To analyze the cell cycle-dependent desmin phosphorylation by Rho kinase, we developed antibodies specifically recognizing the kinase-dependent phosphorylation of desmin at Thr-16, Thr-75, and Thr-76. With these antibodies, phosphorylation of desmin was observed specifically at the cleavage furrow in late mitotic Saos-2 cells. We then found that treatment of the interphase cells with calyculin A revealed phosphorylation at all the three sites of desmin. We also found that an antibody, which specifically recognizes vimentin phosphorylated at Ser-71 by Rho kinase, became immunoreactive after calyculin A treatment. This calyculin A-induced interphase phosphorylation of vimentin at Ser-71 was blocked by Rho kinase inhibitor or by expression of the dominant-negative Rho kinase. Taken together, our results indicate that Rho kinase is activated not only in mitotic cells but also interphase ones, and phosphorylates intermediate filament proteins, although the apparent phosphorylation level is diminished to an undetectable level due to the constitutive action of type 1 protein phosphatase. The balance between intermediate filament protein phosphorylation by Rho kinase and dephosphorylation by type 1 protein phosphatase may affect the continuous exchange of intermediate filament subunits between a soluble pool and polymerized intermediate filaments.

Like other of the Ras superfamily of small GTPases, Rho acts as a molecular switch to control a variety of cellular processes: it regulates signal transduction pathways linking extracellular stimuli to the assembly of actin stress fibers and focal adhesion complexes; it is required for G1 progression and activates serum response factor transcription factor when quiescent fibroblasts are stimulated to grow; and it plays a role in cell cycle and mitotic progression (for a review, see Ref. 2). Although IFs were thought to be relatively stable as compared with other cytoskeletons such as actin filaments and microtubules, intensive in vitro investigations revealed that site-specific phosphorylation by several kinases, such as protein kinase A (PKA), Ca2+/calmodulin kinase II (CaMKII), and cdc2 kinase, dynamically alters their filament structure (26) (for reviews, see Refs. 27–29). Thereafter, some of the above kinases were found to be in vivo IF kinases, using site- and phosphorylation state-specific antibodies that recognize a phosphorylated Ser/Thr residue (30–32). Rho kinase also has been identified as an in vivo IF protein kinase, which site-specifically phosphorylates glial fibrillary acidic protein and vimentin at a cleavage furrow during cytokinesis (22–23). We have shown that Rho kinase plays an essential role in efficient segregation of glial filaments during cytokinesis because mutations in Rho kinase phosphorylation sites impaired segregation of glial filaments into daughter cells and consequently formed an unusually long bridge-like cytoplasmic structure between the daughter cells (24). We clarified that desmin, another type III IF protein, restrictedly expressed in smooth, cardiac, and skeletal muscles, also serves as a substrate for Rho kinase and identified Thr-16, Thr-75, and Thr-76 as the major phosphorylation sites. The intracellular localization of Rho kinase remains to be determined. In nonmuscle cells, this kinase, once activated, was found to translocate from the cytosol to plasma membrane (7). It was also reported that Rho kinase is colocalized with the vimentin filament in serum-starved fibroblasts, and when activated, it translocates to cell
Peripheral regions (34). Therefore, Rho kinase may be active at cell-cell and cell-substrate contact regions in interphase cells. This hypothesis is supported by several lines of experiments showing the Rho kinase-mediated regulation of ezrin, radixin, and moesin proteins (35, 36), adducin (37), and focal complexes (15–17).

In the present work, we newly developed site- and phosphorylation state-specific antibodies for the three Rho kinase phosphorylation sites of desmin in order to analyze the physiological significance of desmin phosphorylation by Rho kinase. Using these antibodies, we found that all the three sites were phosphorylated specifically at the cleavage furrow during cytokinesis. This evidence strongly supports our proposal that Rho kinase acts as a cleavage furrow kinase for IF proteins.

Although various kinases are activated spatiotemporally during the cell cycle and phosphorylate IF proteins, it has been suggested that protein phosphatase is also important for maintenance of the filament structure and plasticity because the IF structure is immediately altered when the cells are treated with protein phosphatase inhibitors (38–41).

We also investigated novel functional aspects of Rho kinase on desmin and vimentin in interphase cells, using the phosphatase inhibitors calyculin A (CA) and okadaic acid (OA). Rho kinase is active to some extent in interphase cells, as well as mitotic cells, although the phosphorylation is apparently masked due to effects of type 1 protein phosphatase (PP1). Our data allow for a new proposal regarding dynamic exchange of type III IF subunits between a soluble pool and the polymerized IFs: Rho kinase- and PP1-mediated IF protein phosphorylation and dephosphorylation, respectively, may influence steady state equilibrium.

**EXPERIMENTAL PROCEDURES**

**Materials and Chemicals—** Recombinant desmin was prepared and purified from *Escherichia coli*, as described (33). GST-CAT (the catalytic domain of Rho kinase, amino acids 6–553) was prepared and purified, as described (13). Desmin was phosphorylated by GST-CAT, as described (33). pEF-BOS-Myc-CAT, pEF-BOS-Myc-RB/PH(TT) (the mutated Rho-binding domain with PH-domain of Rho kinase) (32), and CAKII (Ser-82) respectively, were developed, as described (29, 43). CA and OA were specific sites by cdc2 kinase (Ser-55), PKC (Ser-33), and CaMKII (Ser-4A4, YT33, and MO82, which recognize vimentin phosphorylation at 421–701) were constructed, as described (15, 20). Anti-Rho kinase and anti-PP1 antibodies were generated against the synthetic phosphopeptides PD16, PD75, and PD76, respectively. HA1077 and Y-27632 were kindly provided by Amersham Pharmacia Biotech (Oxford, UK). In the present work, we newly developed site- and phosphorylation state-specific antibodies for the three Rho kinase phosphorylation sites of desmin in order to analyze the physiological significance of desmin phosphorylation by Rho kinase. Using these antibodies, we found that all the three sites were phosphorylated specifically at the cleavage furrow during cytokinesis. This evidence strongly supports our proposal that Rho kinase acts as a cleavage furrow kinase for IF proteins.

**Preparation and Characterization of Antibodies—** Desmin peptides were synthesized, such as PD75, D75, PD76, D76, and PD75–76. Likewise, synthetic peptide D16 or other phosphopeptides/nonphosphopeptides to analyze the epitope specificity of these antibodies. As shown in Fig. 1, inhibition assays were carried out using synthetic peptides to analyze the epitope specificity of these antibodies. The immunoreactivity of α-PD16 was prevented by preincubation with phosphopeptide PD16 but not nonphosphopeptide D16 or other phosphopeptides/nonphosphopeptides, such as PD75, PD76, and PD76. The reaction of α-PD76 was blocked by preincubation with phosphopeptide PD76. The immunoreactivity of α-PD76 was prevented by preincubation with phosphopeptide PD76 and partly blocked with PD75–76, thus α-PD16 and α-PD76 specifically recognized the phosphorylation of desmin at Thr-16 and Thr-76, respectively. On the other hand, α-PD76 was found to recognize double phosphorylation at Thr-75 and Thr-76 as well as the monophosphorylation of desmin at Thr-76.

**Phosphorylation of Desmin**—To investigate the phosphoryla-
Phosphorylation of Desmin-Thr-16, Thr-75, and Thr-76 in vitro

Phosphorylation at Thr-16, Thr-75, and Thr-76 of desmin in Saos-2 cells expressing constitutive active Rho kinase. Saos-2 cells were transfected with pEF-BOS-Myc-CAT and double-stained, as described under "Experimental Procedures" with 9E10 for Myc epitope-tagged RhoK-CAT (left panel) and α-Des (A, right panel), α-PD16 (B, right panel), α-PD75 (C, right panel), or α-PD76 (D, right panel). Scale bar, 20 μm.

Specific Phosphorylation of Desmin-Thr-16, Thr-75, and Thr-76 at Cleavage Furrow during the Cell Cycle—In the next set of experiments, based on the foregoing biochemical and immunocytochemical observation that Thr-16, Thr-75, and Thr-76 of desmin are in vivo phosphorylation sites by Rho kinase, the spatial and temporal distribution of the three phosphorylated sites in Saos-2 cells was analyzed using α-PD16, α-PD75, and α-PD76. Under the conditions used, all the immunoreactivity of α-PD16, α-PD75, and α-PD76 was detected only in late mitotic cells and specifically at the cleavage furrow (Fig. 3) but not in interphase cells (Fig. 4A) or in early mitotic cells, such as prometaphase or metaphase (data not shown). When desmin was stained with α-Des, which reacts with phosphorylated and unphosphorylated desmin, the filamentous structures were observed in mitotic daughter cells (Fig. 3) and in interphase cells (Fig. 4A). In late mitotic Saos-2 cells, Rho kinase also accumulates specifically at the cleavage furrow, as illustrated in Fig. 3, findings that strongly suggest a direct interaction of the kinase with desmin. From these results, we conclude that Rho kinase acts as desmin kinase at the cleavage furrow during cytokinesis. On the basis of the in vitro observation that phosphorylation of desmin by Rho kinase inhibits filament formation (33), we propose that desmin filaments are phosphorylated by Rho kinase and depolymerized at the cleavage furrow during cytokinesis, and hence the segregation of desmin into daughter cells is efficient.

Phosphorylation of Thr-16, Thr-75, and Thr-76 of Desmin in Interphase Cells Treated with Calyculin A—Phosphorylation of IF proteins may play a role in IF structural organization. Rho kinase was found to be co-localized with IF filament in interphase cells (34). To assess the possible function(s) of Rho kinase in IF reorganization, we next analyzed the activation state of Rho kinase on desmin in interphase cells. Intact interphase
cells showed a characteristic network of desmin filaments spanning the cytoplasm, determined using an anti-desmin antibody (Fig. 4A, α-Des, left panel). After treatment with the phosphatase inhibitor CA (20 nM) for 20 min, cells began to round up, the organization of desmin filament was altered, and the IF network collapsed to form a desmin-containing arc near the perinuclear region (Fig. 4A, α-Des, right panel). The immunoreactivity of α-PD16, α-PD75, and α-PD76 appeared in interphase cells treated with CA (Fig. 4A, right panels). Although the staining patterns varied, the filamentous structure was clearly disrupted and the desmin filaments had collapsed. To confirm that Thr-16, Thr-75, and Thr-76 of desmin were phosphorylated in response to CA, immunoblot analysis was performed. As shown in Fig. 4B, α-PD16, α-PD75, or α-PD76-immunoreactive band was observed when interphase cells were treated with CA. Taken together, it is most likely that Rho kinase is to some extent activated even in interphase cells, and an unidentified CA-sensitive phosphatase causes the rapid phosphate turnover on IFs.

Phosphorylation of Vimentin by Rho Kinase in Response to Calyculin A—Because we determined that Ser-71 of vimentin is a specific site for Rho-kinase, and Ser-33, Ser-55, and Ser-82 residues of vimentin are sites specific for PKC, cdc2 kinase, and CaMKII, respectively (29), vimentin is a suitable substrate to verify that Rho kinase, rather than other kinases, phosphorylates IF proteins and regulates their structure and plasticity in interphase cells. We next examined phosphorylation states of vimentin, widely expressed in culture cells, in response to CA, using several types of site- and phosphorylation state-specific antibodies for vimentin. We found that the phosphorylation of Ser-71 appeared in the vimentin filament (Fig. 5) determined using an antibody, GK71, that recognizes phosphorylation of vimentin-Ser-71 (23). On the other hand, the phosphorylation of Ser-33, Ser-55, and Ser-82 was not observed, and it did not increase in interphase cells treated with CA, respectively (Fig. 5), indicating that PKC and cdc2 kinase are not involved in vimentin phosphorylation in interphase Saos-2 cells. CaMKII activity was to some extent detectable but the level was less altered by CA treatment. Moreover, we found an increase in the phosphorylation level of other Rho kinase substrates, such as the myosin binding subunit of myosin phosphatase and myosin light chain (data not shown). Collectively, we concluded that Rho kinase is mainly responsible for IF protein phosphorylation in interphase Saos-2 cells.

To determine whether CA-induced vimentin phosphorylation by Rho kinase could be observed in other cells, MDCK cells and NIH3T3 cells were used (Fig. 6, B and C). When MDCK

---

**Fig. 3.** Immunofluorescence staining of Saos-2 cells with α-PD16, α-PD75, and α-PD76. Confocal microscopic images of Saos-2 cells stained with α-PD16, α-PD75, α-PD76, α-desmin, or α-Rho kinase (green). DNAs were stained with propidium iodide (red). Images represent projections of Z series scans. Inset depicts magnified view at the cleavage furrow area. Scale bar, 10 μm.

**Fig. 4.** Phosphorylation of desmin by Rho kinase in response to CA in Saos-2 cells. A, Saos-2 cells were treated with 20 nM CA for 20 min (right panels). Control cells (left panels) were untreated. Cells were fixed and stained with α-desmin, α-PD16, α-PD75, or α-PD76. Scale bars, 10 μm. B, cells were untreated (control) or treated with 20 nM CA for 20 min (CA) and analyzed by immunoblotting. Desmin (D) is indicated by the arrowhead.
and NIH3T3 cells were treated with 25 nM CA for 20 min and 20 nM CA for 15 min, respectively, the morphology of the vimentin-Ser-71 were analyzed. Fig. 6, B and C, shows that these cells also began to round up, and the vimentin organization was altered. GK71 reacted with vimentin in these interphase cells (Fig. 6, B and C). In Fig. 6D, the CA-induced vimentin-Ser-71 phosphorylation was also confirmed by immunoblot analysis. As in Saos-2 cells, the phosphorylation of Ser-33 and Ser-55 was not observed in MDCK and NIH3T3 cells treated with CA (data not shown). The weak phosphorylation of Ser-82 was less altered (data not shown). These results suggest that Rho kinase-mediated phosphorylation of vimentin in response to CA is a general feature in vimentin-expressing cells.

Different Dose Response Effects of Calyculin A and Okadaic Acid on the Immunoreactivity of GK71—We then used immunoblots to examine the appearance of vimentin phosphorylation by Rho kinase in NIH3T3 cells using OA, another potent inhibitor of PP1 and type 2A protein phosphatase (PP2A). There were obvious differences between the dose-response effects of CA and OA (Fig. 7). The immunoreactivity of GK71 appeared first at a dose range of 10–20 nM in CA-treated cells, but at 3–10 μM in OA-treated cells. An approximately 100-fold difference in inhibitory sensitivity would mean that OA has a 50–100-fold weaker effect than CA on PP1. OA and CA are reported to inhibit PP2A with a similar potency (45, 46). In the course of this experiment, we found that CA-treated cells became round and readily detached from culture dishes, as compared with findings in case of OA treatment. Thus, the GK71 immunoreactivity detected in the above experiments may reflect morphological change of the cells rather than phosphatase inhibition. To rule out this possibility, we did the same experiments using suspended and round NIH3T3 cells instead of those attached to culture dishes. Consequently, CA and OA showed comparable effects on GK71 reactivity of suspended and round NIH3T3 cells to findings in the cells attached to culture dishes (data not shown). From these data, taken together, we conclude that PP1 dephosphorylates Ser-71 of vimentin in intact interphase cells.

Effects of Rho Kinase Inhibitors and Expression of the Dominant Negative Form of Rho Kinase on Vimentin Phosphorylation in Response to Calyculin A—Recently, it has been reported that chemical compounds such as HA1077 and Y-27632 selectively inhibit the activity of Rho kinase (47–49). To confirm that Ser-71 of vimentin is phosphorylated by Rho kinase and not other kinases in interphase cells treated with CA, we investigated the effects of the two Rho kinase inhibitors. NIH3T3 cells were preincubated with these inhibitors (10 μM) for 30 min and then treated with CA and double-stained with α-vimentin and G7K1. When cells were preincubated with the inhibitors, CA-induced immunoreactivity of GK71 was completely blocked.
Phosphorylation/Dephosphorylation of Desmin and Vimentin

Phosphorylation of IF proteins in interphase cells. Our results strongly suggest that the constitutive phosphorylation of vimentin at Ser-71, in response to CA in interphase cells. By PP1, vimentin was found to be phosphorylated at Ser-71, in response to CA. In the present study, using newly developed site- and phosphorylation state-specific antibodies for Rho kinase phosphorylation sites, we have shown that Rho kinase phosphorylates vimentin in response to CA. We concluded that Rho kinase is recruited to the cleavage furrow and is sufficient for exerting its role in cytokinesis.

DISCUSSION

We earlier clarified that desmin can serve as a good substrate for Rho kinase in vitro (33). In the present study, using newly developed site- and phosphorylation state-specific antibodies for Rho kinase phosphorylation sites of desmin, Rho kinase was found to phosphorylate desmin specifically at the cleavage furrow during cytokinesis, as is the case with other type III IF proteins, glial fibrillary acidic protein, and vimentin (22, 23). We have found that Rho kinase itself accumulates at the cleavage furrow in Saos-2 human osteoblast cells, as was noted in U251 human glioma cells (42). Thus, it is a common phenomenon for all type III IF proteins that Rho kinase acts as a cleavage furrow kinase, and it is most likely that Rho kinase regulates the organization of these IFs during cytokinesis via a common molecular mechanism, which ensures effective segregation of IFs into daughter cells. Citron kinase, another Rho target protein with a kinase domain homologous to that of Rho kinase, was also found to localize to the cleavage furrow and the midbody of Hela cells (50). Citron kinase is thought to work in the contractile process rather than act reorganization (50). The different and redundant functions of Rho kinase and citron kinase in cytokinetic process remain to be elucidated. These two kinases may participate in different steps during cytokinesis.

CA and OA are potent inhibitors for PP1 and PP2A. CA and OA inhibit PP1 with a similar potency, whereas OA is 50–100-fold weaker than CA as a PP1 inhibitor. CA and OA are weakly inhibited by PP2A with a similar potency, whereas OA is 50–100-fold weaker than CA as an inhibitor. CA and OA inhibit PP2A with a similar potency, whereas OA is 50–100-fold weaker than CA as an inhibitor.

It has been reported that phosphatases play an essential role for the maintenance and structural integrity of IFs in interphase cells, determined using the protein phosphatase inhibitors CA and OA. CA and OA are potent inhibitors for PP1 and PP2A and are often used to demonstrate the involvement of PP1 and PP2A in a biological cellular processes. CA and OA inhibit PP2A with a similar potency, whereas OA is 50–100-fold weaker than CA as a PP1 inhibitor. CA and OA are weakly sensitive and completely insensitive to other phosphatases, protein phosphatases 2B and 2C, respectively. In the course of experiments on desmin/vimentin phosphorylation with site- and phosphorylation state-specific antibodies, we investigated the effects of CA and OA and determined that PP1 functions as a desmin/vimentin phosphatase. PP1 is known to play a pivotal role in the cell cycle and is regulated by the interaction of the catalytic subunit with a variety of regulatory proteins that have as an important role to localize the enzyme and to determine substrate specificity. The involvement of PP1 in IF integrity was suggested for vimentin when CA and OA were used (38). In neuronal cells, PP2A was identified to be a neurofilament-associated phosphatase that may preserve the filament

Fig. 8. Effects of Rho kinase inhibitors on vimentin phosphorylation in response to calyculin A. A, after preincubation of NIH3T3 cells with buffer (top panel) or 10 μM each of HA1077 and Y-27632 for 30 min (middle and bottom panels), cells were treated with 20 nM CA for 15 min and double-stained with α-vimentin (α-Vim) and GK71, as described under “Experimental Procedures.” Scale bars, 10 μm. B, dose-dependent effects of HA1077 and Y-27632 on the immunoreactivity of GK71. NIH3T3 cells were preincubated with indicated doses of HA1077 or Y-27632 for 30 min and then treated with 20 nM CA for 15 min. The cells were then collected and analyzed by immunoblotting.

Fig. 9. Effects of RB/PH(TT) on vimentin Ser-71 phosphorylation in response to CA. COIL (A) (as a control) or RB/PH(TT) (B) was microinjected into the nuclei of cells. After a 24-h culture, cells were treated with 20 nM CA for 15 min, as described under “Experimental Procedures,” and double-immunostained with 9E10 for Myc epitope-tagged RB/PH(TT) and COIL (middle panels) and GK71 (bottom panels). Top panels are transmittance images of the same frame as the middle and bottom panels. Arrows indicate cells expressing RB/PH(TT) or COIL, whereas arrowheads indicate neighboring, unmicroinjected cells. Scale bars, 10 μm.
tous structure of neurofilament (51). As for keratin 8/18, PP1 and/or PP2A is suggested to function in disassembly and reorganization (39, 40). Although protein phosphatases responsible for IF organization have been noted in some cell systems, as mentioned above, the kinase(s) that phosphorylates IFs in interphase cells is unknown. Only CaMK may be a candidate for a major role in keratin 8/18 phosphorylation in interphase cells (39). Utilizing a series of site- and phosphorylation state-specific antibodies, we have shown here that Rho kinase phosphorylates desmin and/or vimentin in interphase Saos-2, NIH3T3, and MDCK cells, but the phosphorylation was evident only after CA treatment. In comparison, vimentin phosphorylation by PKC and cdc2 kinase did not appear in NIH3T3 cells even after CA treatment, and the phosphorylation level by CaMKII was not altered by CA treatment. Furthermore, we confirmed that Rho kinase induces phosphorylation of vimentin in CA-treated interphase NIH3T3 cells, using Rho kinase inhibitors (47) and expression of the dominant-negative form of Rho kinase, RB/PH(TT) (20); the phosphorylation of the Rho-kinase specific site, Ser-71, on vimentin was completely blocked by pretreatment with Y-27632, HA1077 or the ectopic expression of RB/PH(TT). Thus, Rho kinase functions as an interphase IF kinase (although dephosphorylation activity of PP1 on desmin and vimentin is thought to be higher than the phosphorylation activity by Rho kinase in interphase cells).

The present study sheds light on the tightly balanced activity of Rho kinase and PP1 in interphase cells. As for the biological significance of IF protein phosphorylation by Rho kinase observed here, one can speculate the modulation of IF protein dynamics. Ectopically expressed or microinjected IF proteins were found to be incorporated into preexisting IFs along their entire surface (52, 53). Consistent with these observations, transient expression of assembly-deficient mutant keratin in epithelial cells (54) and microinjection of peptides derived from IF sequence motifs essential for IF assembly (55) disrupted the endogenous IF system. These results clearly indicate that IFs are highly dynamic, and a continuous exchange of subunit proteins occurs on the entire filament surface between a soluble pool and the polymerized IFs, reaching a steady state equilibrium (56). Mechanisms regulating the equilibrium state are unknown. Based on the results observed in this study, we consider that Rho kinase is one candidate that, together with PP1, modulates the equilibrium between a soluble IF protein pool and a polymerized protein pool. The balance of Rho kinase and PP1 activities seems essential not only for modulating IF structure and plasticity but also for cell-substrate adhesion, cell motility, and actin reorganization in interphase cells. Further analyses are required to clarify the molecular mechanism of the concerted action of Rho kinase and PP1 in individual cellular processes, including IF protein dynamics in interphase.

As for the effects of CA and OA observed on Rho kinase activity, it must be noted that CA and OA act as strong tumor promoters. It is possible that CA, and maybe OA, causes tumor promotion by disturbing the balance between Rho kinase and PP1 activities. If such is indeed the case, increments in Rho kinase activity by diminishing PP1 may explain some characteristic features of cancer cells, including abnormal growth, invasion, and metastasis.

Acknowledgments—We thank Yoshitomi Pharmaceutical Industries, Ltd. for kindly providing Y-27632, Drs. H. Tsuiki and H. Saya (Kumamoto University) for providing Saos-2 cells, and Dr. H. Kosako (our laboratory) for preparing the anti-Rho kinase antibody. We are grateful to K. Kuromiya for secretarial services, K. Hara for technical assistance, and M. Ohara for a critique of the manuscript.

REFERENCES
1. Van Aelst, L., and D’Souza-Schorey, C. (1997) Genes Dev. 11, 2295–2322
2. Hall, A. (1998) Science 279, 509–514
3. Kaibuchi, K., Kuroda, S., and Amano, M. (1999) Annu. Rev. Biochem. 68, 459–486
4. Amano, M., Mukai, H., Ono, Y., Chikara, K., Mastui, T., Hamajima, Y., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1996) Science 271, 648–650
5. Watanabe, G., Saito, Y., Chikara, K., Fujisawa, K., Morii, N., Mukai, H., Ono, Y., Kikizuka, A., and Narumiya, S. (1996) Science 271, 645–648
6. Matsui, T., Amano, M., Yamamoto, T., Chikara, K., Nakafuku, M., Ito, M., Nakano, T., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1996) EMBO J. 15, 2208–2216
7. Leung, T., Manser, E., Tan, L., and Lim, L. (1995) J. Biol. Chem. 270, 29051–29054
8. Ishizaki, T., Maekawa, M., Fujisawa, K., Okawa, K., Iwamatsu, A., Fujita, A., Watanabe, N., Saito, Y., Kikizuka, A., Morii, N., and Narumiya, S. (1996) EMBO J. 15, 1885–1893
9. Madanlur, P., Furuyashiki, T., Reid, T., Ishizaki, T., Watanabe, G., Morii, N., and Narumiya, S. (1995) FEBS Lett. 377, 243–248
10. Reid, T., Furuyashiki, T., Ishizaki, T., Watanabe, G., Nakafuku, N., Matsui, T., and Kaibuchi, K. (1996) Science 273, 13556–13560
11. Watanabe, N., Madanlur, P., Reid, T., Ishizaki, T., Watanabe, G., Kikizuka, A., Saito, Y., Nakano, K., Jockusch, B. M., and Narumiya, S. (1996) EMBO J. 15, 2202–2208
12. Kimura, K., Ito, M., Amano, M., Chikara, K., Fukuta, Y., Nakafuku, M., Yamamoto, B., Fung, J., Nakano, T., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1996) Science 273, 1208–1211
13. Leung, T., Manser, E., Tan, L., and Lim, L. (1995) Mol. Cell. Biol. 16, 5313–5327
14. Ishizaki, T., Saito, Y., Fujisawa, K., Maekawa, M., Watanabe, N., Saito, Y., and Narumiya, S. (1995) FEBS Lett. 384, 131–134
15. Kosako, H., Amano, M., Chikara, K., Kimura, K., Fukuta, Y., Nakafuku, N., Yano, T., Shihata, M., Tokui, T., Ichikawa, H., Ikebe, R., Ikebe, M., and Kaibuchi, K. (1997) J. Biol. Chem. 272, 25121–25127
16. Leung, T., Chen, X.-Q., Manser, E., and Lim, L. (1996) Mol. Cell. Biol. 16, 29051–29054
17. Kimura, K., Ito, M., Amano, M., Chikara, K., Fukuta, Y., Nakafuku, M., Yamamoto, B., Fung, J., Nakano, T., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1996) Science 273, 13556–13560
18. Amano, M., Chikara, K., Nakamura, N., Fukuta, Y., Yano, T., Shihata, M., Ikebe, M., and Kaibuchi, K. (1998) Genes to Cells 3, 177–188
19. Hirase, M., Ishizaki, T., Watanabe, N., Uehata, M., Kranenburg, O., Moolenaar, W. H., Matsunaga, F., Matsumoto, M., Bitto, H., and Narumiya, S. (1998) J. Cell Biol. 141, 1625–1636
20. Hirasawa, M., Kikuzaki, K., Nakamura, H., Saito, Y., Chikara, K., and Kaibuchi, K. (1996) J. Biol. Chem. 271, 11728–11736
21. Yasui, Y., Amano, M., Nagata, K., Inagaki, N., Nakafuku, N., Saya, H., Nakano, T., and Kaibuchi, K. (1998) J. Cell Biol. 143, 1249–1258
22. Yoshii, K., Saito, Y., Nakamura, H., Saya, H., Fujisawa, K., and Kaibuchi, K. (1995) J. Biol. Chem. 270, 2795–2798
23. Goto, H., Kosako, H., Tanabe, K., Yanagida, M., Sakurai, M., Amano, M., and Saito, Y. (1995) J. Cell Biol. 133, 141–149
24. Inagaki, M., Sakurai, M., and Saito, Y. (1997) J. Biol. Chem. 272, 25195–25199
25. Inagaki, M. (1994) Science 264, 647–652
26. Eriksson, J. E., Opel, P., and Goldman, R. D. (1992) Curr. Opin. Cell Biol. 4, 99–104
27. Fujisawa, K., and Inagaki, M. (1996) Science 270, 647–652
28. Toivola, D. M., Omary, M. B., Ku, N-O., Peltola, O., Baribault, H., and Eriksson, J. E. (1998) J. Cell Biol. 140, 647–657
29. Fujikawa, K., Inagaki, M., Saka, H., Fujisawa, K., and Kaibuchi, K. (1996) J. Biol. Chem. 271, 245–248
44. Nishizawa, K., Yano, T., Shibata, M., Ando, S., Saga, S., Takahashi, T., and Inagaki, M. (1991) J. Biol. Chem. 266, 3074–3079
45. Ishihara, H., Martin, B. L., Brautigan, D. L., Karaki, H., Ozaki, H., Kato, Y., Fusetani, N., Watabe, S., Hashimoto, K., Uemura, D., and Hartshorne, D. J. (1989) Biochem. Biophys. Res. Commun. 159, 871–877
46. Takai, A., Sasaki, K., Nagai, H., Mieskes, G., Isobe, M., Isono, K., and Yasumoto, T. (1995) Biochem. J. 306, 657–663
47. Uehata, M., Ishizaki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, T., Tamakawa, H., Yamagami, K., Inui, J., Maekawa, M., and Narumiya, S. (1997) Nature 389, 990–994
48. Itoh, K., Yoshikawa, K., Akedo, H., Uehata, M., Ishizaki, Y., and Narumiya, S. (1999) Nat. Medicine 5, 221–225
49. Sahai, E., Ishizaki, T., Narumiya, S., and Treisman, R. (1999) Curr. Biol. 9, 136–145
50. Madaule, P., Eda, M., Watanabe, N., Fujisawa, K., Matsuoka, T., Bito, H., Ishizaki, T., and Narumiya, S. (1998) Nature 394, 491–494
51. Saito, T., Shima, H., Osawa, Y., Nagao, M., Hemmings, B. A., Kishimoto, T., and Hisanaga, S. (1995) Biochemistry 34, 7376–7384
52. Ngai, J., Coleman, T. R., and Lazarides, E. (1990) Cell 60, 415–427
53. Sarria, A. J., Nordeen, S. K., and Evans, R. M. (1990) J. Cell Biol. 111, 553–565
54. Albers, K., and Fuchs, E. (1987) J. Cell Biol. 105, 791–806
55. Goldman, R. D., Khun, S., Chou, Y. H., Opal, P., and Steinert, P. M. (1996) J. Cell Biol. 134, 971–983
56. Foisner, R. (1997) BioEssays 19, 297–305
Balance between Activities of Rho Kinase and Type 1 Protein Phosphatase Modulates Turnover of Phosphorylation and Dynamics of Desmin/Vimentin Filaments

Hiroyasu Inada, Hideaki Togashi, Yu Nakamura, Kozo Kaibuchi, Koh-ichi Nagata and Masaki Inagaki

J. Biol. Chem. 1999, 274:34932-34939.
doi: 10.1074/jbc.274.49.34932

Access the most updated version of this article at http://www.jbc.org/content/274/49/34932

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 56 references, 34 of which can be accessed free at http://www.jbc.org/content/274/49/34932.full.html#ref-list-1