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A Cytoplasmic Factor Required for Contraction of the Cleavage Furrow in *af* Mutant Eggs of *Xenopus laevis*

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ABSTRACT—The *abnormal furrow* (*af*) gene was identified through a maternal effect mutation that causes a defect in cell division in *Xenopus* embryos. In eggs obtained from mutant females (*af* eggs), polar body formation and cytokinesis are completely inhibited while nuclear division continues uninterrupted. Cleavage furrows are recognized as unpigmented narrow bands on the egg surface but they do not contract. Transfer of cytoplasm from wild-type eggs into *af* eggs partially rescues contraction of the cleavage furrow. The factor responsible for contraction promoting activity was present in wild-type eggs throughout the first cell division cycle and induced contraction in a dose dependent manner. The factor was characterized as a high molecular weight protein complex that was associated with particulate fraction in the extract. The recovery of contraction was associated with accumulation of filamentous actin and WGA-binding sites within the cleavage furrow of the *af* eggs which were normally not enriched for these components. Formation and contraction of a filamentous actin ring during cortical wound healing in *af* eggs was indistinguishable from that of wild-type eggs. From these results, the *af* gene product may be specifically required for reorganization of the actin filaments and WGA-binding sites in the cleavage furrow and contraction through these structures during cytokinesis.

INTRODUCTION

Formation of the cleavage furrow is one of the characteristics of cell division in animal cells. It has been proposed that the position of the cleavage furrow is specified by a signal called the cleavage stimulus emanating from the mitotic apparatus (Rappaport, 1986). Filamentous actin (F-actin), myosins and some other proteins are incorporated into the cleavage furrow and the interaction between F-actin and myosins is thought to generate contractile forces (Mabuchi,1986; Satterwhite and Pollard, 1992; Fishkind and Wang, 1995). Recently, phosphorylation and dephosphorylation of certain proteins were shown to be associated with cytokinesis (Larochelle and Epel, 1993; Yamakita *et al.*, 1994; Sekimata *et al.*, 1996; Walker *et al.*, 1997). Furthermore, the Rho family of small GTP-binding proteins (Kishi *et al.*, 1993; Mabuchi *et al.*, 1993; Dutartre *et al.*, 1996; Drechsel *et al.*, 1996) and downstream components of Rho (Kosako *et al.*, 1997) have also been implicated in the process of cytokinesis. Despite these advances, regulation of cytokinesis at the molecular level is largely unknown.

Genetic studies in yeast have contributed a great deal to our understanding of nuclear division cycle. This approach may also provide a useful means to investigate another aspect of the cell cycle, cytokinesis. A number of mutations resulting in defective cytokinesis have been identified not only in *Saccharomyces cerevisiae* (Hartwell, 1971) and *Schizosaccharomyces pombe* (Fankhauser and Simanis, 1994; Chang and Nurse, 1996) but also in *Drosophila* (Miller and Kiehart, 1995), *Dictyostelium* (Larochelle *et al.*, 1996; Adachi *et al.*, 1997), *Tetrahymena* (Watanabe *et al.*, 1990) and *Aspergillus nidulans* (Harris *et al.*, 1994). Some of the genes identified through these mutations appear to be structurally and functionally conserved among eukaryotes. The isolation of such mutants and their use in combination with biochemical studies should allow elucidation of the molecular mechanism of cytokinesis.

A mutation in the *abnormal furrow* (*af*) gene of *Xenopus laevis* was previously described (Kubota *et al.*, 1991; Asada-Kubota and Kubota, 1991). *af* mutant females lay eggs (*af* eggs) which cannot cleave but undergo nuclear division. The cleavage furrows appear at the correct place and time while they do not contract. Elimination of the second polar body is also inhibited. Other contractile events which propagate through the surface of the eggs, such as activation waves (Hara and Tydeman, 1979), an activation contraction (Stewart-Savage and Grey, 1982), post-fertilization waves (Hara *et al.*, 1977) and surface contraction waves (Hara, 1971) are unaffected (Asada-Kubota and Kubota,1991; Kubota unpublished observation). Thus, mutation in *af* gene causes a specific defect in cytokinesis among the contractile events occurring on the cell surface. Cytoplasmic transfer from fertilized wild-type eggs into fertilized *af* eggs provokes contraction of the cleav-

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age furrow. In the present study, a factor responsible for this activity was extracted from electrically activated wild-type eggs and assayed by microinjection into fertilized *af* eggs. The factor appears to be a high molecular weight protein complex which reorganize cytoskeletal elements within the cleavage furrow and promote contraction during cytokinesis.

MATERIALS AND METHODS

Preparation of eggs

Eggs were obtained from females by injecting human chorionic gonadotropin (Teikoku Zoki Ltd., Tokyo). For artificial fertilization, a sperm suspension from freshly minced testes in MSS (Steinberg's solution buffered to pH 7.5 with Hepes in place of Tris) was added to the eggs. Eggs were dejellied with 1.5% sodium thioglycollate in MSS (pH 9.5).

Assay of contraction promoting activity

Groups of 10 *af* eggs were microinjected with 40 nl of samples. Injected eggs were cultured in MSS at 22–23°C and eggs that showed contraction of the first cleavage furrow were scored. A unit of activity was defined as the amount that caused contraction at first and second cleavages in 50% of injected *af* eggs.

Preparation of extracts

Dejellied eggs were activated by a pulse of 12 V of alternating current and then incubated in MSS. Extracts were made 30 min following activation except for the experiment shown in Fig.3B. Eggs were washed three times with cold extraction buffer (0.1 M KCl, 0.5 mM DTT, 0.1 mM PMSF, 1 μg/ml leupeptin and 20 mM Tris, pH 8.0) and packed by centrifugation at 600 × g at 4°C for 30 sec. After excess buffer had been withdrawn, 0.4 volume of extraction buffer was added and eggs were homogenized by pipetting. The homogenates were centrifuged at 10,000 × g at 4°C for 15 min. The cytoplasmic layer between lipid cap and yolk pellet was collected and supplemented with PMSF and leupeptin at 0.1 mM and 1 μg/ml, respectively, and stored at –80°C.

Enzymatic hydrolysis

Extracts for enzyme treatments were obtained as described above except that the buffer used for RNase treatment was composed of 25 mM KCl, 0.1 mM PMSF, 1 μg/ml leupeptin and 25 mM Tris, pH 7.5 and that for subtilisin treatment was composed of 25 mM KCl, 1000 U/ml ribonuclease inhibitor (TOYOBO Co., Ltd., Japan) and 25 mM Tris, pH 8.0. Polyvinyl beads-bound subtilisin and RNase A (Mo Bi Tec GmbH, Gottingen, Gemary) were previously equilibrated with the corresponding buffer. Two hundred-microliters of extract were mixed with bead-bound subtilisin (0.2 U) or RNase A (1 U) and incubated for 20 min at 23°C with occasional agitation. For control experiment, boiled subtilisin-beads were used. Samples were centrifuged at 10,000 × g for 5 min at 4°C and supernatants were used for the assay.

Fractionation of extracts

For fractionation of cytoplasmic extracts, activated eggs were washed with extraction buffer supplemented with 1 mM $MgCl_2$ and 1 mM EGTA, and packed at 600 × g at 4°C for 30 sec. After excess buffer was removed, eggs were directly crushed by centrifugation at 10,000 × g at 4°C for 15 min. The cytoplasmic fractions were diluted 8 times with buffer A (1 mM EGTA, 0.5 mM DTT, 0.1 mM PMSF and 20 mM Tris, pH 8.0) and cytochalasin B was added to 10 μg/ml, and then the extracts were centrifuged at 105,000 × g at 4°C for 60 min. Supernatant and membrane fractions were dialyzed against buffer A containing 0.1 M KCl and concentrated with Ultrafree-15 (Millipore, Bedford, MA, USA) to obtain the original volume of the extracts. Precipitates were suspended in buffer A containing 0.1 M KCl to obtain the original volume of the extract and were dialyzed against the same buffer.

In order to solubilize the activity, the pellets were suspended in buffer A plus one of the following additions: 1 M NaCl; 0.1 M KI; 0.3 M KI; 0.1 M Na_2CO_3 (pH 10.5); or 1% CHAPS. The samples were incubated at 4°C for 30 min and then centrifuged at 105,000 × g at 4°C for 60 min. Supernatants were dialyzed against buffer A containing 0.1 M KCl and concentrated as above. Precipitates were suspended in buffer A containing 0.1 M KCl to give the original volume of the extract and dialyzed against the same buffer.

Sucrose density gradient centrifugation and gel filtration

For sucrose density gradient centrifugation, the KI-solubilized material from 2 ml of extract was concentrated using Ultrafree-15 to give a final volume of 500 μl and then loaded onto a 10 ml of 5–30% sucrose gradient made with buffer A containing 0.1 M KCl and 1 mM CHAPS. Protein standards were loaded onto separate, parallel gradients. They were centrifuged in a HITACHI RPS40T rotor at 28,000 rpm for 14.5 hr at 4°C. One-milliliter fractions were diluted 2 times with buffer A containing 0.1 M KCl and 1 mM CHAPS and then concentrated with Ultrafree CL-PLUS (Millipore, Bedford, MA, USA). The same fractions from two separate preparations were combined and concentrated to 50 μl for assay. The protein concentration of each fraction was determined by the protein assay kit (Bio-Rad, Hercules, CA, USA) using bovine serum albumin as a standard.

For gel filtration, KI-solubilized material from 4 ml of extract was dialyzed and concentrated as described, and then loaded onto a Superose 6 column (Pharmacia Biotech AB, Sweden) equilibrated with buffer A plus 0.1 M KCl and 1 mM CHAPS. One-milliliter fractions were collected and concentrated as above.

Cytology

Fertilization envelopes were removed from the eggs with forceps. To make a wound, a portion of cortex was removed with forceps. Dividing or wounded eggs were fixed in 10% formaldehyde in fixation buffer (5 mM EGTA, 1 mM $MgCl_2$ and 80 mM Pipes, pH 6.8) for 12–16 hr at room temperature. Eggs were washed extensively with phosphate buffered saline (PBS), and then treated with 0.1 M NH_4Cl in PBS for 60 min. Eggs were stained with 5 μg/ml FITC-WGA (Vector Lab. Inc., Burlingame, CA, USA) in PBS for 30 min. After washing with PBS, eggs were permeabilized with 0.5% Tween 20 in PBS for 30 min. Eggs were then stained with 10 U/ml rhodamine-phalloidin (Molecular Probes, Inc., Eugene, OR, USA) in PBS for 30 to 60 min and examined under an epifluorescence microscope.

RESULTS

Extraction and characterization of the factor which promotes contraction of the cleavage furrow in *af* eggs

The appearance of *af* eggs at first cleavage is shown in Fig. 1A. An unpigmented narrow band runs along the surface of the egg. This band resides in the cleavage plane and marks the position of the cleavage furrow, so this band will be referred to as the cleavage furrow. It does not contract and ingression of the cleavage furrow does not occur. Previous report showed that transfer of cytoplasm from fertilized wild-type eggs into fertilized *af* eggs provoked partial contraction of the cleavage furrow in the recipient *af* eggs (Kubota *et al.*,1991). For further characterization of this activity, a cytoplasmic extract was made from unfertilized wild-type eggs. Before extraction, eggs were activated with an electric shock to inactivate cytostatic factor which would otherwise arrest cleavage of injected eggs at metaphase (Meyerhof and Masui, 1979). Cytoplasmic

extracts from these eggs, when injected into fertilized *af* eggs, caused contraction of the cleavage furrow (Fig. 1B). Contraction along the unpigmented band was recognized as the appearance of many folds. The surface of the egg ingressed as contraction proceeded. Thus, the activity responsible for contraction was extractable from artificially activated wild-type eggs. The *af* eggs rescued in this way, however, could not complete cytokinesis.

When cytoplasmic extracts were diluted with the same extraction buffer and injected at a constant volume, increasing dilution of the extracts resulted in less frequent induction of contraction at the first cleavage (Fig. 2). The extent of contraction decreased as dilution increased (data not shown). The frequency of contraction also decreased when the injected volume was reduced (Fig. 2). These results indicate a dose dependent response of contraction in *af* eggs and suggest that contraction promoting activity could be assayed by measuring the frequency at which contractions are promoted in injected *af* eggs.

To determine which molecule exerts the activity, extracts were treated with polyvinyl beads-bound subtilisin or RNase A (Table 1). Boiled subtilisin-beads were used for control experiments. Contraction promoting activity in the extracts was significantly reduced by exposure to subtilisin-beads, on the other hand, there was virtually no loss of activity following treatment with RNase A-beads. Thus, contraction promoting activity is associated with the presence of a protein but not with the presence of RNA.

Next, the effects of divalent cations and phosphatase inhibitors on the activity were tested. As shown in Table 2, different concentrations of $MgCl_2$, EDTA, EGTA or phosphatase inhibitors was added to the extraction medium and the activity was assayed in each extract. Ca^{2+} was omitted because extracts prepared with a buffer containing milimolar levels of Ca^{2+} caused degeneration of injected eggs. Activity was found to be reduced by the presence of 20 mM EDTA in the extraction buffer. Lower concentrations of EDTA had no effect on the activity. Phosphatase inhibitors neither increased nor decreased the activity.

To examine whether the activity changes with the cell cycle, extracts were made at different times after activation by electric shock. As shown in Fig. 3A, the activity did not change during the first cell cycle.

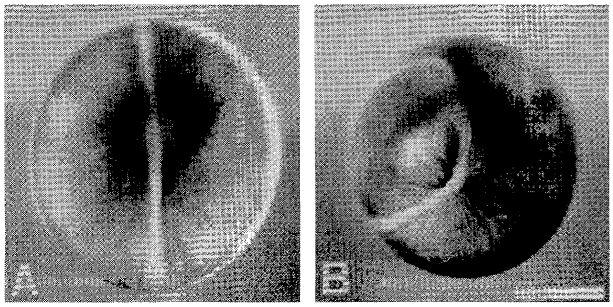

Fig. 1. Appearance of an *af* egg and a rescued *af* egg at first cleavage. (**A**) A control, untreated *af* egg. A narrow band of unpigmented surface in the animal hemisphere represents the cleavage furrow. (**B**) An *af* egg injected with cytoplasmic extract from wild-type eggs. Contraction of the unpigmented thin band was induced and many folds were visible in the contracting region. Bar, 0.5 mm.

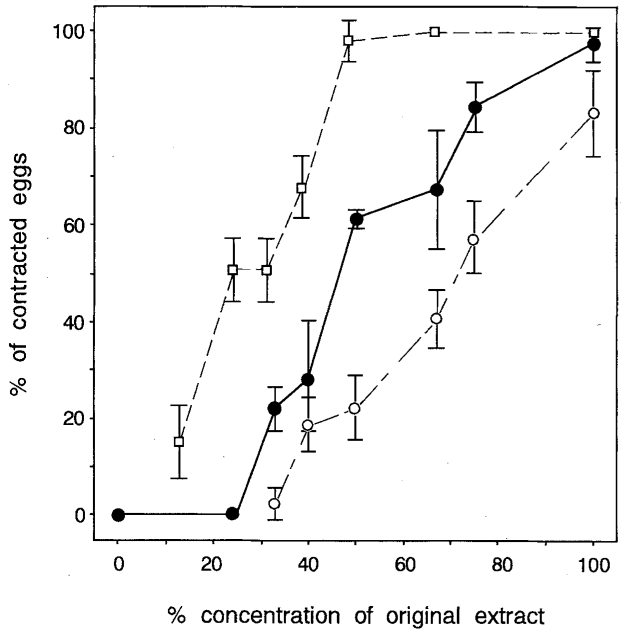

Fig. 2. Dependence of contraction frequency on the concentration and injected volume of cytoplasmic extracts. Fertilized *af* eggs were injected with 20 nl (○), 40 nl (●) or 80 nl (□) of extract diluted to the indicated concentrations with extraction buffer. Ordinate, percentage of *af* eggs which showed contraction at first cleavage. Abscissa, concentration in percentage of the original extracts. Values represent mean values ± SE of 3 experiments.

Table 1. Effects of enzyme treatments on the contraction-promoting activity

treatment	contracted eggs/injected eggs	contracted (%)
control	29/30	97
subtilisin	5/30	17
RNase A	25/30	83

Table 2. Effects of a divalent cation, chelators and phosphatase inhibitors on the contraction-promoting activity

add to the extraction buffer		contracted eggs/ injected eggs	contracted (%)
no addition		25/30	83
$MgCl_2$	2 mM	29/30	97
	10 mM	29/30	97
	20 mM	23/30	93
EDTA	2 mM	28/30	93
	10 mM	28/29	97
	20 mM	6/30	20
EGTA	2 mM	30/30	100
	10 mM	27/28	96
	20 mM	24/30	80
phosphatase inhibitors*		26/28	93

* 80 mM sodium β-glycerophosphate, 0.1 M NaF and 20 mM sodium pyrophosphate.

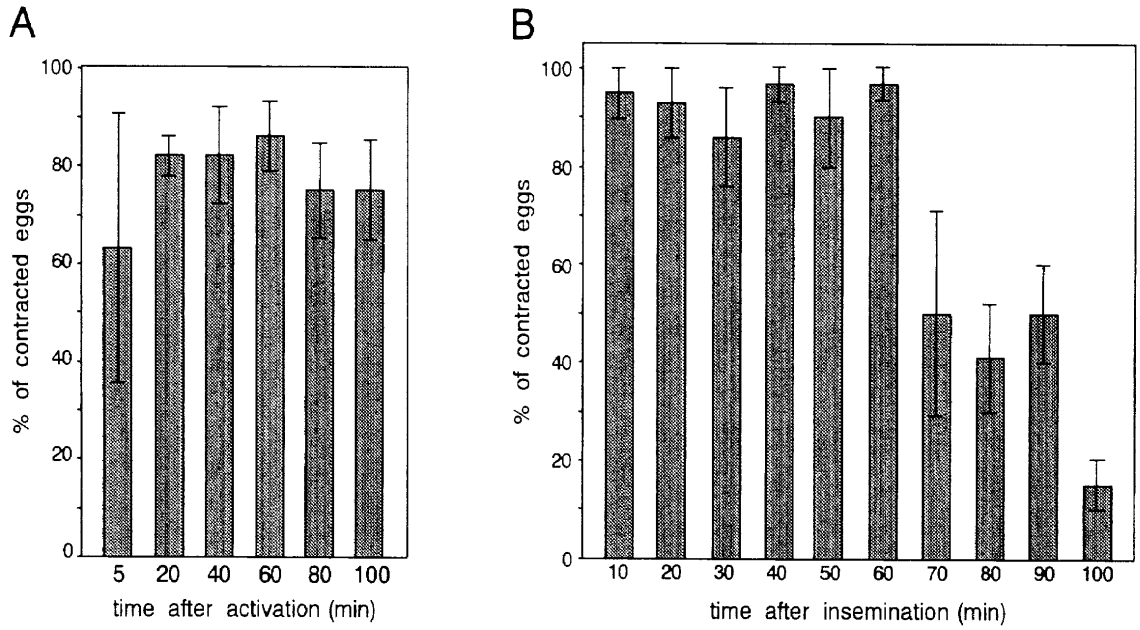

Fig. 3. (**A**) Presence of the activity during the first cell division. Extracts were made at the indicated times after activation with an electric shock. (**B**) Responsiveness of *af* eggs to the activity during the first cell division. Identical extracts were injected at 10 min intervals following insemination. The onset of the first cleavage was 80-85 min in these experiments (23°C). Bars indicate mean values ± SE of 3 experiments.

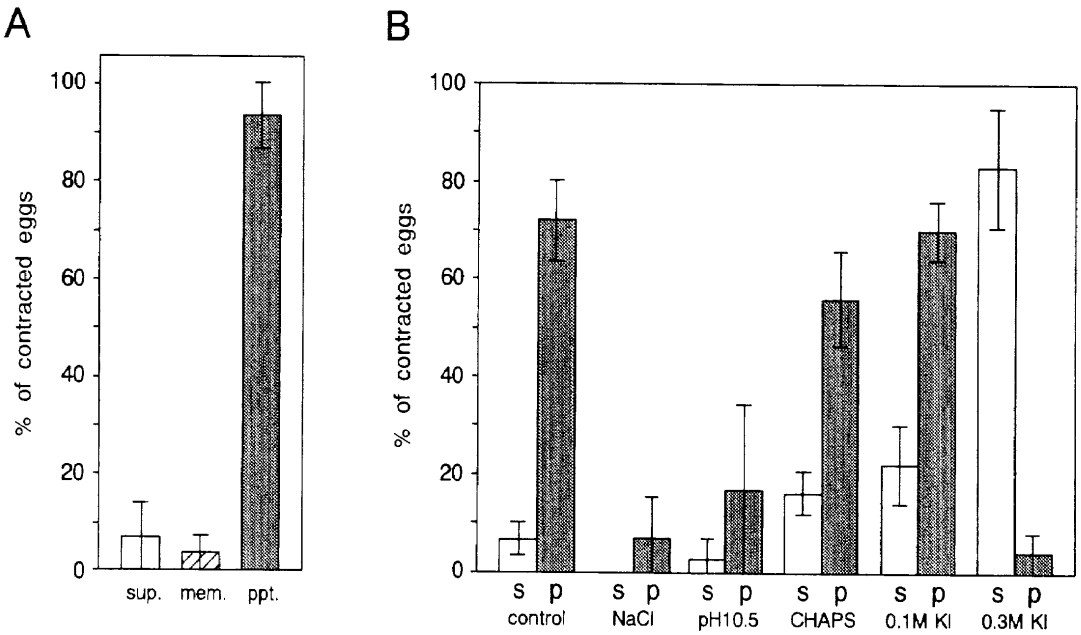

Fig. 4. (**A**) Subcellular localization of the activity. Extracts were separated into supernatant (sup.), membrane (mem.) and precipitate (ppt.) fractions by centrifugation at 105,000 × g for 60 min. Each fraction was restored to its original volume and assayed. (**B**) Precipitates were resuspended and extracted with 1 M NaCl, 0.1 M Na_2CO_3 (pH 10.5), 1 % CHAPS, 0.1 M KI or 0.3 M KI, then centrifuged to separate solubilized materials (s) and precipitates (p). Each fraction was restored to its original volume of the extract and assayed. Bars indicate mean values ± SE of 3 experiments.

To test for the possibility that the responsiveness of *af* eggs to the activity could change during the cell cycle, the same extract was injected into *af* eggs at various times after insemination and the contraction of the first cleavage furrow was assessed (Fig. 3B). The onset of the first cleavage was 80–85 min after insemination under these experimental conditions (23°C). During the first 60 min after insemination, almost all the *af* eggs showed contraction at the first cleavage. However, injection at 70 min after insemination and thereafter resulted in a reduced frequency of contraction at the first cleavage. This was not due to a decline in the activity during these experiments because the activity of the factor in the crude extracts was stable for at least several days at 0–4°C. For reproducible detection of the activity, injection was always finished before 60 min following insemination, that is, before 0.7 in normalized time (NT) where 0.0 was defined as the

moment of insemination and 1.0 as the time of onset of the first cleavage (Kirschner *et al.*, 1980).

The subcellular localization of the activity was examined by high speed centrifugation at 105,000 × g for 60 min. After centrifugation, extracts were separated into clear soluble fraction, membrane fraction and golden precipitate. The majority of the activity was recovered in the precipitate (Fig. 4A). In order to solubilize the activity, the precipitates were incubated under different extraction conditions and then centrifuged at 105,000 × g to separate the solubilized and unsolubilized material. As shown in Fig. 4B, only 0.3 M KI was effective in solubilizing the activity in the precipitate. Treatment with 0.1 M KI or 1% CHAPS had little effect on solubilization. The activity could be recovered neither in the supernatant nor in the precipitate after treatment with 1 M NaCl or 0.1 M Na_2CO_3 (pH 10.5). The activity could not be restored by mixing of supernatant and precipitate fractions obtained by either of these treatments.

The complexity of the solubilized activity was examined by sucrose gradient centrifugation and gel filtration. KI-solubilized materials were fractionated on a 5–30% sucrose gradient. The activity sedimented at a position corresponding to approximately 19S (Fig. 5A). For gel filtration, KI-solubilized materials were chromatographed on a Superose 6 column. The activity migrated with a molecular mass of higher than 670 kDa (Fig. 5B).

The factor promotes accumulation of F-actin and WGA-binding sites in the cleavage furrow

In order to examine the distribution of F-actin in *af* and rescued *af* eggs, eggs were fixed during the first cleavage and stained with rhodamine-phalloidin. FITC-WGA was also used because it binds to the bottom of the cleavage furrow (Tencer, 1978).

Most eggs showed less obvious accumulation of fluorescence at the cleavage furrow because the autofluorescence of yolk that emanated from the less pigmented surface largely interfered with that of rhodamine-phalloidin. In wild-type eggs, rhodamine-phalloidin stained the center of the cleavage furrow, the entire cortex and the border between the preexisting and newly inserted membrane in the animal hemisphere (Fig. 6A). A similar staining pattern was obtained by FITC-WGA staining (Fig. 6D), while some of the WGA-binding sites on the bottom of the furrow were lost during permeabilization. In *af* eggs, both rhodamine-phalloidin and FITC-WGA were evenly distributed throughout the cortex and the surface, respectively (Fig. 6B, E). Rescued *af* eggs were also stained evenly, however, accumulation of rhodamine-phalloidin and FITC-WGA was now observed in the contracting region to various degrees, probably reflecting the extent of furrowing at fixation (Fig. 6C, F). Intense staining for both rhodamine-phalloidin and FITC-WGA were observed at the boundary between the preexisting and unpigmented surface at the contracting region. However, concentration of both fluorescence beyond the contracting region could hardly be seen in rescued *af* eggs.

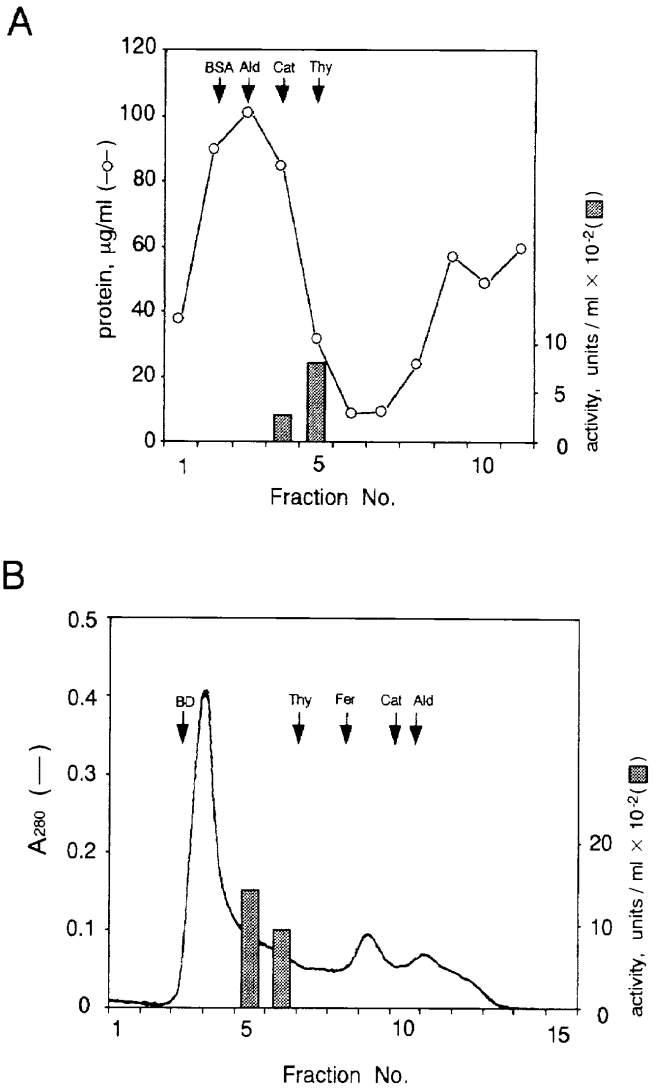

Fig. 5. Fractionation of KI-solubilized proteins. (**A**) KI-solubilized proteins were separated on 5–30 % sucrose gradient. The markers used are bovine serum albumin (BSA, 4.5 S), aldolase (Ald, 7.3 S), catalase (Cat, 11 S) and thyroglobulin (Thy, 19 S). (**B**) Elution profile of KI-solubilized proteins from Superose 6 gel filtration chromatography. The markers are blue dextran (BD, 2000 kDa), thyroglobulin (Thy, 669 kDa), ferritin (Fer, 440 kDa), catalase (Cat, 232 kDa) and aldolase (Ald, 158 kDa).

Cortical wound healing in *af* eggs

When eggs are injured on their cortex, microfilamentous bundles are formed at the edge of the lesion (Bluemink, 1972; Geuskens and Tencer, 1979b) and close the wound. To examine the ability of *af* eggs to form and contract an F-actin ring , a portion of animal cortex was dissected with forceps to make a wound and then stained with rhodamine-phalloidin and FITC-WGA. Closure of the wound took place both in wild-type and *af* eggs. Figure 7A-C show a sequence of cortical wound healing in an *af* egg. A basal level of cortical F-actin and surface WGA-binding sites were visible at the injury soon after dissection (Fig. 7D, G). As closure proceeded, rings of intense fluorescence corresponding to those of both rhodamine-phalloidin and FITC-WGA appeared around the wound and gradually became smaller in diameter (Fig. 7E, H). When the

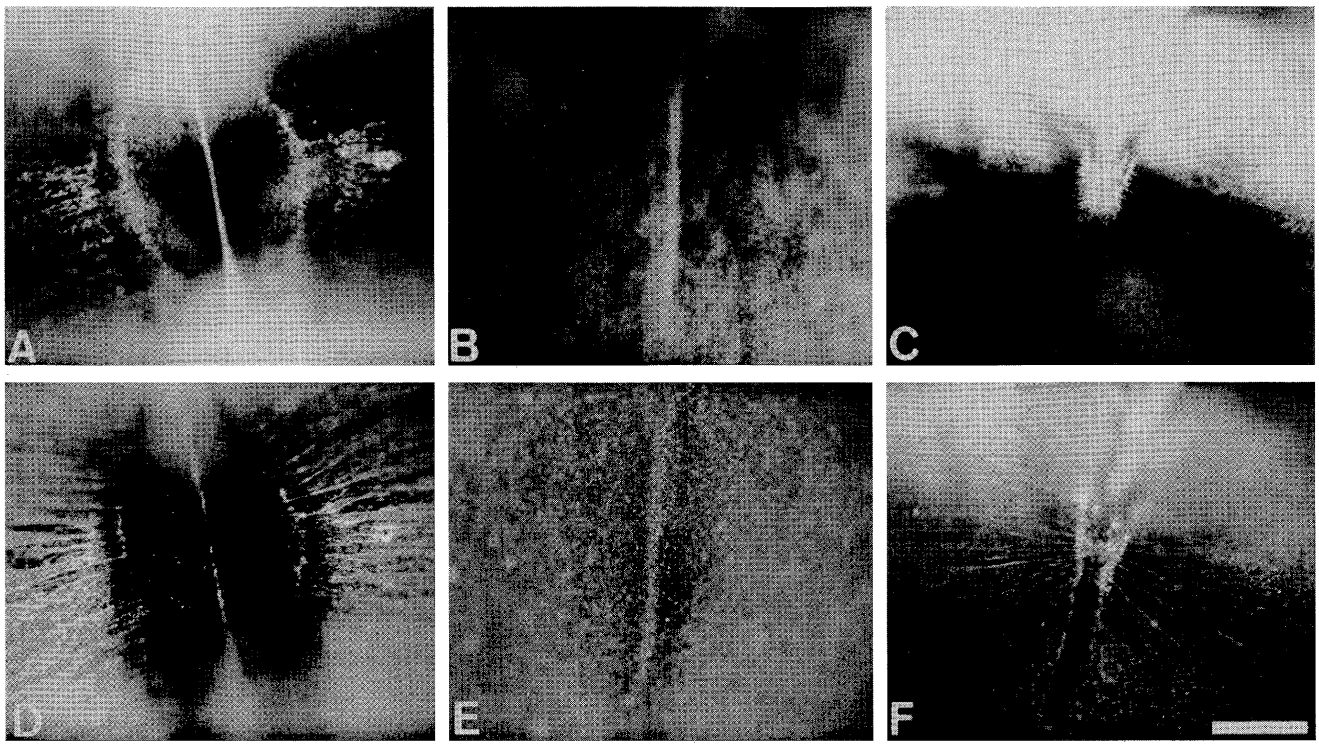

Fig. 6. Rhodamine-phalloidin and FITC-WGA staining of wild-type, *af* and partially rescued eggs. Eggs were inseminated at the same time and then fertilization envelopes were removed. A fraction of *af* eggs were injected with extract from wild-type eggs to promote contraction of the cleavage furrow. Eggs were fixed when the wild-type eggs had proceeded half-way into cytokinesis and were then stained with rhodamine-phalloidin and FITC-WGA. (**A**, **D**) Cleavage furrow of a wild-type egg. Rhodamine-phalloidin and FITC-WGA were enriched in the center of the cleavage furrow and the boundary between the preexisting and newly inserted membrane. (**B**, **E**) Unpigmented band region of an *af* egg. Both rhodamine-phalloidin and FITC-WGA were evenly distributed. Fluorescence in the cleavage furrow and in the less-pigmented surface is mainly due to the autofluorescence of yolk. (**C**, **F**) Cleavage furrow of a partially rescued *af* egg. Both rhodamine-phalloidin and FITC-WGA accumulated at the contracting region of the cleavage furrow. Fluorescence in non-contracting region of the cleavage furrow and in the less-pigmented surface is mainly due to the autofluorescence of yolk. Bar, 0.2 mm.

wound was closed, small rings of rhodamine-phalloidin and FITC-WGA fluorescence were observed to surround the scar (Fig. 7F, I). These results show that the machinery required for the formation and contraction of the F-actin ring in wound healing is functional in the cortex of *af* eggs suggesting a clear distinction between the activity required for cytokinesis and wound healing.

DISCUSSION

Biochemical characteristics of contraction promoting activity

In a previous study, Kubota *et al.* (1991) reported that two proteins with molecular masses of about 78 kDa and 38 kDa were specifically lost in *af* eggs. N-terminal amino acid sequence of the 78 kDa protein is highly homologous (85%) to enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase which is involved in fatty acid β-oxidation (T. Ueno, unpublished data). However, a monoclonal antibody raised against the 78 kDa protein recognized a couple of protein bands and revealed that some wild-type frogs lay eggs with the same blotting pattern as *af* eggs (Ueno, unpublished data). Thus, the 78 kDa protein appears to be an isozyme. Furthermore, there are significant variations in the position of the protein spots around the 38 kDa protein prepared from different frogs (Kubota, unpublished data). From these observations it was concluded that the differences in the levels of 78 kDa and 38 kDa proteins do not correlate with the *af* mutation. At present, the most reliable method to characterize the molecule deficient in *af* eggs is to inject biochemically separated fractions from wild-type egg extract into *af* eggs and to monitor contraction of the cleavage furrow in the injected eggs. In the present work, this approach allowed the isolation and characterization of a factor that promotes contraction of the cleavage furrow.

Most of the activity was recovered in the particulate fraction after high speed centrifugation. Fractionation of the KI-solubilized proteins by sucrose density gradient centrifugation and gel filtration suggests that the factor is a high molecular mass complex. This protein complex, which may contain the *af* gene product, is designated as cleavage furrow contraction promoting factor (CPF).

CPF activity could be extracted at any time during the first cell cycle. This indicates that changes in the amount of CPF does not occur during the cell cycle. The responsiveness of *af* eggs to CPF fell after 0.7 NT. The reason why the *af* eggs do not respond to CPF is not clear. However, it is possible that CPF is extracted in an inactive form and that

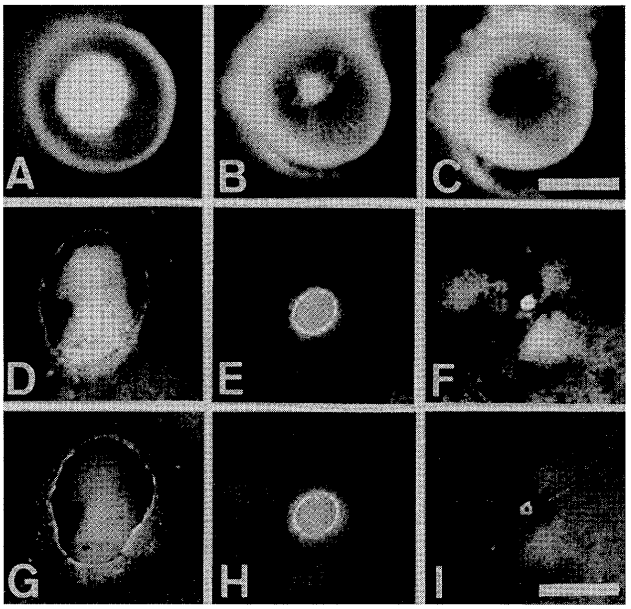

Fig. 7. (**A**–**C**) Cortical wound healing of an *af* egg. (**A**) 2, (**B**) 8.5 and (**C**) 21.5 min after dissection of the cortex in the animal pole region. Bar, 0.5 mm. (**D**–**I**) Distribution of F-actin and WGA-binding sites during cortical wound healing of *af* eggs. Wounded *af* eggs were fixed at different time points and stained with rhodamine-phalloidin (**D**–**F**) and FITC-WGA (**G**–**I**). Soon after dissection of the cortex (**D**, **G**), fluorescence of rhodamine-phalloidin and FITC-WGA became visible but were not enriched for around the wounded cortex. During closure of the wound (**E**, **H**), both rhodamine-phalloidin and FITC-WGA accumulated around the injury. (**F**, **I**) A closed wound. A small scar was stained by rhodamine-phalloidin and FITC-WGA. Bar, 0.3 mm.

there is not enough time to activate CPF injected after 0.7 NT. Alternatively, as CPF is a large molecule and is associated with the particulate fraction in the extract, CPF could not diffuse to the appropriate place in the egg when the extract is injected after 0.7 NT.

F-actin bundle formation in *af* eggs

F-actin co-localized with WGA-binding sites in dividing and wounded *Xenopus* eggs (Tencer, 1978; Geuskens and Tencer, 1979a, b; this work). WGA-binding sites may have some relation to F-actin anchoring points on the cell membrane, as suggested in sea urchin eggs (Mabuchi, 1994). In *af* eggs, wounds made on their cortices closed completely and accumulation of both F-actin and WGA-binding sites around the wounds was associated with these processes. On the other hand, neither F-actin nor WGA-binding sites accumulate to the cleavage furrow of *af* eggs. These results suggest that the mechanisms of F-actin bundle formation and contraction in cytokinesis are different from those of cortical wound healing. This conclusion argues against the prediction that F-actin bundles in cortical wound healing are structurally and functionally similar to those of cytokinesis (Merriam and Christensen, 1983). When furrowing was induced in *af* eggs, both F-actin and WGA-binding sites concentrated in the contracting region. The *af* gene product seems to be specifically required for cell division and reorganize F-actin and WGA-binding sites within the cleavage furrow in response to the cleavage stimulus from the mitotic apparatus. Incomplete accumulation of both components along the cleavage furrow and failure in cytokinesis of rescued *af* eggs may be due to the small amount of functional CPF because of restricted volume for injection.

Role of CPF and other factors in the cleavage of amphibian eggs

In amphibian eggs, some factors have been implicated in the establishment of the cleavage furrow. Furrow inducing cytoplasm (FIC) underlies the cortex only along the cleavage plane and induces a furrow-like dent when transplanted into the cortex outside the cleavage plane (Sawai,1972,1983). In response to FIC, the competent cortex forms a furrow or furrow-like dent (Sawai, 1972, 1983). This cortical area roughly overlaps with surface contraction wave (SCW)-2 and both of them shift as rings from the animal hemisphere towards the vegetal hemisphere (Sawai, 1972, 1982, 1983; Yoneda *et al.*, 1982).

Previous reports showed that SCWs are detected normally, and that determination of the cleavage planes is not affected in *af* eggs (Asada-Kubota and Kubota, 1991; Kubota *et al.*, 1991). These observations suggest that part of the function of FIC and the response of the cortical factor are also normal in *af* eggs. CPF may be a component of FIC and promote contraction of the cleavage furrow or furrow-like dent after their formation sites are determined by other component (s) involved in FIC. It is worth investigating whether CPF activity is restricted to the cleavage furrow region during cytokinesis.

The appearance and behavior of *af* mutants are normal, and *af* eggs can be used repeatedly. Therefore, the *af* gene product is not required for cell division in adult tissues and oogonia, but functions in oocytes and early embryos. To understand the mechanisms of cleavage furrow formation and differences between cytokinesis in embryonic and somatic cells, it will now be necessary to purify CPF and investigate its function and regulation during the cell cycle.

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