.5 cm long, was made in a glass tube (2 mm in diameter, and 7 cm long). The samples were first resolved by either isoelectric focusing (IEF) or non-equilibrium pH gradient gel electrophoresis (NEpHGE). IEF was carried out at 100 V for 10 min at first, then at 300 V for 2.5-3 h, and finally at 400 V for 10 min. NEpHGE was carried out at 200 V for 1 h. IEF and NEpHGE gel rods were then placed tandemly on a second dimensional gel slab (12.5% acrylamide; 0.8 mm thick, 7 cm wide, and 5 cm high). After sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli (*18*), the slab gel was stained with Coomassie Brilliant Blue R-250 (CBB) or a silver staining kit (Wako Pure Chemical Industries, Osaka). The molecular size markers used were myosin heavy chain, β-galactosidase, phosphorylase-*b*, BSA, actin, and carbonic anhydrase. The pH gradient formed during IEF or NEpHGE was determined according to O'Farrell (*15, 16*). Tubulin from porcine brain was a generous gift from Dr. Natsumi Hosoya.

RESULTS

Isolation of Cleavage Furrows—We previously reported a mass isolation method for cleavage furrows from eggs of the sand dollar, *C. japonicus*, involving IM-A (*10*). We

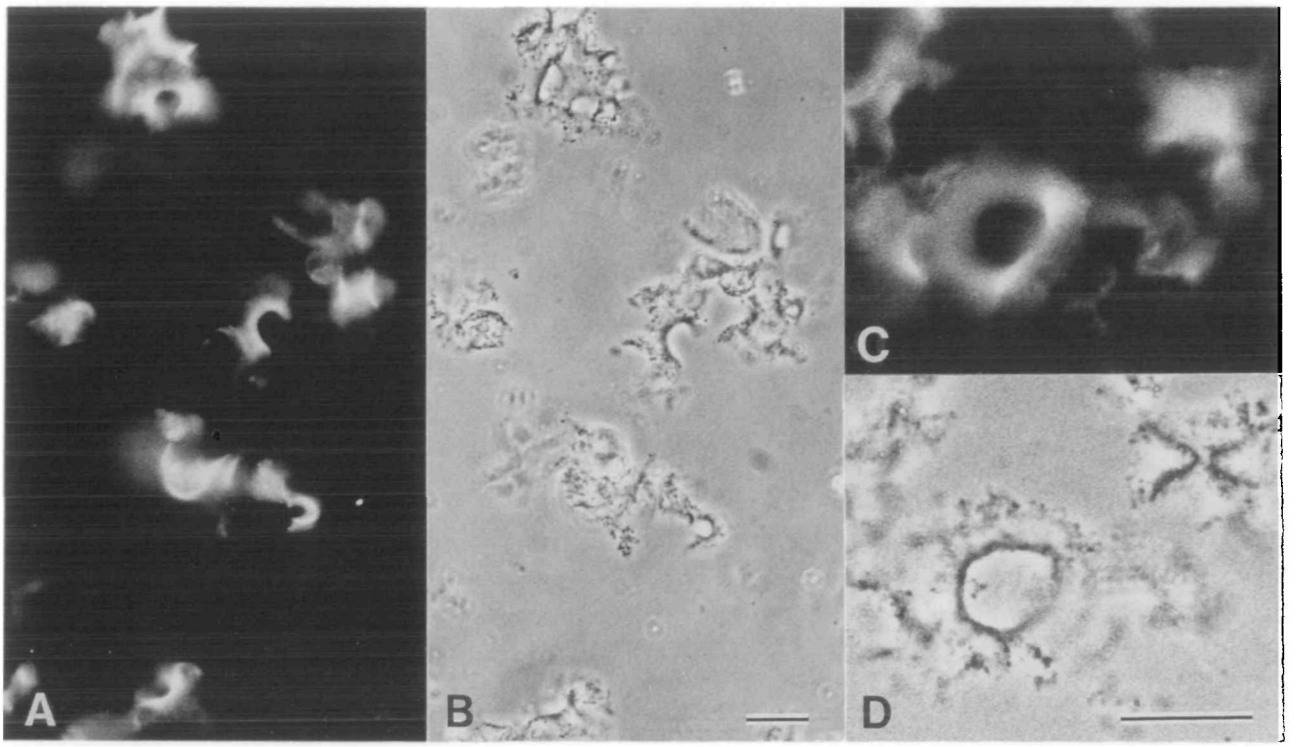

Fig. 1. **Cleavage furrows of *Hemicentrotus pulcherrimus* eggs isolated with IM-B.** Fluorescence (A, C) and phase-contrast (B, D) micrographs of cleavage furrows stained with Rh-ph are presented here. The left and right images in C and D are the top and side views of isolated furrows, respectively. Picture C is focused on the right side of the left furrow so that other parts including the right furrow are out of focus. Bar, 50 μm.

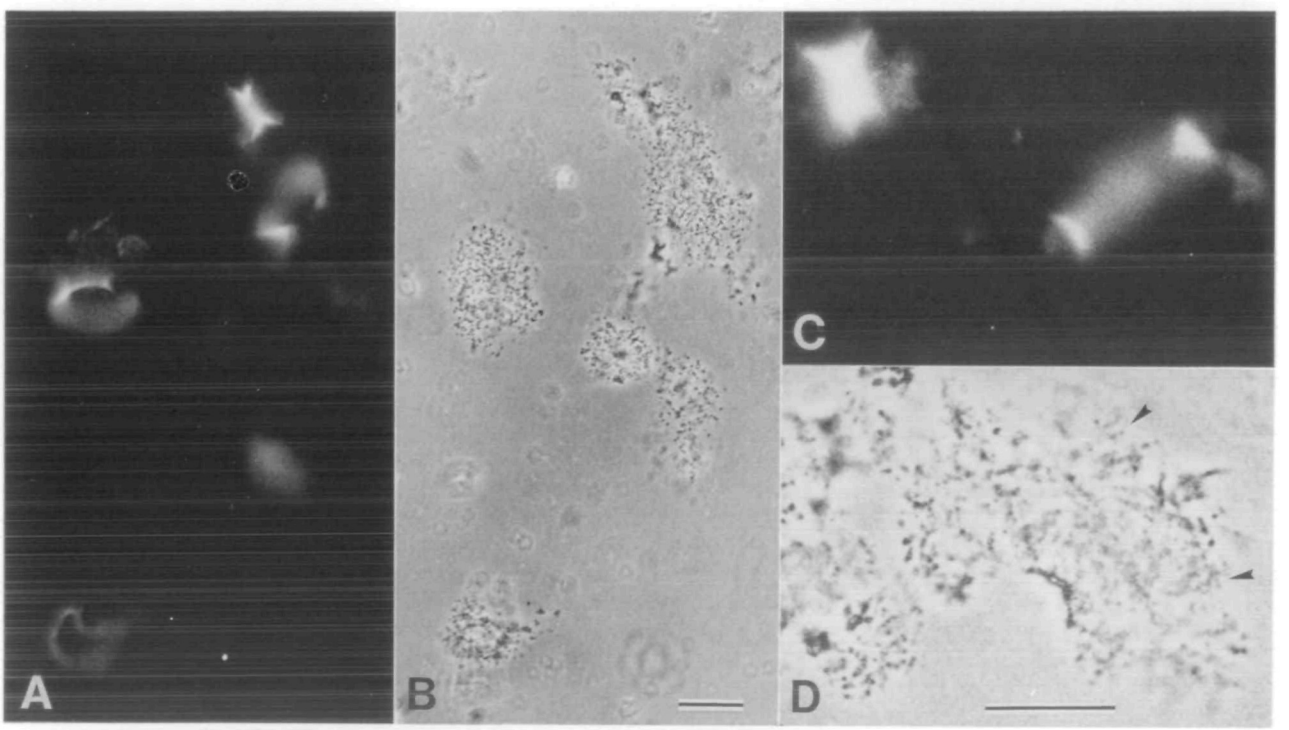

Fig. 2. **Cleavage furrows of *Hemicentrotus pulcherrimus* eggs isolated with IM-C.** Fluorescence (A, C) and phase-contrast (B, D) micrographs of cleavage furrows stained with Rh-ph are presented here. Note that these furrows are associated with the mitotic apparatus (arrowhead in D). Bar, 50 μm.

tried at first to isolate cleavage furrows using this method from eggs of other kinds of sand dollars or regular sea urchins. We were able to isolate cleavage furrows from the eggs of another kind of sand dollar, *S. mirabilis*. However, we could not obtain cleavage furrows from those of regular sea urchins. Therefore, we modified this isolation method in several points in order to isolate cleavage furrows from the eggs of the typical Japanese regular sea urchin, *H. pulcherrimus*. First, we examined the type and concentration of detergent in the isolation medium. We found that 0.2% (w/v) DOC was the most suitable detergent. At DOC concentrations greater than this (for example, 0.25 or 0.3%), fewer intact furrows were isolated. When the dividing eggs were treated with 0.1% (w/v) NP-40 or 0.1% (w/v) Triton X-100 instead of 0.2% DOC, the eggs were not disrupted well. While, at higher concentrations (0.2-0.4%) of Triton X-100, the cortices of the eggs tended to break and aggregate with each other. Second, we examined the temperature during the treatment of the eggs with the isolation medium. Treatment at 0°C gave a good yield. When we treated the eggs at room temperature, the cortices of the eggs including the cleavage furrow region tended to break into pieces. Third, we examined the centrifugation conditions for the suspension that was obtained on treatment of the eggs with the isolation medium. We found that cleavage furrows were recovered at the interface between 0.3 and 1.5 M of the sucrose step gradient after centrifugation at $40 \times g$ for 1 h. When the suspension was centrifuged at a higher speed and for a shorter period (for example, at $400 \times g$ for 30 min), the furrows tended to break into pieces and the amount of cortical contaminants increased. Finally, we examined the components of the isolation medium. We found that a medium consisting of 0.1 M KCl, 2 mM $MgCl_2$, 5 mM EGTA, 0.5 mM DTT, 10%(v/v) DMSO, 5 μg/ml leupeptin, 0.5 mM PMSF, and 10 mM MOPS, pH 7.3 (IM-B), was the most effective. When DMSO was omitted from the isolation medium, the furrows tended to break into pieces.

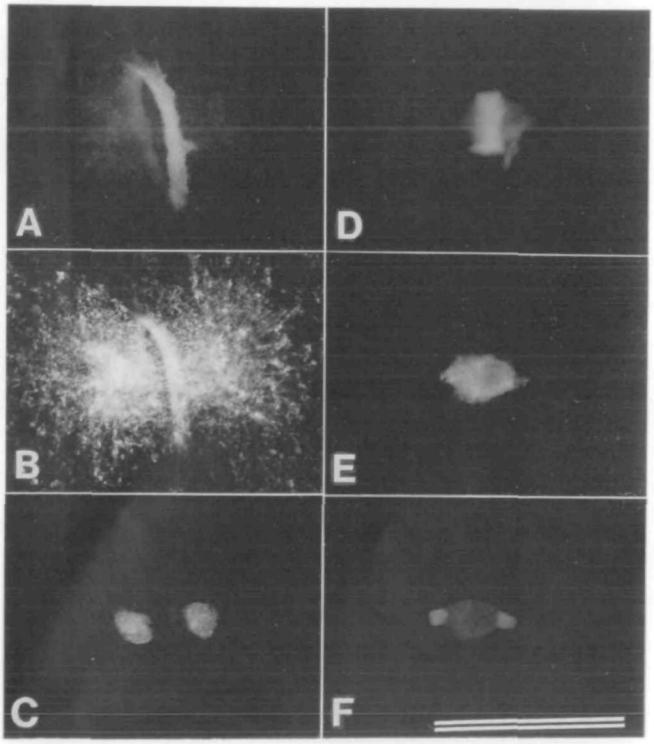

Fig. 3. **Mitotic apparatus attached to the cleavage furrow visualized on indirect immunofluorescence microscopy.** Cleavage furrows isolated with IM-C were stained with Rh-ph (A and D), and an anti-α-tubulin antibody (B and E). DNA was visualized by DAPI (C and F). A-C, a typical mitotic apparatus; D-F, a mitotic spindle. In B, rhodamine fluorescence was not perfectly cut out by the filter used, so the contractile ring can be seen in addition to the mitotic apparatus. Bar, 50 μm.

Figure 1 shows the cleavage furrows isolated from eggs of *H. pulcherrimus* with IM-B. Upon staining of F-actin with Rh-ph, the contractile rings were clearly observed. A phase contrast image of the same area revealed only a few cytoplasmic and cortical contaminants.

Thus we established a procedure for the isolation of cleavage furrows, as described under "MATERIALS AND METHODS." However, sometimes we were not able to isolate furrows because they broke into pieces during the isolation. This might be due to the condition of the eggs. Finally, we were constantly able to isolate cleavage furrows with IM-C that contained NaF and β-glycerophosphate (Fig. 2), which are potent inhibitors of protein phosphatases (*19*). We found that there was a significant difference in appearance between the furrows isolated with IM-C and those isolated with IM-B, which did not contain protein phosphatase inhibitors; a structure reminiscent of the mitotic apparatus was usually attached to the cleavage furrows isolated with IM-C (compare Figs. 1D and 2D). This was confirmed by indirect immunofluorescence microscopy with an anti-α-tubulin antibody (Fig. 3). The structure attached to the isolated cleavage furrow was stained by this technique. The majority of the cleavage furrows were associated with the mitotic apparatus (Fig. 3B), while some of them were associated with the mitotic spindle without astral microtubules (Fig. 3E). The fibrous nature of the microtubules was not clearly observed, suggesting that the microtubule structure might be damaged to some extent during the isolation of the furrows. Weak birefringence was detected for these structures on polarized light microscopy (not shown).

The procedure with IM-B yielded an isolated furrow fraction containing 100-200 μg of protein from 1 ml of packed eggs, while the procedure with IM-C yielded a furrow fraction containing 10-20 μg of protein from 1 ml of packed eggs.

Using the method with IM-C, we succeeded in isolating cleavage furrows from eggs of other kinds of sea urchins, *S. nudus* and *P. depressus*. In addition, we were also able to isolate furrows with IM-C from eggs of the sand dollars, *C. japonicus* and *S. mirabilis*.

Protein Constituents—We investigated the protein constituents of isolated cleavage furrows by IEF/SDS-PAGE and NEpHGE/SDS-PAGE two-dimensional gel electrophoreses. IEF is suitable for the analysis of acidic proteins (*15*), while NEpHGE is suitable for the analysis of basic proteins (*16*).

At first we compared the protein constituents of the cleavage furrows from *C. japonicus* with those of egg cortices before cleavage (Fig. 4, A and B). We confirmed that three acidic proteins, 32, 35, and 37 kDa ones (pIs of these proteins, below 4.5), were specifically present in the cleavage furrow preparation, as we reported previously

(*10*). We newly found that five proteins, 51 kDa (pI, above 8), 49 kDa (pI, about 8.0), 45 kDa (pI, about 7.5), 40 kDa (pI, about 8.0), and 36 kDa (pI, about 8.0) ones, were also specifically present in the furrow preparation (Fig. 4A).

We then compared the protein constituents of the furrow preparation from *C. japonicus* with those in the other

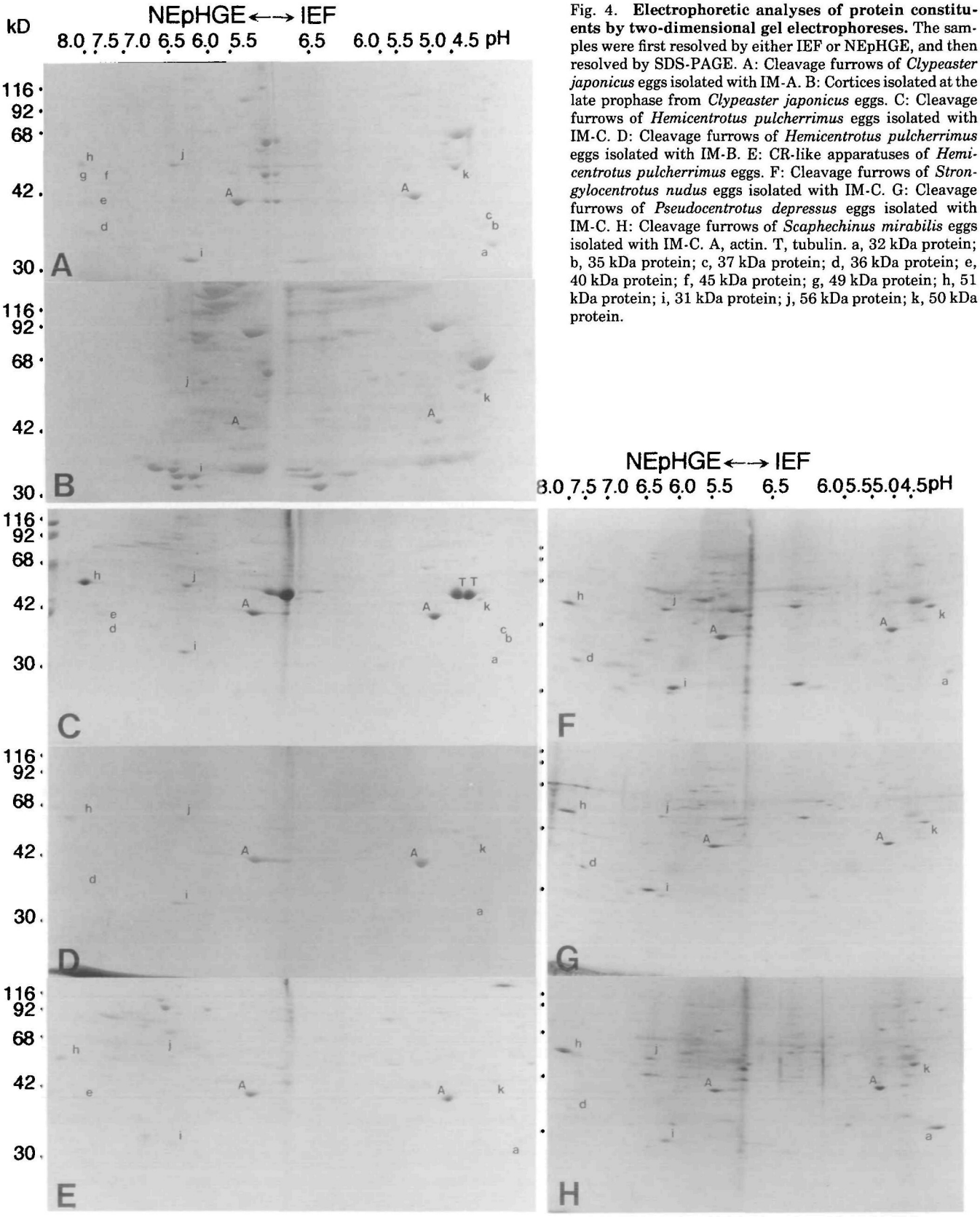

Fig. 4. **Electrophoretic analyses of protein constituents by two-dimensional gel electrophoreses.** The samples were first resolved by either IEF or NEpHGE, and then resolved by SDS-PAGE. A: Cleavage furrows of *Clypeaster japonicus* eggs isolated with IM-A. B: Cortices isolated at the late prophase from *Clypeaster japonicus* eggs. C: Cleavage furrows of *Hemicentrotus pulcherrimus* eggs isolated with IM-C. D: Cleavage furrows of *Hemicentrotus pulcherrimus* eggs isolated with IM-B. E: CR-like apparatuses of *Hemicentrotus pulcherrimus* eggs. F: Cleavage furrows of *Strongylocentrotus nudus* eggs isolated with IM-C. G: Cleavage furrows of *Pseudocentrotus depressus* eggs isolated with IM-C. H: Cleavage furrows of *Scaphechinus mirabilis* eggs isolated with IM-C. A, actin. T, tubulin. a, 32 kDa protein; b, 35 kDa protein; c, 37 kDa protein; d, 36 kDa protein; e, 40 kDa protein; f, 45 kDa protein; g, 49 kDa protein; h, 51 kDa protein; i, 31 kDa protein; j, 56 kDa protein; k, 50 kDa protein.

TABLE I. **Proteins specific to cleavage furrow preparations.**

	Proteins (kDa)								Reference
	32	35	37	36	40	45	46	51	
C. japonicus cleavage furrows isolated with IM-A	+	+	+	+	+	+	+	+	*10*, this study
C. japonicus cortices	−	−	−	−	−	−	−	−	*10*, this study
H. pulcherrimus cleavage furrows isolated with IM-C	+	+	+	+	+	−	−	+	this study
H. pulcherrimus cleavage furrows isolated with IM-B	+	−	−	+	−	−	−	+	this study
S. nudus cleavage furrows isolated with IM-C	+	−	−	+	−	−	−	+	this study
P. depressus cleavage furrows isolated with IM-C	−	−	−	+	−	−	−	+	this study
S. mirabilis cleavage furrows isolated with IM-C	+	−	−	+	−	−	−	+	this study
H. pulcherrimus CR-like apparatus	+	−	−	−	+	−	−	+	this study

+, protein spot visible in the two-dimensional gel. −, protein spot not visible in the two-dimensional gel.

species. (1) When we compared *C. japonicus* furrows with the furrows from *H. pulcherrimus* eggs isolated with IM-C (Fig. 4C), we found that the three acidic proteins, the 32, 35, and 37 kDa ones, and three basic proteins, the 36, 40, and 51 kDa ones, were common in the two-dimensional gel patterns. (2) When we compared *C. japonicus* furrows with the furrows from *H. pulcherrimus* eggs isolated with IM-B (Fig. 4D), we found that the 32, 36, and 51 kDa proteins were common. (3) Next, we compared *C. japonicus* furrows with the furrows from eggs of other sea urchins or sand dollars isolated with IM-C (Fig. 4, F, G, and H). We found that the 32, 36, and 51 kDa proteins were common in the furrow preparations of *S. nudus* (Fig. 4F), *P. depressus* (Fig. 4G), and *S. mirabilis* (Fig. 4H), while in the furrow preparation from *P. depressus*, the 32 kDa protein was less clear (Fig. 4G).

Tosuji *et al.* (*14*) previously reported that CL-A, a protein phosphatase inhibitor, induces the formation of a CR-like apparatus in an unfertilized sea urchin egg. This apparatus was found to contain actin filaments and myosin, as judged on immunohistochemistry and SDS-PAGE. We compared the protein constituents of the CR-like apparatus isolated from *H. pulcherrimus* eggs with those of the furrow preparation from *C. japonicus* eggs (Fig. 4E). We found that the 32, 40, and 51 kDa proteins were also present in the CR-like apparatus.

The results of the comparison of protein constituents described above are summarized in Table I. The 51 and 36 kDa proteins were common in the preparations from 5 echinoid species. The 32 kDa protein was also common in the preparations from 4 echinoid species, however, it was not clear in the preparation from *P. depressus*. The 35, 37, and 40 kDa proteins were common in the cleavage furrows of *C. japonicus* eggs isolated with IM-A and those of *H. pulcherrimus* eggs isolated with IM-C.

In addition, all the preparations including the cortices of *C. japonicus* eggs contained a neutral 31 kDa protein (pI, about 6.5), a neutral 56 kDa protein (pI, about 6.5), and an acidic 50 kDa protein (pI, about 4.5) (i, j, and k in Fig. 4, respectively).

On comparison of the protein constituents of the furrows isolated from *H. pulcherrimus* eggs with IM-C with those of the furrows with IM-B, two 50 kDa protein spots were detected in the IEF gel pattern of the furrow preparation with IM-C (Fig. 4, C and D), while these spots were not detected for the furrows isolated with IM-B. These spots were found to be tubulin subunits, which was confirmed by co-electrophoresis with porcine brain tubulin. This is reasonable because the cleavage furrows isolated with IM-C were associated with the mitotic apparatus. We were also able to obtain a furrow fraction from the eggs of *C. japonicus* with IM-C as described above. We found that the two spots of tubulin subunits were also present in the IEF gel pattern of the furrows isolated with IM-C (data not shown), which were not detected for the furrows isolated with IM-A (*10*, Fig. 4A).

DISCUSSION

We succeeded in isolating cleavage furrows containing the contractile ring from the eggs of regular sea urchins after modification of several points of the previously reported method (*10*). We carefully examined the types and concentrations of detergents, the centrifugation conditions, the temperature, and the components of the isolation medium. Finally, we found the optimum conditions for the isolation of cleavage furrows, as described under "MATERIALS AND METHODS."

We found that there were differences with regard to morphology between the cleavage furrows isolated with IM-C and those isolated with IM-B. The furrows isolated in the presence of NaF and β-glycerophosphate (IM-C) were usually accompanied by an attached mitotic apparatus. Tubulin spots were therefore detected in the two-dimensional gel pattern of these furrow preparations. On the other hand, mitotic apparatuses were not found in the furrow preparations isolated in the absence of NaF and β-glycerophosphate (IM-B).

Larochelle and Epel described a procedure for the isolation of cleavage furrows from the eggs of the sea urchin, *Lytechinus pictus* (*20*), with sodium vanadate, a tyrosine phosphatase inhibitor, in the isolation medium. The furrows were also associated with the mitotic apparatus. The complex of the cleavage furrow and the mitotic apparatus is similar in appearance to that obtained in this study. This may suggest that there are structural relationships between the cleavage furrow and the mitotic apparatus, and that the association of these structures or stability of the mitotic apparatus requires the phosphorylation of protein(s). It has been proposed that the mitotic apparatus determines the position of the cleavage furrow at the end of the anaphase (*21-23*). Furthermore, Wheatley and Wang (*24*) recently reported that cleavage progression and completion appear to require continuous interaction of the cortex with a subset of microtubules in cultured epithelial cells. It would be interesting to determine how these structures are associated with each other.

We also succeeded in isolating cleavage furrows without the mitotic apparatus in the absence of phosphatase inhibitors, although the furrows were more fragile and the

yield was lower than in the case of the presence of NaF and β-glycerophosphate.

The present results indicate that the contractile ring may be stabilized by the phosphorylation of protein(s) and/or association with the mitotic apparatus. We have proposed that the phosphorylation of protein(s) would be necessary for the formation of the contractile ring (*25*). A strong inhibitor of protein phosphatase, CL-A, induces the formation of a CR-like apparatus, which contains actin filaments and myosin, in an unfertilized sea urchin egg (*14*). This apparatus then contracts to cleave the egg into two fragments. This CR-like apparatus persists for a long period after contraction. In addition, when fertilized sea urchin eggs are treated with ML-9, an inhibitor of protein kinases that preferentially inhibits myosin light chain kinase activity, the contractile ring formation does not occur (*12*). These facts have indicated that the state of phosphorylation of protein(s) might be involved in both the formation and stabilization of the contractile ring. The results of the present study are consistent with this idea.

We found that 51 and 36 kDa proteins were common in the cleavage furrow preparations from various echinoid eggs. We also found that a 32 kDa protein was common in the cleavage furrow preparations that we isolated, except that its presence was not clear in the preparation from *P. depressus*. Furthermore, we found that the 51 and 32 kDa proteins were also present in the CR-like apparatus isolated from *H. pulcherrimus* eggs. Therefore, these proteins must play important roles in cytokinesis. We are currently investigating the nature of these proteins.

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