

## Bovine Liver Chromatin Fraction Contains Actin Polymerization Activity Inducing Micronuclei Formation when Injected into Prometaphase Cultured Cells

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**ABSTRACT.** We previously reported that exogenous histone H1, when injected into mitotic cells, disrupts the synchronous progression of mitotic events by delaying chromosome decondensation. This strategy was utilized to determine whether any other interphase proteins are also able to disrupt normal mitotic processes, when introduced into the mitotic phase. We found that a chromatin subfraction from bovine liver nuclei induced postmitotic micronuclei formation in a dose-dependent manner when injected into the prometaphase of rat kangaroo kidney epithelial (PtK<sub>2</sub>) cells. Close observation showed that, in the case of injected mitotic cells, the mitotic spindles were disrupted, chromosomes became scattered throughout the cytoplasm, and actin filaments were organized ectopically. In addition, when the fraction was injected into interphase cells, extra actin filaments were formed and microtubule organization was affected. In order to determine whether the micronuclei formation resulted from the ectopic formation of actin filaments, we examined the effect of the actin polymerization inhibitor, cytochalasin D. The results showed that the drug inhibited micronuclei formation. From these findings, we concluded that this chromatin subfraction contains actin polymerization activity, thus causing the disruption of mitotic spindles.

**Key words:** cell cycle/chromosome segregation/actin filament polymerization/mitotic spindle

The cell nucleus of higher eukaryotes undergoes dramatic structural and functional changes during the cell cycle, with such significant cell functions as replication of DNA, transcription and maturation of mRNA occurring in interphase. In this phase, chromosomes decondense and are surrounded by a nuclear envelope that is perforated by nuclear pores, through which nucleo-cytoplasmic transport occurs.

In the mitotic phase, the higher order structures of chromosomes are changed into compact ones that are suitable for correct segregation. The interphase microtubules (MTs) disassemble to form a mitotic spindle (MS), which is a bipolar structure composed of microtubules and their associated proteins. The sister chromatids, the centromeres of which bind to the MS extending from bipolar microtubule organizing centers (MTOCs), segregate from one another. The nuclear envelope, including nuclear pore complexes,

disassembles and some of its components are dispersed throughout the cytoplasm. At this phase, no DNA and little RNA are synthesized. At the end of mitosis, chromosomes decondense, the nuclear envelope reassembles to form two daughter nuclei, and the MS dissociates and is reassembled into interphase MTs.

We previously demonstrated that chromosome condensation is prolonged, sister chromatid separation is inhibited and the synchronous progression of mitotic events is disrupted when the intracellular level of histone H1 is elevated at mitosis by the injection of exogenous H1 (Matsuoka *et al.*, 1994). In this study, we report that a chromatin subfraction derived from bovine liver nuclei is capable of inducing micronuclei formation by disrupting the MS. Furthermore, the findings herein show that the fraction that induces ectopic actin filament formation that might also cause the disorganization of the MS. The activity of the fraction was found to be heat- and trypsin-sensitive, suggesting that the activity is composed of proteinaceous factors.

It is known that actin filament assembly and turnover are controlled by numerous actin polymerization regulators (Bear *et al.*, 2001; Ayscough, 1998). While actin filaments are involved in a number of cytoplasmic events such as cell

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Abbreviations: H1fr, a histone H1-enriched fraction; H2fr, a histone H2A+H2B-enriched fraction; H3fr, a histone H3+H4-enriched fraction; MS, mitotic spindle; MT, microtubule; MTOC, microtubule organizing center.

motility and cytokinesis, recent studies have provided evidence for important roles of actin in nuclear processes such as chromatin remodeling and splicing (Rando *et al.*, 2000). Consistent with these findings, several regulators of actin polymerization have been shown to be localized in the nucleus or are translocated into the nucleus in a signal-regulated manner (Rando *et al.*, 2000). It was found that some of these nuclear regulators actually have actin polymerization activity *in vitro*; however, it has not been demonstrated whether or how they act in living cells. The micronuclei-inducing activity identified in this study may consist of member(s) of the family of nuclear actin polymerization regulators and its activity may be regulated in a cell cycle-dependent manner.

## Materials and Methods

### Antibodies and reagents

Polyclonal anti-tubulin antibodies (rabbit) were purchased from ICN Biomedicals (Costa Mesa, CA). Rhodamine-labeled phalloidin was purchased from Molecular Probes (Eugene, OR). Cytochalasin D was purchased from Sigma (St. Louis, MO).

### Cell culture

PtK<sub>2</sub> (rat kangaroo kidney epithelium) cells were cultured as monolayers at 37°C in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS) and non-essential amino acid solution under 5% CO<sub>2</sub> in air. Since these cells maintain a flat morphology even at mitosis and have only a few chromosomes, the state of their chromosomes after microinjection can easily be observed. In order to depolymerize the actin filaments, cytochalasin D (1 µg/ml) was added to the culture medium. Although under normal culture conditions, micronuclei-containing cells are sometimes observed in this cell line, the frequency is less than 1%.

### Partial purification of micronuclei-inducing activity

No protease inhibitors were used, because they might inhibit the proteolysis of certain proteins which are required for mitotic progression such as metaphase-anaphase transition when the samples were injected into mitotic cells. Nuclei were prepared from adult bovine livers as described previously (Yamaizumi *et al.*, 1978). We fractionated chromatin proteins from the nuclei, based on the methodology used to purify histone pairs (Simon and Felsenfeld, 1979). A nuclear fraction (H2fr.) that largely contains histone pairs H2A and H2B was prepared from 12 ml of packed nuclei. H2fr. was dialyzed against 50 mM NaCl in 20 mM potassium phosphate (pH 6.7) and then applied to a 1.5×1.2 cm CM-cellulofine column. The column was washed with 200 mM NaCl in 20 mM potassium phosphate (pH 6.7) and eluted with 1 M NaCl in 20 mM potassium phosphate (pH 6.7). The eluate was dialyzed against 400 mM KCl, 20 mM Tris-HCl (pH 8.6), and then loaded on to a 1.5×7.8 cm DNA-cellulose column (Sigma; protein amount (mg) / gel bed vol-

ume (ml) was adjusted to 1). We concentrated each fraction to the appropriate concentration level by means of Centricon 30 or Microcon 30 filters. During the concentration, the salt concentration was adjusted to below 500 mM. When the protein concentration of a fraction was below 0.1 mg/ml, bovine serum albumin (BSA) was added (final concentration of 0.1 mg/ml) before the initiation of the concentration procedure.

### Microinjection

PtK<sub>2</sub> cells (5×10<sup>4</sup>) were plated on coverslips with small circles cut with a diamond knife in the form of 35 mm dishes 4 or 5 days before use. Microinjection was carried out into 20–50 prometaphase or interphase PtK<sub>2</sub> cells within the small circles with a Narishige micromanipulator. For the identification of injected cells, each sample was mixed with 1/5 vol. of rhodamine-conjugated or fluorescein isothiocyanate (FITC)-conjugated (when actin filaments were labeled with the rhodamine-phalloidin) affinity purified goat anti-mouse immunoglobulin G (IgG) (Organon Teknika, West Chester, PA). In the case of double labeling experiments using the anti-tubulin antibody and the rhodamine-phalloidin, all the cells in circles were injected. At 3 hr after injection, the cells were fixed with 4% paraformaldehyde in phosphate buffered saline (PBS: 137 mM NaCl, 3 mM KCl, 8 mM Na<sub>2</sub>HPO<sub>4</sub> and KH<sub>2</sub>PO<sub>4</sub>) and stained with Hoechst 33342. Of the injected cells, those that contained more than three nuclei were counted.

### Heat or trypsin treatment

For heat treatment, a H2fr.-containing tube was placed in a boiling water bath for 5 min. For trypsin treatment, H2fr. was exposed to 11.7 U of trypsin for 1 hr at 37°C, followed by 30 µg of soybean trypsin inhibitor. As a control, trypsin was premixed with trypsin inhibitor.

### Immunofluorescence microscopy

Cells were fixed by incubating the coverslips for 10 minutes at room temperature in 4% paraformaldehyde in PBS. After washing with PBS, they were then permeabilized with 0.5% Triton X-100 in PBS for 5 minutes at room temperature. After washing with PBS, they were incubated for 30 minutes at room temperature with PBS containing 0.5% BSA and 2% goat serum to block nonspecific binding sites, and then for 2 hours at room temperature with rabbit anti-tubulin antibodies (1:30 dilution). They were then washed with PBS and incubated for 1 hour at room temperature with FITC-conjugated goat anti-rabbit IgG (Organon Teknika, West Chester, PA). They were then washed again with PBS, incubated for 5 minutes with Hoechst 33342 (1 µg/ml in PBS) and mounted in Mowiol containing triethylenediamine. For the rhodamine-phalloidin labeling of actin filaments, the rhodamine-phalloidin was added to the secondary antibody solution (1:20 dilution).

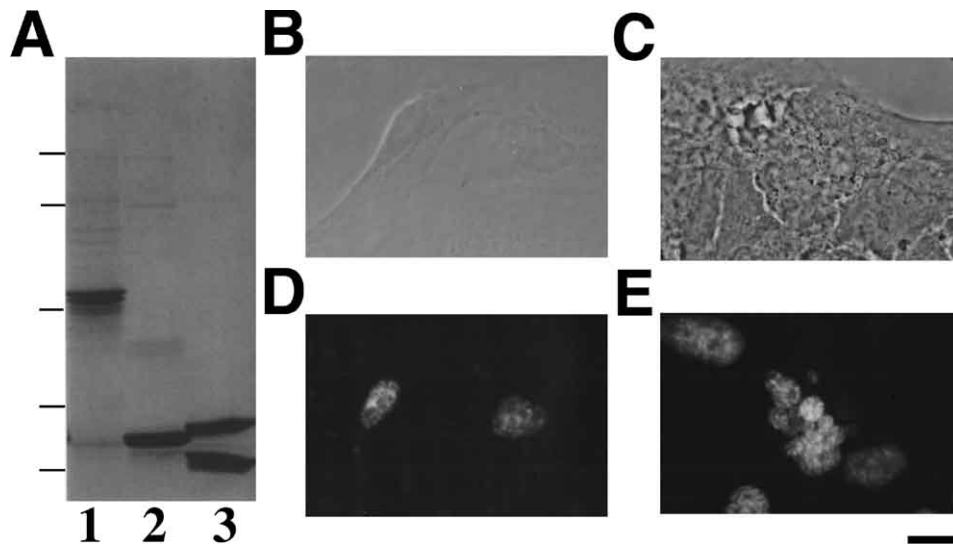
## Results

We previously found that exogenous histone H1 prolonged

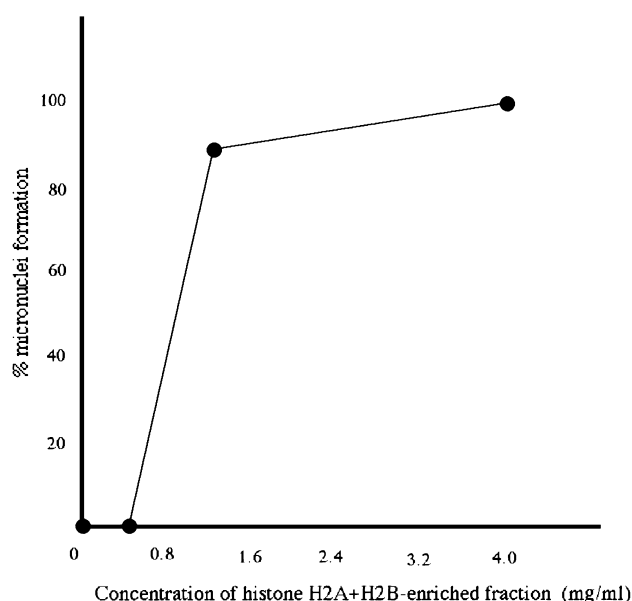
chromosome condensation and disrupted the synchronous progression of mitotic events when injected into mitotic PtK<sub>2</sub> cells (Matsuoka *et al.*, 1994). We also found that the reassembly of the nuclear transport-competent structure was dependent on the existence of chromosomes, but not on chromosome decondensation. Thus, it was shown that the microinjection experiments in living mitotic cells would be of use in the analysis of mitotic events. Since chromatin changes its structure and function during the cell cycle the function of chromatin components may be regulated in a cell cycle-specific manner. Therefore, by using the same *in vivo* system, we attempted to determine whether other interphase chromatin proteins in addition to histone H1 disrupt the normal mitotic processes when introduced into the mitotic phase. To accomplish this, we initially prepared and fractionated the chromatin proteins from bovine liver nuclei. Since nearly all liver cells are arrested at the G<sub>0</sub> phase, they represent a potentially good source of interphase nuclei.

We fractionated the chromatin proteins based on methodology used to purify histone pairs (Simon and Felsenfeld, 1979) (Fig. 1A). This method does not require the addition of DNase which is toxic to cells when injected intracellularly. We obtained three fractions, a histone H1-enriched fraction (H1fr.), a histone H2A+H2B-enriched fraction (H2fr.) and a histone H3+H4-enriched fraction (H3fr.), and injected them via needle into mitotic PtK<sub>2</sub> cells at a concentration of 2 mg/ml. As a control, lysozyme was injected at the same concentration, resulting in the appearance of two daughter

cells usually within 45 min after the injection (Fig. 1B and 1D). When an excess volume was introduced into the mitotic cells, abnormal cell divisions including the production of anucleated cells, were observed (data not shown). We assumed that these phenomena were the results of mechanical damage due to the injections, since the same observation was obtained when the control protein, lysozyme or even only buffer was injected with almost the same frequency (less than 10% of injected cells) as for the sample injection. As shown previously (Matsuoka *et al.*, 1994), H1fr. injection prolonged chromosome condensation and inhibited sister chromatid segregation. When we injected H3fr., we also observed that chromosome condensation was prolonged and sister chromatid segregation did not occur normally (data not shown). Although this phenotype was similar to that induced by H1fr., the effects of H3fr. appeared to be weaker than those of H1fr. In contrast, we found that injected H2fr. induced the formation of some micronuclei in the injected cells after division (Fig. 1C and 1E). The micronuclei-containing cells appeared at 90 min to 2 hours after injection, while normal daughter cells were observed within 45 min after the injection of lysozyme, indicating that the injected H2fr. delayed the progression of mitosis. Each of these micronuclei contained DNA, as evidenced by Hoechst staining. In addition, we injected various concentrations of H2fr. into prometaphase PtK<sub>2</sub> cells and 3 hours after the injection we observed the state of the DNA by means of Hoechst staining. As shown in Fig. 2, micronuclei formation was reproduced in a protein concentration-dependent manner.



**Fig. 1.** H2fr. induced micronuclei formation. (A) Histone enriched chromatin fractions used in this study. Lane 1, H1fr.; lane 2, H2fr.; lane 3, H3fr. One  $\mu$ g of each fraction was subjected to SDS-PAGE and stained with Coomassie brilliant blue. H1fr., H2fr. and H3fr. predominantly contain histones H1A+H1B, H2A+H2B, and H3+H4, respectively. The size of the molecular weight markers is indicated on the left: 67, 43, 30, 20, and 14.4 kDa. (B–E) The effects of H2fr. on mitotic cells. Two mg/ml of H2fr. (C and E), or 2 mg/ml of lysozyme (B and D) were injected into prometaphase PtK<sub>2</sub> cells. Three hours (H2fr.) or 45 minutes (lysozyme) later, the cells were fixed and DNA was stained with Hoechst 33342. (B and C) Phase contrast images; (D and E) Hoechst images. Bar, 10  $\mu$ m.



**Fig. 2.** Concentration-dependent induction of micronuclei formation by H2fr. H2fr. was injected into prometaphase PtK<sub>2</sub> cells at concentrations of 0.4, 1.3, and 4.0 mg/ml. After 3 hours, the cells were fixed and stained with Hoechst 33342. The rate of micronuclei formation was determined by counting the number of cells which contained micronuclei and the total number of cells injected. The injection was performed into a total of 20–30 cells for each data point.

To determine whether the micronuclei-inducing activity of H2fr. consists of proteinaceous factors or not, the H2fr. was treated with heat or trypsin and then injected into prometaphase PtK<sub>2</sub> cells. As a control for trypsin treatment, trypsin inhibitor was pre-mixed with trypsin. As shown in Table I, the activity was diminished by both heat and trypsin treatment, indicating that proteinaceous factors are required for the activity.

We next attempted to purify the activity. The activity was monitored by examining whether micronuclei formation occurred when each fraction was injected into prometaphase PtK<sub>2</sub> cells. For the first step, dialyzed H2fr. was subjected to ion exchange chromatography on a CM-cellulofine column. The column was then washed with 200 mM NaCl in 20 mM potassium phosphate buffer (pH 6.7). The bound materials were eluted with 1 M NaCl in the same buffer (Fig. 3A, lane 1). Most of the activity was recovered in this eluted fraction.

**Table I.** THE MICRONUCLEI-INDUCING ACTIVITY WAS DIMINISHED BY BOTH HEAT OR TRYPSIN TREATMENT

	micronuclei formation	normal
untreated	12	2
boiled	0	11
trypsin	0	21
trypsin + trypsin inhibitor	17	0

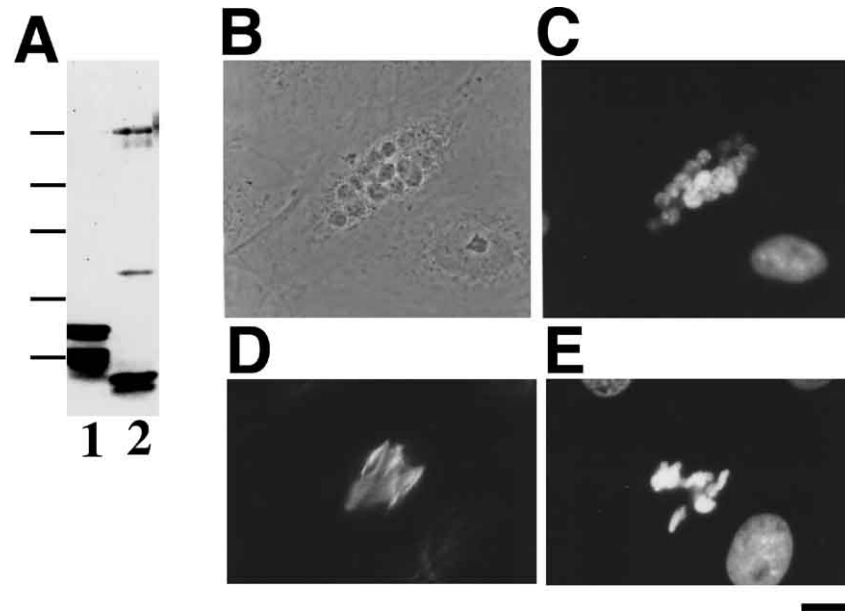
Since histones H2A and H2B, which were the major components of H2fr., were contained in the same fraction, the eluate was dialyzed and then applied to a DNA-cellulose column. The activity was primarily recovered in the void fraction, although most of the proteins, including histones H2A and H2B bound to the column (Fig. 3A, lane 2), suggesting that the core components of activity are neither H2A nor H2B (Fig. 3B and 3C).

In order to examine how the micronuclei are formed after injection, we next examined the state of the mitotic spindles (MS) at 20 min after injection of the void fraction from the DNA-cellulose, because it is known that the integrity of MS is required for proper chromosome segregation (Amon, 1999). As shown in Fig. 3D, the shape of the MS in the injected cells was found to be multipolar. In these cells, chromosomes were scattered throughout the cytoplasm (Fig. 3E). These results suggest that the fraction that possessed the MS disrupting activity, also caused the abnormal segregation of chromosomes. Thus, it is most likely that micronuclei were formed around the dis-segregated chromosomes.

Furthermore, when this fraction was injected into the cytoplasm of interphase cells, it was observed that extra actin filament formation was induced, as shown in Fig. 4C (see Fig. 4F as a control), and that the radial organization of MTs appeared to be altered to a parallel one (Fig. 4B, see Fig. 4E as a control). We also found that ectopic actin filament formation occurred in the mitotic cells, when the fraction was injected into prometaphase PtK<sub>2</sub> cells (Fig. 4K, see Fig. 4I as a control). These results suggest that the ectopic actin filament formation causes the disruption of MS (Fig. 4L, see Fig. 4J as a control). To confirm this, we injected the fraction into prometaphase cells in the presence of an actin polymerization inhibitor, cytochalasin D. As shown in Figs. 5A, 5B and 5C, micronuclei formation was blocked and simultaneously cytokinesis was inhibited by the drug, leading to non-dividing cells containing two nuclei. Collectively these data lead to the conclusion that the fraction contains actin polymerizing activity, and that the ectopic formation of actin filaments in mitosis induces the disruption of the MS. Moreover, when the same fraction was injected into the cytoplasm of cells other than PtK<sub>2</sub> cells, such as mouse NIH3T3 cells (Fig. 4G) and bovine MDBK cells (Fig. 4H), at interphase, extra actin filament formation was also induced in the cytoplasm, indicating that this type of activity is conserved among species.

## Discussion

The findings herein show that a chromatin fraction isolated from bovine liver nuclei contained micronuclei formation-inducing activity when injected into mitotic cells. We also found that the fraction-injected cells usually did not perform cytokinesis. It is well known that a round of DNA replication in a cell usually requires several hours and the cell cy-



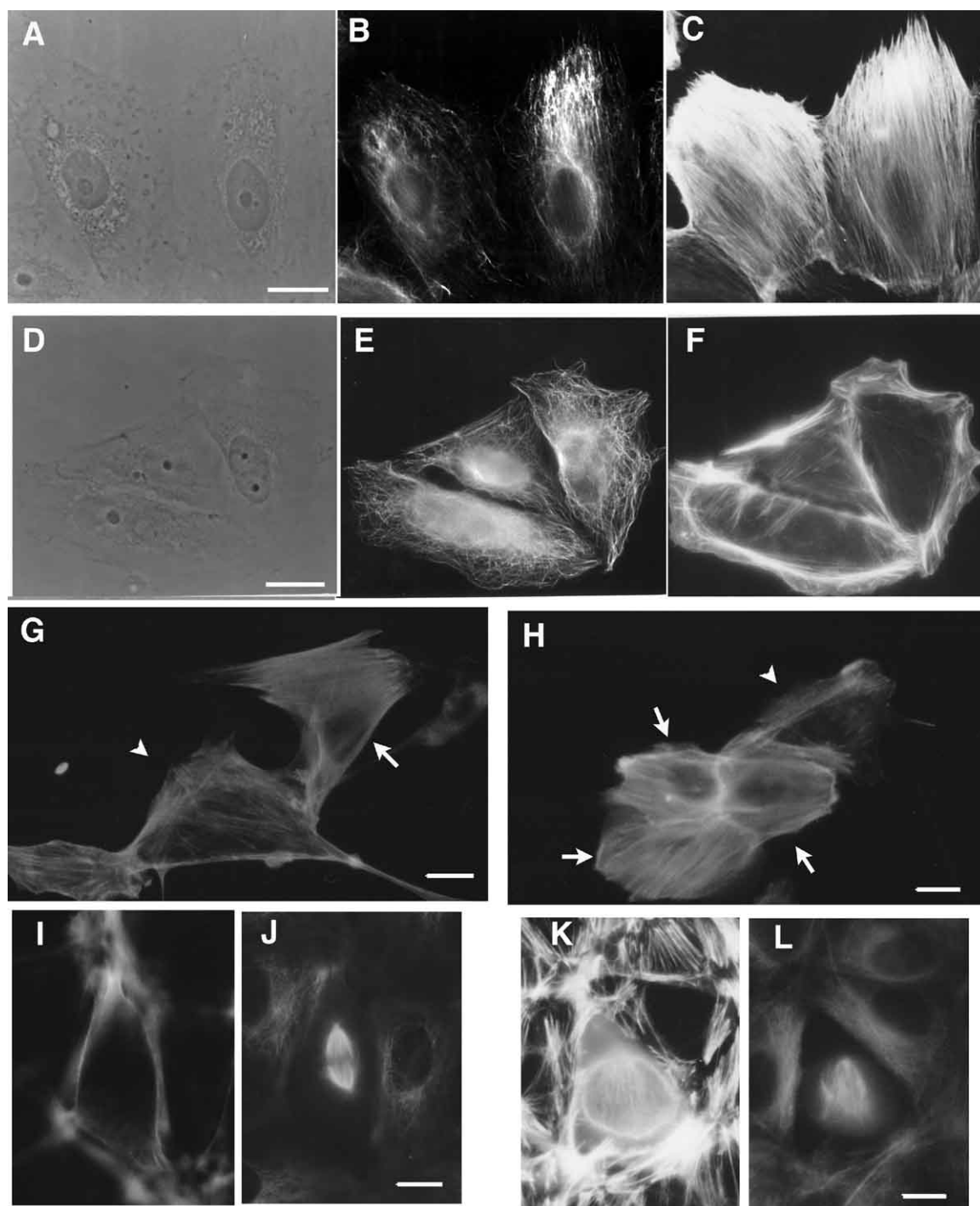
**Fig. 3.** Partial purification of the micronuclei formation inducing activity. (A) SDS-PAGE profiles of the fractions of each purification step. Four  $\mu\text{g}$  of the 1 M NaCl eluate of CM-cellulofine (lane 1) and 1  $\mu\text{g}$  of the void fraction of DNA-cellulose (lane 2) were subjected to SDS-PAGE and stained with Coomassie brilliant blue. The bands whose molecular masses were estimated to be 66kDa, 25 kDa, 10 kDa and 9kDa were enriched in these steps. The size of the molecular weight markers is indicated on the left: 67, 43, 30, 20, and 14.4 kDa. (B and C) The activity of the void fraction of DNA-cellulose column chromatography. The void fraction of the DNA-cellulose column chromatography was concentrated and injected into prometaphase PtK<sub>2</sub> cells. After 3 hours, the cells were fixed and stained with Hoechst 33342 (C). The phase contrast image is shown in B. (D and E) The disruption of microtubules by the void fraction of DNA-cellulose. The void fraction of the DNA-cellulose column chromatography was concentrated and injected into prometaphase PtK<sub>2</sub> cells. After 20 minutes, the cells were fixed and stained with anti-tubulin antibodies (D) and Hoechst 33342 (E). Bar, 10  $\mu\text{m}$ .

cle progression requires protein synthesis. It was found that the micronuclei formation was accomplished within 2 hr after the injection even when *de novo* protein synthesis was inhibited (data not shown). Thus, it seems unlikely that in the injected cells, the nuclear division was repeated without cytokinesis. Therefore, it is most likely that chromosomes were not segregated properly in the cells and that, when the cells entered interphase, the scattered chromosomes decondensed and micronuclei were formed around the scattered chromosomes. In addition, we believe that the failure of chromosome segregation inhibited cytokinesis.

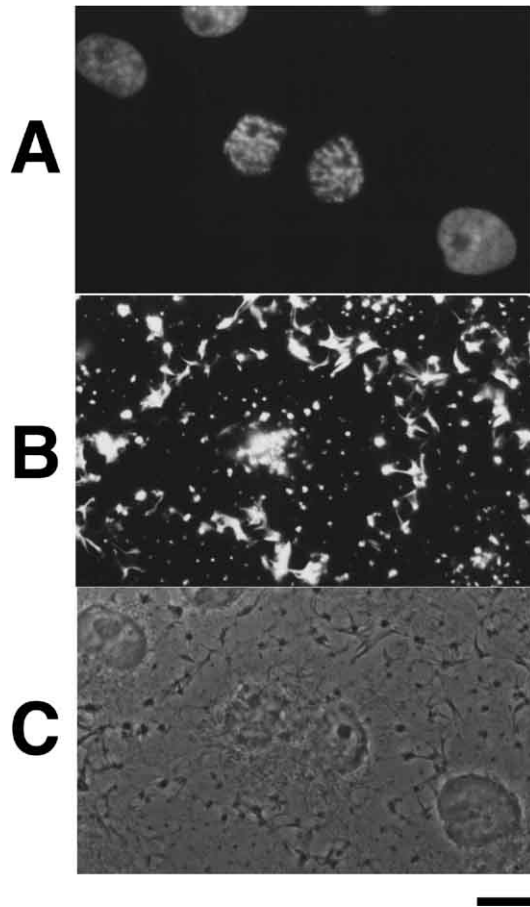
The fraction-injected mitotic cells showed ectopic actin filaments and disorganized multipolar MS. In interphase cells, the extra actin filament formation was also induced and the radial organization of MTs was changed to the parallel one. On the other hand, the micronuclei-inducing activity was completely blocked by the actin polymerization inhibitor, cytochalasin D. Taken together, this activity primarily affected actin molecules to induce ectopic actin filament formation. Although actin filaments and MTs had been viewed as separate cytoskeletal systems with distinct functions, a growing body of evidence has shown that the two systems functionally cooperate in a variety of cellular processes, including vesicle and organelle transport, cleavage furrow placement, directed cell migration, spindle rota-

tion, and nuclear migration (Goode *et al.*, 2000). Since we demonstrated that the aberrant interaction between these two systems induced in mitosis results in the failure of normal chromosome segregation, the interaction must be strictly regulated both temporally and spatially, at least in mitosis.

It has recently been demonstrated that actin shuttles between the nucleus and cytoplasm (Wada *et al.*, 1998), and that several regulators of actin polymerization are localized in the nucleus (Rando *et al.*, 2000). In addition, actin has been reported to play important roles in nuclear processes such as chromatin remodeling and splicing (Papoulas *et al.*, 1998; Schroder *et al.*, 1987). The micronuclei formation-inducing activity may contain a member of such nuclear regulators of actin polymerization and may be involved in some of these nuclear processes. Although several proteins are present in the final fraction used in this study (the void fraction of DNA-cellulose column chromatography), one of the prominent bands was a 25 kDa protein. Since the molecular mass of the Rho GTPase family, a well-known actin polymerization regulator, is around 25 kDa (Takai *et al.*, 2001), we originally suspected that this band is a member of the Rho family. We excised the band from the SDS-PAGE gel and determined its partial amino acid sequence. As a result, it was found that the band was, in fact, not a member of the



**Fig. 4.** Induction of ectopic actin filament formation in interphase cells as well as in mitotic cells. (A, B, C, D, E and F) Induction of extra actin filament formation in interphase cells. The void fraction (0.2 mg/ml) of the DNA-cellulose column chromatography (A, B, and C) or 0.2 mg/ml of lysozyme (D, E, and F) was injected into all the interphase PtK<sub>2</sub> cells shown in the panels. After 3 hours, the cells were fixed and stained with anti-tubulin antibodies (B and E) and rhodamine-phalloidin (C and F). The phase contrast image is shown in A and D. (G and H) Induction of extra actin filaments in interphase cells other than PtK<sub>2</sub> cells. The same fraction was injected into the interphase NIH3T3 cells (G) or MDBK cells (H). After 3 hours, the injected cells (arrows) and normal cells (arrowheads) were fixed and stained with rhodamine-phalloidin. (I and K) Induction of ectopic actin filament formation in mitotic cells. The same fraction was injected into the prometaphase PtK<sub>2</sub> cells (K and L). As a control, 0.2 mg/ml of lysozyme was injected (I and J). After 45 minutes, the cells were fixed and stained with rhodamine-phalloidin (I and K) and anti-tubulin antibodies (J and L). Bars, 10 μm.



**Fig. 5.** The effect of cytochalasin D on the micronuclei-inducing activity. The void fraction of the DNA-cellulose column chromatography (0.2 mg/ml) was injected into the prometaphase PtK<sub>2</sub> cells in the presence of cytochalasin D (1 µg/ml) in the culture medium. After 3 hours, the cells were fixed and stained with Hoechst 33342 (A) and rhodamine-phalloidin (B). The phase contrast image is shown in C. Bar, 10 µm.

Rho family but a degradation product of histone macroH2A (Pehrson and Fried, 1992) (data not shown). However, thus far, we have failed to detect any actin polymerization activity for histone macroH2A. The other three prominent bands whose molecular weight are 66 kDa, 10 kDa, and 9kDa, respectively, were not co-eluted with the micronuclei-inducing activity on a gel-filtration column (data not shown). Therefore, a relatively minor protein in the void fraction of DNA-cellulose column chromatography may be responsible for the micronuclei-inducing activity. We believe that the

molecular identification and characterization of the activity will help us to better understand the role of molecular interactions between actin and tubulin networks as well as the nuclear function of the actin network.

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## References

- Amon, A. 1999. The spindle checkpoint. *Curr. Opin. Genet. Dev.*, **9**: 69–75.
- Ayscough, K.R. 1998. In vivo functions of actin-binding proteins. *Curr. Opin. Cell Biol.*, **10**: 102–111.
- Bear, J.E., Krause, M., and Gertler, F.B. 2001. Regulating cellular actin assembly. *Curr. Opin. Cell Biol.*, **13**: 158–166.
- Goode, B.L., Drubin, D.G., and Barnes, G. 2000. Functional cooperation between the microtubule and actin cytoskeletons. *Curr. Opin. Cell Biol.*, **12**: 63–71.
- Matsuoka, Y., Takechi, S., Nakayama, T., and Yoneda, Y. 1994. Exogenous histone H1 injection into mitotic cells disrupts synchronous progression of mitotic events by delaying chromosome decondensation. *J. Cell Sci.*, **107**: 693–701.
- Papoulas, O., Beek, S.J., Moseley, S.L., McCallum, C.M., Sarte, M., Shearn, A., and Tamkun, J.W. 1998. The *Drosophila* trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. *Development*, **125**: 3955–3966.
- Pehrson, J.R. and Fried, V.A. 1992. MacroH2A, a core histone containing a large nonhistone region. *Science*, **257**: 1398–1400.
- Rando, O.J., Zhao, K., and Crabtree, G.R. 2000. Searching for a function for nuclear actin. *Trends Cell Biol.*, **10**: 92–97.
- Schroder, H.C., Trolltsch, D., Wenger, R., Bachmann, M., Diehl-Seifert, B., and Muller, W.E. 1987. Cytochalasin B selectively releases ovalbumin mRNA precursors but not the mature ovalbumin mRNA from hen oviduct nuclear matrix. *Eur. J. Biochem.*, **167**: 239–245.
- Simon, R.H. and Felsenfeld, G. 1979. A new procedure for purifying histone pairs H2A + H2B and H3 + H4 from chromatin using hydroxylapatite. *Nucleic Acids Res.*, **6**: 689–696.
- Takai, Y., Sasaki, T., and Matozaki, T. 2001. Small GTP-binding proteins. *Physiol. Rev.*, **81**: 153–208.
- Wada, A., Fukuda, M., Mishima, M., and Nishida, E. 1998. Nuclear export of actin: a novel mechanism regulating the subcellular localization of a major cytoskeletal protein. *EMBO J.*, **17**: 1635–1641.
- Yamaizumi, M., Uchida, T., Okada, Y., Furusawa, M., and Mitsui, H. 1978. Rapid transfer of non-histone chromosomal proteins to the nucleus of living cells. *Nature*, **273**: 782–784.

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