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.) 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) 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., 1994a,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., 1994a). 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., 1994b). The other type is GFAP phosphorylation at Thr7, Ser13, and Ser34, which appeared at metaphase-anaphase transition, was maintained until telophase and decreased...
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'-ACAGGCTCTATGAGAGGGAGACG-Y), then the mutated coding segment was subcloned into Escherichia coli strain BL21(DE3)pLysS (Novagene, Madison, WI).

#### Peptide Synthesis and Production of Hybridomas

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

#### Immunoblotting

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) (BIOSOURC/E, 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., 1994b). 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<sup>5</sup> 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 with purified GFAP (0.15 mg/ml) as an exogenous substrate in 1 mM ATP, 0.1 μM cAMP, 3 mM Mg<sub>Cl</sub>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 (Amerham 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<sup>13</sup>-Tyr-Val-Ser-Ser-Leu) and PG34 (Cys-Pro-Gly-Pro-Arg-Leu-phosphoSer<sup>34</sup>-Leu-Ala-Ala-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.

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.

increased 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
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
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 Ca2+/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., 1994b) 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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References

Ando, S., K. Tsujiura, Y. Matsuoka, T. Tokui, S.-i. Hisanaga, E. Okumura, M. Uchiyama, T. Kishimoto, H. Yasuda, and M. Inagaki. 1993. Phosphorylation of synthetic vimentin peptides by cdc2 kinase. Biochem. Biophys. Res. Commun. 195:837-843.

Beavo, J.A., P.J. Bechtel, and E.G. Krebs. 1974. Preparation of homogeneous cyclic AMP-dependent protein kinase(s) and its subunits from rabbit skeletal muscle. Methods Enzymol. 38:299-308.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Chou, Y.-H., J.R. Bischoff, D. Beach, and R.D. Goldman. 1990. Intermediate filament reorganization during mitosis is mediated by p34cdc2 phosphorylation of vimentin. Cell 62:1063-1071.

Czernik, A.J., J.-A. Giraudat, A.C. Nairn, J. Chen, G. Snyder, J. Kebabian, and P. Greengard. 1991. Production of phosphorylation state-specific antibodies. Methods Enzymol. 201:264-283.

Evans, R.M. 1988. Cyclic AMP-dependent protein kinase-induced vimentin filament disassembly involves modification of the N-terminal domain of intermediate filament subunits. FEBS Lett. 234:73-78.

Fenton, B., and D.M. Glover. 1993. A conserved mitotic kinase active at late anaphase-telephase in syncytial Drosophila embryos. Nature (Lond.). 365:.

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Franke, W.W., E. Schmid, and C. Grund. 1982. Intermediate filament proteins in nonfilamentous structures: transient disintegration and inclusion of subunit proteins in granular aggregates. Cell. 30:103–113.

Goldstein, R.M., S.J. Schultz, J. Bartek, A. Ziemiecki, T. Ried, and E.A. Nigg. 1994. Cell cycle analysis and chromosomal localization of human Plk1, a putative homologue of the mitotic kinases Drosophila polo and Saccharomyces cerevisiae Cdc5. J. Cell Sci. 107:1509–1517.

Goldstein, R.M., K.E. Mundt, A.M. Fry, and E.A. Nigg. 1995. Cell cycle regulation of the activity and subcellular localization of Plk1, a human protein kinase implicated in mitotic spindle function. J. Cell Biol. 129:1617–1628.

Hosoya, N., H. Hosoya, S. Yamashiro, H. Mohri, and F. Matsuura. 1993. Localization of caldesmon and its dephosphorylation during cell division. J. Cell Biol. 121:1075–1082.

Hunt, T. 1989. Maturation promoting factor, cyclin and the control of M-phase. Trends Biochem. Sci. 14:445–447.

Inagaki, M., Y. Gouda, M. Matsuyama, K. Nishizawa, S. Ando, and C. Sato. 1987. Site-specific phosphorylation induces disassembly of vimentin filaments in vitro. Nature (Lond.). 328:649–652.

Inagaki, M., Y. Gonda, M. Matsuyama, K. Nishizawa, Y. Nishi, and C. Sato. 1988. Intermediate filament reconstitution in vitro; the role of phosphorylation on the assembly-disassembly of desmin. J. Biol. Chem. 263:5970–5976.

Inagaki, M., Y. Gonda, K. Nishizawa, S. Kitamura, C. Sato, S. Ando, K. Tanabe, K. Kikutchi, S. Tsuiki, and Y. Nishi. 1990. Phosphorylation sites linked to gial filament disassembly in vitro locate in a non-a-helical head domain. J. Biol. Chem. 265:4722–4729.

Inagaki, M., Y. Nakamura, M. Takeda, T. Nishimura, and N. Inagaki. 1994a. Gial fibrillary acidic protein: dynamic property and regulation by phosphorylation. Brain Pathol. 4:239–243.

Inagaki, N., N. Iso, T. Nakano, and M. Inagaki. 1994b. Spatiotemporal distribution of protein kinase and phosphatase activities. Trends Biochem. Sci. 19:448–452.

Johnston, J.A., and R.D. Sloboda. 1992. A 62 kD protein required for mitotic progression is associated with the mitotic apparatus during M-phase and with the nucleus during interphase. J. Cell Biol. 119:843–854.

Kitada, K., A.L. Johnson, L.H. Johnston, and A. Sugino. 1993. A multiplicity suppressor gene of the Saccharomyces cerevisiae G, cell cycle mutant gene DBF4 encodes a protein kinase and is identified as CDC5. Mol. Cell. Biol. 13:4445–4457.

Lamb, N.J.C., A. Fernandez, J.R. Feramisco, and W.J. Welch. 1989. Modulation of vimentin containing intermediate filament distribution and phosphorylation in living fibroblasts by the cAMP-dependent protein kinase. J. Cell Biol. 108:257–267.

Lazarides, E. 1980. Intermediate filaments as mechanical integrators of cellular space. Nature (Lond.). 283:249–256.

Matsuoka, Y., K. Nishizawa, T. Yano, M. Shibata, S. Ando, T. Takahashi, and M. Inagaki. 1992. Two different protein kinases act on a different time schedule as gial filament kinases during mitosis. EMBO (Eur. Mol. Biol. Organ.) J. 11:2895–2902.

Nigg, E.A. 1993. Targets of cyclin-dependent protein kinases. Curr. Opin. Cell Biol. 5:187–193.

Nishizawa, K., T. Yano, M. Shibata, S. Ando, S. Saga, T. Takahashi, and M. Inagaki. 1991. Specific localization of phospho-caldesmon kinase protein in the constricted area of dividing cells. J. Biol. Chem. 266:3074–3079.

Norbury, C., and P. Nurse. 1992. Animal cell cycles and their control. Annu. Rev. Biochem. 61:441–470.

Pines, J. 1993. Cyclins and cyclin-dependent kinases: take your partners. Trends Biochem. Sci. 18:195–197.

Pines, J., and T. Hunter. 1990. p34cdk2: the S and M kinase? New Biol. 2:389–401.

Reed, S.I. 1992. The role of p34 kinases in the G1 to S-phase transition. Annu. Rev. Cell Biol. 8:529–561.

Rutka, J.T., S.L. Hubbard, K. Fukiyama, K. Matsuawza, P.B. Dirks, and L.E. Becker. 1994. Effects of antisense gial fibrillary acidic protein complementary DNA on the growth, invasion, and adhesion of human astrocytoma cells. Cancer Res. 54:3267–3272.

Samejima, I., and M. Yanagida. 1994. Identification of caub and cek, a novel protein kinase gene, which complement a fission yeast mutation that blocks anaphase. Mol. Cell. Biol. 14:6361–6371.

Sherr, C.J. 1993. Mammalian G1 cyclins. Cell. 73:1059–1065.

Troy, T.H. and L.H. Johnston. 1994. The Dbf2 and Dbf20 protein kinases of budding yeast are activated after the metaphase to anaphase cell cycle transition. EMBO (Eur. Mol. Biol. Organ.) J. 13:1103–1113.

Tsujimura, K., J. Tanaka, S. Ando, Y. Matsuoka, M. Kusubata, H. Sugiiura, T. Yamauchi, and M. Inagaki. 1994a. Identification of phosphorylation sites on gial fibrillary acidic protein for cdk2 kinase and Ca2+-calmodulin-dependent protein kinase II. J. Biochem. (Tokyo). 116:426–434.

Tsujimura, K., M. Ogawa, Y. Takeuchi, S. Imahoh-Ohmi, M.H. Ha, and M. Inagaki. 1994b. Visualization and function of vimentin phosphorylation by cdk2 kinase during mitosis. J. Biol. Chem. 269:31097–31106.

Yamakita, Y., S. Yamashiro, and F. Matsuura. 1994. In vivo phosphorylation of regulatory light chain of myosin II during mitosis of cultured cells. J. Cell Biol. 124:129–137.

Yano, T., C. Taura, M. Shibata, Y. Hirono, S. Ando, M. Kusubata, T. Takahashi, and M. Inagaki. 1991. A monoclonal antibody to the phosphorylated form of gial fibrillary acidic protein: application to a non-radioactive method for measuring protein kinase activities. Biochem. Biophys. Res. Commun. 175:1144–1151.