Detection of Protein Kinase Activity Specifically Activated at Metaphase-Anaphase Transition

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Abstract. We have previously reported that Ser13 and Ser34 on glial fibrillary acidic protein (GFAP) in the cleavage furrow of glioma cells are phosphorylated during late mitotic phase (Matsuoka, Y., K. Nishizawa, T. Yano, M. Shibata, S. Ando, T. Takahashi, and M. Inagaki. 1992. *EMBO (Eur. Mol. Biol. Organ.) J.* 11:2895–2902). This observation implies a possibility that there is a protein kinase specifically activated at metaphase-anaphase transition. To further analyze the cell cycle–dependent GFAP phosphorylation, we prepared monoclonal antibodies KT13 and KT34 which recognize the phosphorylation of GFAP at Ser13 and Ser34, respectively. Immunocytochemical studies with KT13 and KT34 revealed that the GFAP phosphorylation in the cleavage furrow during late mitotic phase occurred not only in glioma cells but also in human SW-13 and mouse Ltk$^-$ cells in which GFAP was ectopically expressed, thus the phosphorylation can be monitored in a wide range of cell types. Furthermore, we detected kinase activity which phosphorylates GFAP at Ser13 and Ser34 in the lysates of late mitotic cells but not in those of interphase cells or early mitotic cells. These results suggest that there exists a protein kinase which is specifically activated at the transition of metaphase to anaphase not only in GFAP-expressing cells but also in cells without GFAP.

CELL-DIVISION cycle is the fundamental means by which cells duplicate, and comprises a complicated series of cytoplasmic and nuclear events which are elaborately coordinated under the control. It has become increasingly evident that the cell cycle control system is based on protein phosphorylation (for reviews see Nurse, 1990; Norbury and Nurse, 1992). Accumulating evidence has revealed that mitotic cyclins, G1 cyclins, and cyclin-dependent protein kinases (cdks)[1] play critical roles in controlling the cell cycle at G2-M and G1-S transitions (for reviews see Hunt, 1989, 1991; Reed, 1992; Pines, 1993; Sherr, 1993; Nigg, 1993). On the other hand, the mechanisms that govern the cell cycle from metaphase-anaphase transition to the end of mitosis are largely unknown. Some proteins were reported to be phosphorylated during late mitotic phase, indicating that protein kinases may contribute to the execution and control of the events (Johnston and Sloboda, 1992; Yamakita et al., 1994; Toyn and Johnston, 1994). However, limited information is available on kinases activated during late mitotic phase (Fenton and Glover, 1993; Kitada et al., 1993; Samejima and Yanagida, 1994).

Phosphorylation state-specific antibodies which recognize a phosphorylated serine/threonine residue and its flanking sequence provide a useful tool to analyze phosphorylation of proteins in vivo (Nishizawa et al., 1991; Czernik et al., 1991; Matsuoka et al., 1992; Inagaki et al., 1994*a*,*b*). Glial fibrillary acidic protein (GFAP) is an intermediate filament protein expressed in the cytosol of glial cells (Lazarides, 1980). Recently, we reported two distinct types of GFAP phosphorylation during mitosis of U251 glioma cells, using antibodies that recognize GFAP phosphorylation (Matsuoka et al., 1992). One type is GFAP phosphorylation at Ser8 residue which appeared at G2-M transition, remained until metaphase, and declined in anaphase. The phosphorylation was observed diffusely throughout the cytoplasm. On the basis of evidence that cdc2 kinase activity is maximal at G2-M transition (Nurse, 1990; Pines and Hunter, 1990) and that Ser8 is phosphorylated in vitro by cdc2 kinase, we concluded that cdc2 kinase is responsible for the phosphorylation (Tsujimura et al., 1994*a*). Using a monoclonal antibody specifically recognizing vimentin, another intermediate filament protein, phosphorylated at Ser55, we demonstrated that cdc2 kinase is responsible for phosphorylation of vimentin in the entire cytoplasm at G2-M transition (Tsujimura et al., 1994*b*). The other type is GFAP phosphorylation at Thr7, Ser13, and Ser34, which appeared at metaphase-anaphase transition, was maintained until telophase and decreased

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1. *Abbreviations used in this paper*: CBB, Coomassie Brilliant Blue; cdk, cyclin-dependent protein kinase; CF kinase, cleavage furrow kinase; 2-ME, 2-mercaptoethanol; PI, propidium iodide; PKA, cAMP-dependent protein kinase; PKC, protein kinase C.

at the exit of mitosis. The phosphorylation occurred between the daughter nuclei and in the cleavage furrow. The specific localization of GFAP phosphorylation suggests that it may play an important role in efficient separation of glial filaments to daughter cells. This observation also implies a possible existence of a protein kinase which is specifically activated at metaphase-anaphase transition. However, with polyclonal antibodies it was difficult to obtain clear data for the demonstration of kinase activity, because there was a minor population that recognized unphosphorylated GFAP and additional unknown proteins (Nishizawa et al., 1991; Matsuoka et al., 1992).

To overcome the difficulty, we developed monoclonal antibodies, KT13 and KT34 which recognize the phosphorylation state of GFAP at Ser13 and Ser34, respectively. Immunocytochemical studies with KT13 and KT34 revealed that the cell cycle–dependent phosphorylation of GFAP at Ser13 and Ser34 is observed in a wide range of cell types during late mitotic phase. We also obtained evidence for a kinase activity which phosphorylates GFAP at Ser13 and Ser34 in lysates of late mitotic cells but not in those of interphase cells or early mitotic cells.

Materials and Methods

Purification of Proteins

The catalytic subunit of cAMP-dependent protein kinase (PKA) was prepared from bovine heart by the method of Beavo et al. (1974). A human GFAP cDNA (pBabe-neo-GFAP) was kindly provided by Dr. James T. Rutka (Rutka et al., 1994). For bacterial expression, a 5′ NdeI site was engineered at the beginning of the coding segment by oligonucleotide-directed mutagenesis using a 5′ primer (5′-ACAAGCTTCATATGGAGAGGAGACG-3′), then the mutated coding segment was subcloned into the NdeI–BamHI sites of prokaryotic expression vector, pET-3a (Novagene, Madison, WI). *Escherichia coli* strain BL21(DE3)pLysS (Ogawara et al., 1995) was transformed with the plasmid and the induction of GFAP expression was achieved by 6 h incubation of the bacteria with 0.5 mM isopropyl-β-D-thiogalactopyranoside in LB medium. The transformants were harvested by centrifugation at 8,000 *g* for 15 min and stored at −80°C. Recombinant GFAP was purified as described previously (Inagaki et al., 1990). Protein concentration was determined by the method of Bradford (1976), using bovine serum albumin as a standard.

Phosphorylation of GFAP

After dialysis against 2 mM ethylene glycol-*bis*(β-aminoethyl ether) N,N,N′,N′,-tetraacetic acid (EGTA), 50 mM 2-mercaptoethanol (2-ME), 1 mM PMSF, and 5 mM Tris-HCl (pH 8.8) for 24 h at 4°C, purified GFAP (0.15 mg/ml) was phosphorylated by incubation with 5 μg/ml PKA, 0.1 mM ATP, 3 mM $MgCl_2$, 25 mM Tris-HCl (pH 7.5) for 1 h at 25°C.

Cell Culture and Transfection

Human glioma U251 cells, mouse fibroblastic Ltk⁻ cells, and human adrenal cortex carcinoma-derived SW-13 cells were maintained in DMEM supplemented with 10% FCS and L-glutamine. To express human GFAP in Ltk⁻ and SW-13 cells, the 1,314-bp EcoRI–BamHI fragment of pBabe-neo-GFAP was introduced into the pRc/CMV vector (Invitrogen, San Diego, CA), and then the cells were transfected with the plasmid (4 μg/ml) using 10 μg/ml Lipofectin (GIBCO BRL, Gaithersburg, MD). The cells expressing human GFAP were cloned and maintained in the presence of 500 μg/ml G418 sulfate (GIBCO BRL).

Peptide Synthesis and Production of Hybridomas

GFAP peptides, PG13 (Cys-Ser-Ala-Ala-Arg-Arg-phosphoSer^{13}-Tyr-Val-Ser-Ser-Leu), G13 (Cys-Ser-Ala-Ala-Arg-Arg-Ser-Tyr-Val-Ser-Ser-Leu), PG34 (Cys-Pro-Gly-Pro-Arg-Leu-phosphoSer^{34}-Leu-Ala-Arg-Met-Pro), G34 (Cys-Pro-Gly-Pro-Arg-Leu-Ser-Leu-Ala-Arg-Met-Pro), PG389 (Cys-Gln-Ile-Arg-Glu-Thr-phosphoSer^{389}-Leu-Asp-Thr-Lys-Ser), and G389 (Cys-Gln-Ile-Arg-Glu-Thr-Ser-Leu-Asp-Thr-Lys-Ser) were synthesized and purified as described previously (Tsujimura et al., 1994*b*). Monoclonal antibodies against phosphopeptide PG13 (KT13) and phosphopeptide PG34 (KT34) were produced, following the method described previously (Yano et al., 1991; Tsujimura et al., 1994*b*). The monoclonal antibody MO389 which reacted with both phosphopeptide PG389 and nonphosphopeptide G389 was also developed using the same procedures with phosphopeptide PG389 as the antigen.

Immunoblots

All the procedures have been described in detail elsewhere (Yano et al., 1991; Nishizawa et al., 1991).

Immunofluorescence Microscopy

Cells growing on glass coverslips were fixed in 3.7% formaldehyde in ice-cold PBS for 15 min and permeabilized with 0.1% Triton X-100 in PBS for 10 min at room temperature. The cells were then incubated for 1 h at room temperature with the monoclonal antibodies, KT13 or MO389 (dilution 1:20). For immunostaining with monoclonal antibody KT34, the fixed cells were treated with methanol at −20°C for 10 min instead of Triton X-100. After being washed three times with PBS, they were incubated for 1 h with FITC-conjugated goat anti-mouse antibodies (dilution 1:1,000) (BIOSOURCE, Camarillo, CA), and stained with 25 μg/ml propidium iodide (PI) (Sigma) for 15 min at room temperature.

Preparation of Interphase, Metaphase, and Late Mitotic Cells

Interphase and metaphase U251 cells were prepared as described previously (Nishizawa et al., 1991; Tsujimura et al., 1994*b*). In brief, U251 cells were plated onto 10-cm dishes, and then were arrested in metaphase by the addition of 10 ng/ml colcemid (GIBCO BRL) for 12 h. Metaphase cells were collected by mechanical shake off and the adherent cells were used as interphase cells. Late mitotic U251 cells were prepared as described by Hosoya et al. (1993) but with slight modification. Cells were first cultured for 12 h in normal growth medium containing 50 ng/ml nocodazole (GIBCO BRL). Mitotic cells were detached by gentle pipetting, collected, rinsed twice in DMEM, suspended in nocodazole free growth medium and plated onto the culture dish. After removal of nocodazole, the population of late mitotic cells reached a peak in 30 min.

Detection of Protein Kinase Activities That Phosphorylate Ser13 and Ser34 of GFAP

5×10^6 U251 cells were suspended in an extraction buffer containing 0.5 mM dithiothreitol, 2 mM EGTA, 20 mM β-glycerophosphate, 5 μg/ml leupeptin, 10 mM 2-ME, 100 mM NaCl, 1 mM PMSF, and 20 mM Tris-HCl (pH 8.0), sonicated and centrifuged at 100,000 *g* for 30 min. For the detection of the kinase activity to phosphorylate Ser13 or Ser34, the soluble fraction (0.6 mg protein/ml) was incubated for 1 h using purified GFAP (0.15 mg/ml) as an exogenous substrate in 1 mM ATP, 0.1 μM calyculin A, 3 mM $MgCl_2$, and 25 mM Tris-HCl (pH 7.5) at 25°C. Reactions were stopped by adding an equal volume of sodium dodecyl sulfate (SDS) sample buffer which contained 0.2 mg/ml Bromophenol blue, 10% glycerol, 100 mM 2-ME, 2% SDS, and 62.5 mM Tris-HCl (pH 6.8), and boiled for 1 min. Phosphorylated GFAP was analyzed by Western blotting as described above except that detection was achieved using the enhanced chemiluminescence method (Amersham Corp., Arlington Heights, IL).

Results

Production and Characterization of Monoclonal Antibodies KT13 and KT34

To analyze the phosphorylation of GFAP during late mitotic phase, we developed two monoclonal antibodies KT13 and KT34, raised against the synthetic peptides PG13 (Cys-Ser-Ala-Ala-Arg-Arg-phosphoSer^{13}-Tyr-Val-Ser-Ser-Leu) and PG34 (Cys-Pro-Gly-Pro-Arg-Leu-phosphoSer^{34}-Leu-Ala-Arg-Met-Pro), respectively. The reac-

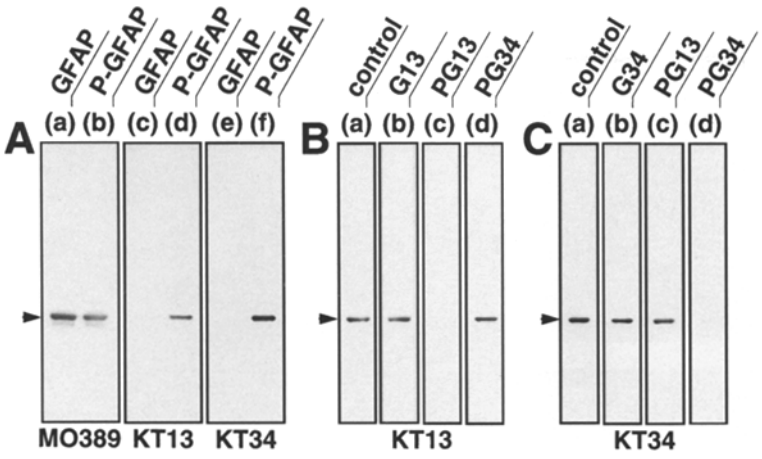

Figure 1. Specificity of monoclonal antibodies KT13 and KT34 analyzed by Western blotting. (*A*) Reactivity of KT13 and KT34 to unphosphorylated and phosphorylated GFAP. Unphosphorylated GFAP (*a*, *c*, and *e*) and GFAP phosphorylated at 1.8 mol phosphate/mol protein by PKA (*b*, *d*, and *f*), were resolved by SDS-PAGE (75 ng in each lane) and transferred onto a polyvinylidene difluoride filter (PVDF) membrane. Both Ser13 and Ser34 are reported to be phosphorylated by PKA in vitro (Inagaki et al., 1990). The blots were then stained with 1 μg/ml MO389 (*a* and *b*), 1 μg/ml KT13 (*c* and *d*), or 1 μg/ml KT34 (*e* and *f*). (*B*) Specificity of KT13 determined by inhibition assay. GFAP phosphorylated by PKA (75 ng in each lane) was stained with 1 μg/ml KT13 preincubated with 133 μg/ml of nonphosphopeptide G13 (*b*), phosphopeptide PG13 (*c*), or phosphopeptide PG34 (*d*). As a control, lane *a* shows the reactivity of KT13 after preincubation with PBS. (*C*) Specificity of KT34 determined by the same procedures as described in *B*. 1 μg/ml KT34 was preincubated with 133 μg/ml of nonphosphopeptide G34 (*b*), phosphopeptide PG13 (*c*), or phosphopeptide PG34 (*d*). As a control, 1 μg/ml KT34 was preincubated with PBS. Arrowheads indicate the position of GFAP.

tivity of KT13 and KT34 to GFAP phosphorylated at Ser13 and Ser34 and to unphosphorylated GFAP was checked by Western blotting. Fig. 1 *A* shows that KT13 and KT34 reacted with the phosphorylated GFAP but not with unphosphorylated GFAP. On the other hand, anti-GFAP antibody MO389 recognized both the phosphorylated and unphosphorylated GFAPs (Fig. 1 *A*). Next, to examine the epitope specificity of these antibodies, competition experiments were carried out using various synthetic peptides. Preincubation of KT13 with phosphopeptide PG13 but not with nonphosphopeptide G13 or phosphopeptide PG34 prevented the binding of KT13 to the phosphorylated GFAP (Fig. 1 *B*). Likewise, preincubation of KT34 with phosphopeptide PG34 but not with nonphosphopeptide G34 or phosphopeptide PG13 prevented the reaction of KT34 with the phosphorylated GFAP (Fig. 1 *C*). Thus, KT13 and KT34 specifically recognize the phosphorylation of GFAP at Ser13 and at Ser34, respectively.

GFAP Phosphorylation during Late Mitotic Phase Was Monitored in Various Types of Cells

Fig. 2, *a* and *b* show U251 glioma cells immunostained with MO389 and KT13, respectively. MO389 detected GFAP distributed throughout the cytoplasm. On the other hand, the KT13 immunoreactivity was observed specifically between the daughter nuclei and in the cleavage furrow of U251 cells. The immunoreactivity appeared at the onset of anaphase was maintained until telophase, and de-

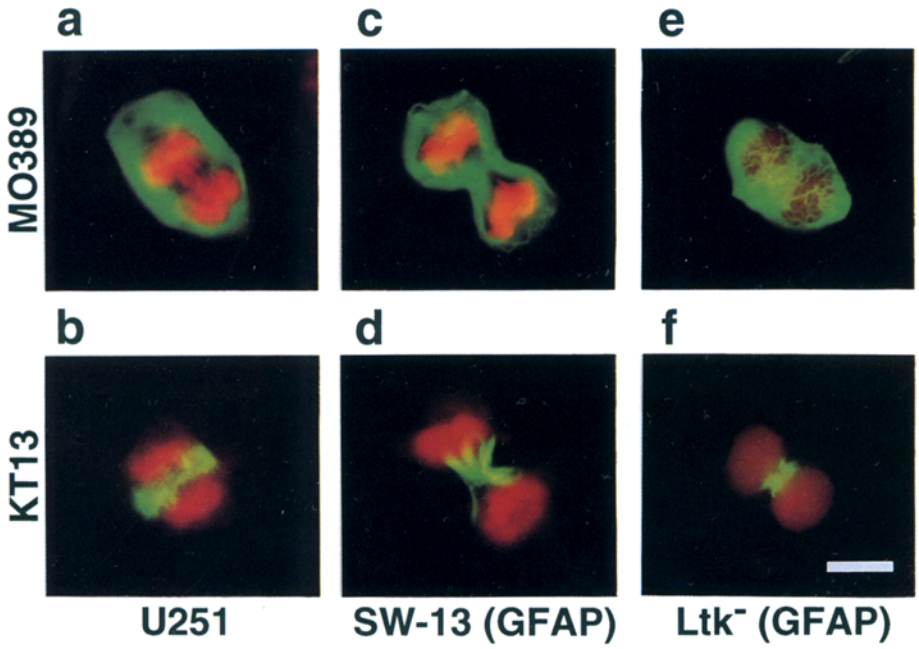

Figure 2. Fluorescence photomicrographs showing MO389 and KT13 immunoreactivity in U251 glioma cells and GFAP-introduced nonglial cells. Human glioma U251 cells (*a* and *b*), human adrenal cortex carcinoma SW-13 cells transfected with the GFAP expression vector (*c* and *d*), and mouse fibroblastic Ltk⁻ cells transfected with GFAP expression vector (*e* and *f*) were fixed, reacted with monoclonal antibody MO389 (*a*, *c*, and *e*) or KT13 (*b*, *d*, and *f*), and visualized by FITC-conjugated second antibody (*green*). The chromosomes were stained with PI (*red*). The cells in *a* and *b* are in anaphase and those in *c–f* are in telophase. Bar represents 10 μm.

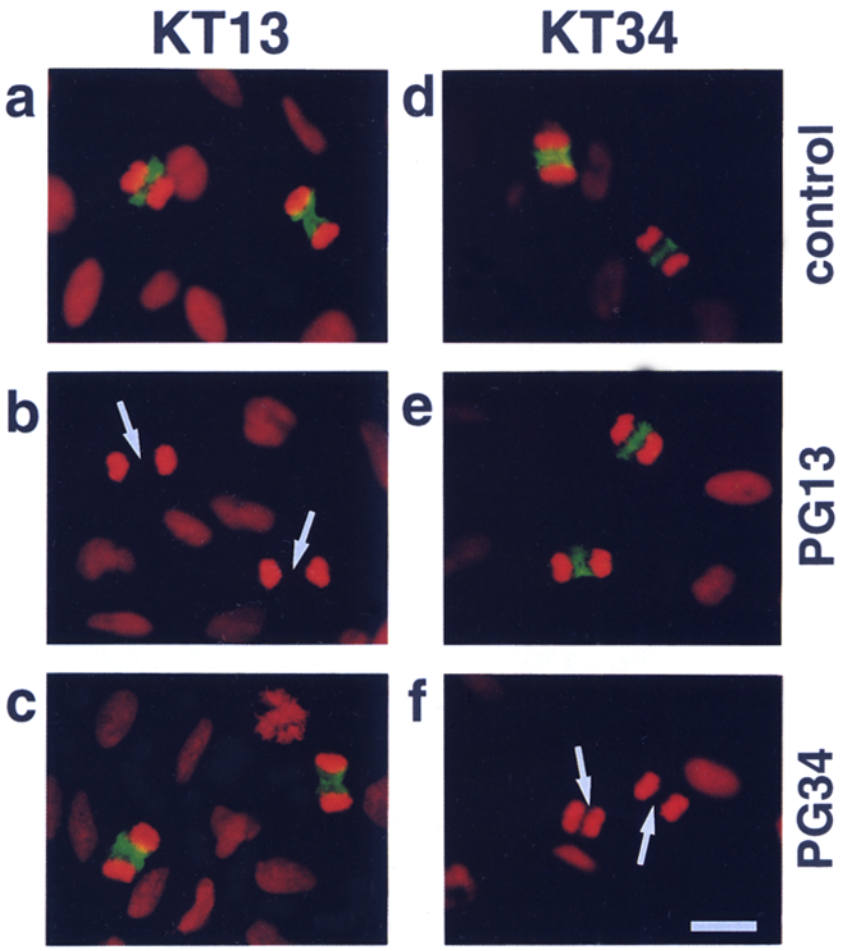

Figure 3. Fluorescence photomicrographs showing the specificity of KT13 and KT34 immunoreactivities in U251 glioma cells. Antibodies KT13 (*a*, *b*, and *c*) and KT34 (*d*, *e*, and *f*) (1 μg/ml) were preincubated with 50 μg/ml of phosphopeptides PG13 (*b* and *e*) or PG34 (*c* and *f*), and then used for immunostaining. As a control, *a* and *d* show the reactivity of these antibodies after preincubation with PBS. Arrows indicate late mitotic cells. Bar represents 20 μm.

creased at the exit of mitosis. These results confirm our previous observations with polyclonal anti-phosphoGFAP antibodies (Nishizawa et al., 1991; Matsuoka et al., 1992).

Next, we asked whether Ser13 of GFAP would be similarly phosphorylated when GFAP was introduced into nonglial cell lines. Human adrenal cortex carcinoma-derived SW-13 cells and mouse fibroblastic Ltk$^-$ cells were stably transfected with the GFAP expression vector and immunostained with MO389 (Fig. 2, *c* and *e*). The expressed GFAP was distributed diffusely in the cytoplasm of SW-13 and Ltk$^-$ cells as in the case of endogenous GFAP in U251 cells. Immunofluorescence studies with KT13 revealed that Ser13 of the ectopic GFAP in SW-13 and Ltk$^-$ cells was also phosphorylated in the cleavage furrow during late mitotic phase (Fig. 2, *d* and *f*). These results clearly demonstrate that the Ser13 phosphorylation of GFAP can be monitored not only in glial cells but also in nonglial cells, regardless of species. Similar results were obtained with KT34 (Fig. 3 *d*, and data not shown).

To examine the specificity of KT13 and KT34 immunoreactivities, competition experiments were carried out. Preincubation of KT13 with phosphopeptide PG13 but not with phosphopeptide PG34 or PBS prevented the immunostaining of KT13 (Fig. 3, *a*, *b*, and *c*). Similarly, the immunostaining with KT34 was abolished by preincubation of KT34 with phosphopeptide PG34 but not with phosphopeptide PG13 or PBS (Fig. 3, *d*, *e*, and *f*). These observations confirm the specificity of KT13 and KT34 immunostainings.

Detection of Cleavage Furrow Kinase Activity in Late Mitotic Cell Lysates

A possible mechanism to explain our findings is that GFAP is phosphorylated by a kinase which is specifically activated in the cleavage furrow at the onset of anaphase. We tentatively termed it CF kinase (Cleavage Furrow kinase) and attempted to identify its activity directly, using GFAP as a substrate. As a first step, we monitored KT13-positive U251 cells after release from mitotic arrest with nocodazole to obtain the cell population expected to contain CF kinase activity. Fig. 4 *a* shows that >60% of the cells were arrested at early stages during mitosis when treated with nocodazole. KT13-positive cells were first observed at 15 min after release from mitotic arrest (Fig. 4 *b*). The population of KT13-positive cells reached a peak (35–40%) between 30 and 45 min (Fig. 4, *c* and *d*) and decreased gradually thereafter (Fig. 4, *e* and *f*). We then analyzed the phosphorylation of endogenous GFAP in the U251 cells at 30 min after release, by Western blotting (Fig. 5). As shown in lane *b* of Fig. 5 *C*, KT13-immunoreactive band at 50 kD corresponding to the position of GFAP (Fig. 5 *B*) was detected only in this cell lysates. No KT13-immunoreactive band was detected in this lysate of

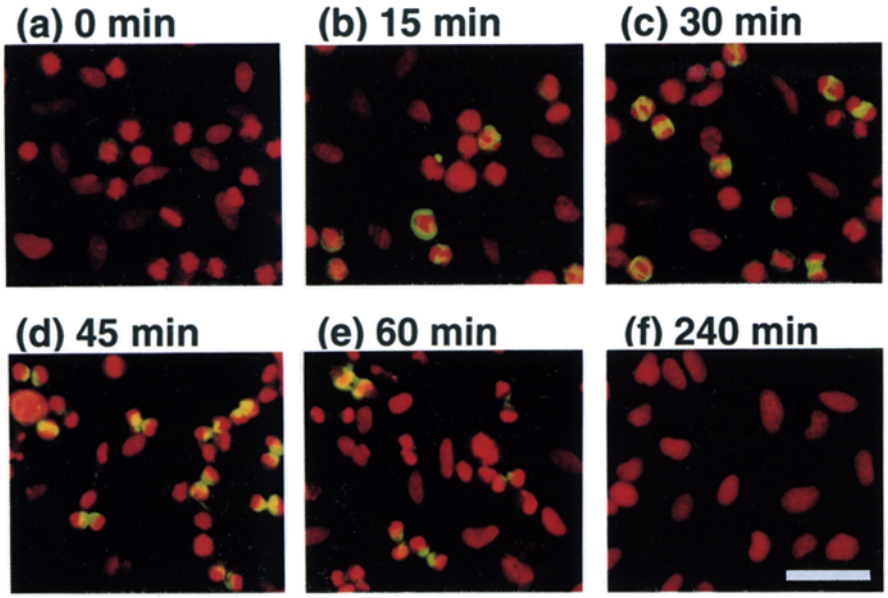

Figure 4. Fluorescence photomicrographs showing KT13-immunoreactive U251 cells after release from mitotic arrest. U251 cells were arrested in metaphase with 50 ng/ml nocodazole. At 0 (*a*), 15 (*b*), 30 (*c*), 45 (*d*), 60 (*e*), and 240 (*f*) min after removal of nocodazole. Cells were fixed and stained with KT13 (*green*) and PI (*red*). Bar represents 50 μm.

metaphase or interphase cells (Fig. 5 *C*, lanes *a* and *c*). Similar observations were obtained with KT34 (data not shown). Therefore, we used the U251 cells at 30 min after release from mitotic arrest to search for CF kinase activity.

Soluble fractions prepared from late mitotic cells were incubated in the presence or absence of purified GFAP as an exogenous substrate. As shown in Fig. 6, this fraction specifically contained the kinase activity phosphorylating Ser13 and Ser34 of GFAP. In contrast, no or only a very faint signal was detected in soluble fractions of metaphase or interphase cells (Fig. 6, *C* and *D*, lanes *b* and *f*). The possibility that the KT13- and KT34-immunoreactive bands (Fig. 6, *C* and *D*, lanes *d*) are derived from endogenous phosphorylated GFAP was ruled out because no MO389-immunoreactive band was detected in soluble fractions without exogenous GFAP (Fig. 6 *B*, lanes *a*, *c*, and *e*). Thus, we detected CF kinase activity which is specifically activated during late mitotic phase.

Discussion

Using phosphorylation state-specific monoclonal antibodies, we identified the phosphorylation of GFAP at Ser13 and Ser34 during late mitotic phase in various types of cells, regardless of species. Furthermore, we detected a protein kinase activity which phosphorylates Ser13 and Ser34 of GFAP specifically in soluble fractions of late mitotic U251 cells but not in those of interphase or

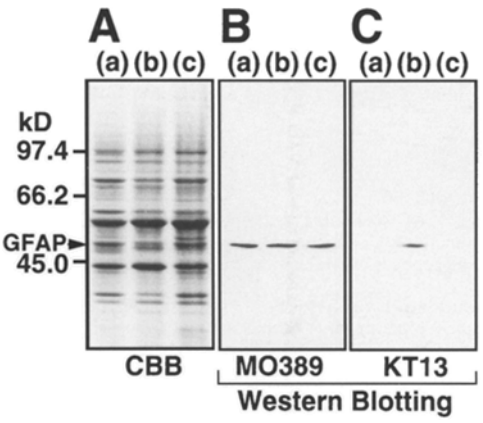

Figure 5. Western blot analysis of GFAP phosphorylation at Ser13 in U251 cell lysates. Lysates of 5×10^4 cells were loaded on lanes, resolved by SDS-PAGE, and transferred onto PVDF membrane. The blots were then stained with Coomassie Brilliant Blue (*CBB*) (*A*), MO389 (*B*), or KT13 (*C*). Lane *a*, metaphase cells; lane *b*, late mitotic phase cells at 30 min after release from mitotic arrest; lane *c*, interphase cells. The position of GFAP is indicated by an arrowhead.

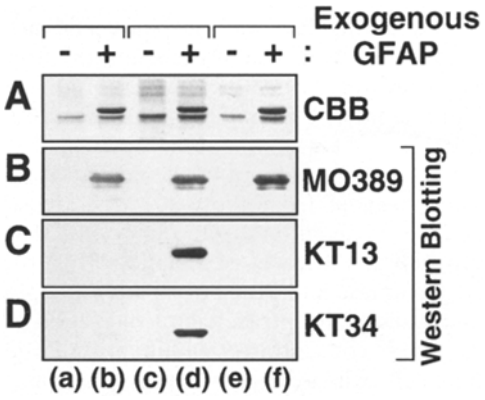

Figure 6. Detection of CF kinase activity in a soluble fraction of U251 cell lysates. Soluble fractions of U251 cell lysate (0.6 mg protein/ml) were incubated for 1 h in the absence (*a*, *c*, and *e*) or presence (*b*, *d*, and *f*) of purified GFAP (0.15 mg/ml) as an exogenous substrate. Each sample was resolved by SDS-PAGE, and transferred onto PVDF membrane. The blots were then stained with CBB (*A*), MO389 (*B*), KT13 (*C*), or KT34 (*D*). Lanes *a* and *b*, soluble fractions of metaphase cells; lanes *c* and *d*, soluble fractions of late mitotic phase cells at 30 min after release from mitotic arrest; lanes *e* and *f*, soluble fractions of interphase cells.

metaphase-arrested cells. These results indicate that a protein kinase is activated at the onset of anaphase.

In contrast to the mechanisms that execute and control cell cycle events at G2-M and G1-S transitions (Hunt, 1989, 1991; Reed, 1992; Pines, 1993; Sherr, 1993; Nigg, 1993), much less is known of mechanisms at metaphase-anaphase transition and during late mitotic phase of eukaryotic cells. This is partly due to difficulties in arresting the cell cycle at metaphase-anaphase transition and in obtaining cells highly enriched in late mitotic cells, for standard biochemical analysis of cell cycle–dependent protein phosphorylation requires synchronized cells. The monoclonal antibodies we developed in the present study overcame these difficulties in two ways. First, immunocytochemical analysis with the antibodies enabled us to detect cell cycle–dependent phosphorylation of GFAP in single cells in nonsynchronized cell preparations. In addition, KT13 and KT34 recognize the site-specific phosphorylation of GFAP. Therefore, the data we obtained are far more specific compared to data obtained by the standard assay using radiolabeled phosphate.

The detection of CF kinase activity in late mitotic cells raises the question of the identity of the kinase. Amino acid sequences flanking Ser13 and Ser34 provide some hints: they do not contain consensus sequence preferentially phosphorylated by the family of proline-directed kinases such as cdc2 kinase, MAP kinase, and other members of the cyclin-dependent protein kinase (cdk) family (Pearson and Kemp, 1991; Ando et al., 1993). Therefore, CF kinase may not belong to these kinases. We reported that both Ser13 and Ser34 on GFAP are phosphorylated by PKA and protein kinase C (PKC) in vitro (Inagaki et al., 1990). However, these kinases also phosphorylate Ser8 on GFAP in vitro (Inagaki et al., 1990) which is not phosphorylated during late mitotic phase (Matsuoka et al., 1992). Therefore, CF kinase appears to be neither PKA nor PKC. Golsteyn et al. (1994) reported cloning of the human Plk1 gene which is closely related to *Drosophila* polo and *Saccharomyces cerevisiae* CDC5. Both polo and CDC5 are activated during late mitotic phase and are thought to be required for cell cycle progression (Fenton and Glover, 1993; Kitada et al., 1993). Human Plk1 kinase activity was low during interphase and high during mitosis (Golsteyn et al., 1995). The kinase was already activated at the G2-M transition, and the kinetics of the activation of Plk1 were indistinguishable from those of cdc2 kinase (Golsteyn et al., 1995). This differs from our results that CF kinase is activated at the onset of anaphase (Fig. 6), and suggests that CF kinase and Plk1 kinase are probably not identical. Interestingly, the intracellular localization of Plk1 was shown to be concentrated within postmitotic bridges of dividing cells when cells go through anaphase. Golsteyn et al. (1995) suggested that Plk1 is required for the dynamic function of the mitotic spindle during chromosome segregation.

KT13 and KT34 were also useful to reveal the intracellular distribution of CF kinase activities. The activity was specifically localized in the cleavage furrow and between the daughter nuclei (Figs. 2 and 3). Mechanisms governing the activation of CF kinase in these restricted areas remain to be elucidated. However, the specific localization of the CF kinase activity in the area where the cytosol is pinched suggests that it may play an important role in separation of the daughter cells. In this context, one possible function of CF kinase activity may be to reorganize intermediate filaments, including GFAP filament, in the cleavage furrow. There is evidence which strongly suggests that phosphorylation of intermediate filament proteins, including GFAP, regulates their morphological organization. In vitro studies revealed that site-specific phosphorylation of intermediate filament proteins induced disassembly of the filament structure (Inagaki et al., 1987, 1988, 1990; Evans, 1988; Peter et al., 1990). In vivo experiments demonstrated that increase in intermediate filament protein phosphorylation by microinjection of the catalytic subunit of PKA (Lamb et al., 1989), expression of the constitutively active form of Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) or PKC (Ogawara et al., 1995), or mitotic arrest with colcemid treatment (Franke et al., 1982; Chou et al., 1990; Tsujimura et al., 1994*b*) led to the collapse of filaments into bundles and granular dot structures. Such reorganization of intermediate filaments may be required for separation of the cytosol in the cleavage furrow. We are now analyzing the influence of the blockade of CF kinase–induced GFAP phosphorylation on the GFAP filament structure and cellular behavior, by introducing a mutant GFAP in which Ser13, Ser34, and Thr7 are changed to alanine into intermediate filament-negative SW-13 cells.

In conclusion, we detected a CF kinase activity which phosphorylates GFAP at the cleavage furrow during late mitotic phase. The phosphorylation state-specific monoclonal antibodies KT13 and KT34 proved to be powerful tools for the detection of CF kinase activity. Probably, CF kinase is not a kinase identified previously. Purification of the kinase is ongoing in order to investigate physiological functions and the mechanism of activation at the onset of anaphase.

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