The formation and directional guidance of neurites involves dynamic regulation of Rho family GTPases. Rac and Cdc42 promote neurite outgrowth, whereas Rho activation causes neurite retraction. Here we describe a role for collapsin response mediator protein (Crmp-2), a neuronal protein implicated in axonal outgrowth and a component of the semaphorin 3A pathway, in switching GTPase signaling when expressed in combination with either dominant active Rac or Rho. In neuroblastoma N1E-115 cells, co-expression of Crmp-2 with dominant active RhoA V14 induced Rac morphology, cell spreading and ruffling (and the formation of neurites). Conversely, co-expression of Crmp-2 with dominant active Rac1 V12 inhibited Rac morphology, and in cells already expressing Rac1 V12, Crmp-2 caused localized peripheral collapse, involving Rho (and Cdc42) activation. Rho kinase was a pivotal regulator of Crmp-2; Crmp-2 phosphorylation was required for Crmp-2/Rac1 V12 inhibition, but not Crmp-2/RhoA V14 induction, of Rac morphology. Thus Crmp-2, regulated by Rho kinase, promotes outgrowth and collapse in response to active Rho and Rac, respectively, reversing their usual morphological effects and providing a mechanism for dynamic modulation of growth cone guidance.

The Rho GTPase family, which regulates the actin cytoskeleton, has been shown to be involved in processes of neurite outgrowth. In neuroblastoma N1E-115 cells, Rac1 promotes the formation of lamellipodia, and Cdc42 promotes the formation of filopodia, which have a sensory role in growth of neurites (1). Cdc42 signaling to Rac1 can also occur (2, 3). Rho acts antagonistically to Rac and Cdc42 in N1E-115 cells inducing growth cone collapse, neurite retraction, and cell rounding (1, 4). In primary neurons and PC12 cells, neurites are formed in response to inhibition of Rho or ROCK1 (5, 6). The directional growth of axons and dendrites in embryonic nervous system is influenced by a series of guidance cues (7). Semaphorin 3A (Sema 3A) induces growth cone collapse in chick dorsal root ganglia (DRG) neurones, a response mediated by Rac 1 (8) acting through its effectors (9). Sema 3A can also act as an attractant for cortical apical dendrites (10). Collapsin response mediator protein-2 (Crmp-2), which is expressed in post-mitotic neurones (11), is implicated in the Sema 3A response (12) and is one of a family of proteins related to the Caenorhabditis elegans Unc 33 gene involved in axonal outgrowth (13). Crmp-2 is a brain substrate of ROCK (14), and it also interacts with α2-chimaerin, a neuronal Rac regulator that promotes neuritogenesis (15).

We have found that expression of Crmp-2 in neuroblastoma N1E-115 cells can alter Rho-GTPase-driven morphology. Co-expression of Crmp-2 with dominant active RhoA V14 promoted cell spreading and neurite formation, mediated by Rac. Co-expression of Crmp-2 with dominant active Rac1 V12 inhibited Rac morphology, an effect that was blocked by mutation of a single ROCK phosphorylation site in Crmp-2 or by kinase-inactive ROCK. Microinjection of Crmp-2 in Rac1 V12 expressing cells with pre-existing Rac morphology led to ROCK-dependent localized collapse of the Rac phenotype. Crmp-2, in combination with active Rho or Rac GTPases, can thus effect a cyclical switch in signaling in which ROCK has a regulatory role, promoting dynamic shape changes.

**EXPERIMENTAL PROCEDURES**

**cDNA Cloning and Site-directed Mutagenesis—**Rat Crmp-2 cDNA was obtained by polymerase chain reaction from rat brain cDNA, the coding sequence (nucleotides 178–1896) was cloned into BamHI/EcoRI sites of pBSII SK+ (Stratagene) and glutathione S-transferase fusion vector pRP281, and insert DNA was sequenced. For mammalian expression Spel/KpnI fragment purified from pBSII SK+ Crmp-2, blunted with T4 DNA polymerase, was cloned into the Smal site of pXJ40HA, pXJ40GFP, and pXJ40FLAG (3, 16). Crmp-2 T555A mutation was made in the pXJ40FLAG construct using the Quik-change mutagenesis kit (CLONTECH). Rac1 V12, RhoA V14, Rac1 N71, RhoA N19, Cdc42 N71, and ROCK constructs in pXJ40 (HA-, GFP-, or FLAG-tagged) were all as described previously (5, 15, 16).

**Cell Culture, Microinjection, Transfection, and Immunocytochemistry—**Neuroblastoma N1E-115 or Swiss 3T3 cells were cultured in Dulbecco’s modified Eagle’s medium, 10% fetal calf serum (2, 17). For transfection N1E-115 cells (105/ml) were plated on glass slides coated in laminin (10 µg/ml) and transfected using LipofectAMINE (15). Swiss 3T3 cells were grown on acid-washed coverslips, coated with poly-L-lysine (5 µg/ml). DNA was injected at 50 µg/ml into the nucleus using an Eppendorf microinjector and Zeiss Axiovert microscope with the CO2 chamber at 37 °C, and cells were fixed and stained after 5 h with rhodamine-conjugated phallolidin for filamentous actin and anti-vinculin (Sigma). Other antibodies used were anti-HA (Roche Molecular Biochemicals), anti-GFP (CLONTECH), anti-FLAG (Sigma), and fluorescein isothiocyanate- and Cy5-conjugated secondary antibodies (Amersham Pharmacia Biotech). Crmp-2 rabbit polyclonal antiserum was generated in rabbits by the Glaxo/Singapore research fund. The costs of publication of this paper were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Crmp-2 Switches RhoA and Rac1 Morphology

M. Brown, G. Ferrari, T. Leung, S. Govind, and C. Hall, unpublished results.

Raised using recombinant Crmp-2 protein thrombin-cleaved from glutathione S-transferase fusion protein. Confocal microscopy was carried out using a Zeiss LSM 410 confocal microscope. For time lapse analysis neuroblastoma cells were microinjected with cDNA constructs (1 μg/ml) and recorded for 2–5 h on an Axiovert 135 microscope (Zeiss) in an atmosphere of humidified air and 5% CO₂ at 37 °C with a Palmax TM-6CN video camera and Sony-u-matic VO5800PS video recorder.

RESULTS

Crmp-2 Alters the Morphology Elicited by Rho Family Proteins—The participation of Crmp-2 in the Rac-dependent Sema 3A signaling pathway (8, 12) and its interaction with α2-chimaerin (15), suggested that Crmp-2 could have a functional relationship with Rac GTPase. This was investigated using neuroblastoma N1E-115 cells in which GTPase-dependent morphology has been characterized (1, 17, 18). On transfection Crmp-2 was present in the cell body with a punctate vesicular or membrane distribution and in peripheral ruffles, together with F-actin (Fig. 1A, a). Crmp-2 cDNA alone had little effect on cell morphology (Fig. 1A, a and e); however, co-transfection of Crmp-2 with constitutionally active Rac1 V12 resulted in inhibition of Rac morphology (Fig. 1A, e). Cells were flattened with microspikes and varied peripheral morphology (Fig. 1A, c and d) at the expense of the typical ruffled Rac1 V12 phenotype (Fig. 1A, b). There was no increase in formation of neurites (in cells grown in serum).

Dynamic Effects of Crmp-2 on Rac1-dependent Morphology—To examine the dynamic relationships between Crmp-2 and Rac1 V12, cells were microinjected with the appropriate cDNAs and examined for a period of 5–7 h. Co-injection of Crmp-2 cDNA with Rac1 V12 cDNA inhibited formation of the usual Rac phenotype (result not shown).

In cells first transfected (or microinjected) with GFP-Rac1 V12 cDNA to establish Rac morphology, the subsequent injection of Crmp-2 cDNA led to selective, localized collapse of peripheral ruffling with the formation of branched, fine processes and long microspikes (see Fig. 1B, a; cf. Fig. 1A, d). This regionalized collapse generating microspikes and minor filopodial outgrowth occurred over a short time scale (within 20–30 min) at an interval of 2 h after injection with Crmp-2 cDNA, suggesting that Crmp-2 acts downstream of Rac activation. To determine whether these changes in morphology involved activity of the other Rho family GTPases, GFP-Rac1 V12-expressing cells were microinjected with Crmp-2 cDNA in the presence of either dominant inactive RhoA N19 or Cdc42 N17 (Fig. 1B). Co-injection of Crmp-2 cDNA with RhoA N19 cDNA into cells exhibiting Rac1 V12 morphology led to the formation of numerous small microspikes, but there was no localized peripheral collapse (Fig. 1B, b), indicating that activation of RhoA is involved in Crmp-2 effects on Rac morphology. Co-injection of Crmp-2 cDNA, together with Cdc42 N17 cDNA, into Rac1 V12-expressing cells led to retraction of the peripheral ruffle and cell rounding (a Rho effect), but no microspikes were formed (Fig. 1B, c). Microinjection of either RhoA N19 or Cdc42 N17 cDNA alone did not significantly affect pre-existing Rac morphology (Fig. 1B, d and e). It appears that in cells exhibiting Rac morphology, expression of Crmp-2 induces activation of Rho pathways and also of Cdc42.

Crmp-2 Switches RhoA Signaling to Rac—Crmp-2 had a marked effect when co-expressed with dominant active RhoA V14. Instead of the round N1E-115 cell morphology typical of RhoA V14 expression (Fig. 2A, a), co-transfection of Crmp-2 with RhoA V14 led to cell spreading and the formation of ruffles, microspikes, and neurites (Fig. 2A, b–e), indicative of activation of Rac (and Cdc42). RhoA V14 and Crmp-2 co-localized, together with F-actin, in neurites and in peripheral ruffles of the typical ruffled Rac1 V12 phenotype (Fig. 2A, b). There was no increase in formation of neurites (in cells grown in serum).

The dynamic effects of Crmp-2 and RhoA V14 were investigated in microinjected cells by time lapse microscopy (Fig. 2B). Co-injection of Crmp-2 and RhoA V14 led to cell spreading and the formation of a Rac/Cdc42 phenotype, and cells underwent peripheral shape changes over several hours. Initially, there was also rapid extrusion and retraction of membrane protuberances (Fig. 2R, arrow). Cells co-expressing RhoA V14 and Crmp-2 showed stronger Crmp antibody staining, particularly in regions of membrane protrusion (result not shown).

The effect of Crmp-2 on Rho signaling was also investigated in Swiss 3T3 cells in which Rho activation is indicated...
Crmp-2 Switches RhoA and Rac1 Morphology

FIG. 2. Co-expression of Crmp-2 with RhoA V14 promotes Rac morphology. A, N1E-115 cells transfected with pXJ40FLAG-RhoA V14 cDNA (a) or with FLAG-RhoA V14 and GFP-Crmp-2 (b and c) were stained with anti-FLAG antibody, Cy5-conjugated secondary antibody, and TRITC-phalloidin as described (15). Scale bar, 10 μm. Quantification of the formation of Rac phenotypes (black columns) (d and e) and neurites >1 cell diameter (open columns) (d) are shown. The effects of co-transfected dominant negative Rac1 N17 and dominant negative Cdc42 N17 in combination with RhoA V14/Crmp-2 on the Rac phenotype (black columns) were quantified (e). Results were obtained from three separate experiments. Error bars represent S.E. B, time lapse analysis (phase contrast) of N1E-115 cell microinjected with GFP-RhoA V14 and pXJ40HA-Crmp-2. Cells were injected at time 0 and observed for 5.5 h.

Crmp-2 Activity Is Regulated by Phosphorylation—Crmp-2 is a substrate of Rho kinase, which phosphorylates Crmp-2 at Thr-555 (14). Crmp-2 is also slightly phosphorylated in the presence of activated Rac and Cdc42 (14). To determine whether the effects of Crmp-2 in response to RhoA V14 required its phosphorylation by ROK, we substituted Crmp-2 threonine 555 with alanine (shown to eliminate ROK phosphorylation (14)). Surprisingly, the T555A mutated Crmp-2 completely blocked the Crmp-2/Rac1 V12 inhibition of Rac morphology (Fig. 3C) but not Crmp-2/RhoA V14 promotion of Rac morphology (Fig. 3D) in N1E-115 neuroblastoma cells. Similarly in Swiss 3T3 cells the T555A mutated Crmp-2 failed to inhibit the Rac1 V12-induced loss of stress fibers/focal adhesions (Fig. 3B). In combination with RhoA V14, Crmp-2 T555A was as effective as Crmp-2 in promoting Cdc42/Rac morphology (microspikes, ruffles, and loss of stress fibers) (Fig. 3A). Co-expression of kinase-inactive ROK (6) with Rac1 V12/Crmp-2 also abolished the inhibitory effect on Rac inhibition (Fig. 3C), indicating that phosphorylation by ROK can regulate Crmp-2. RhoA N19 co-transfected with Rac1 V12/Crmp-2 had a more limited effect on Rac inhibition (Fig. 3C). Taken together these results indicate that Crmp-2, in combination with active Rho/Rac GTPases, can reciprocally exchange Rho and Rac signaling and is subject to regulation by ROK.

FIG. 3. ROK phosphorylation of Crmp-2 is required for its inhibition of Rac1 V12 but not RhoA V14. A, Swiss 3T3 fibroblasts in low serum were microinjected with Rho A V14 alone, with RhoA V14 + Crmp-2, with RhoA V14 + Crmp-2 T555A, or with Crmp-2 alone, and filamentous actin was stained with TRITC-phalloidin. Microinjected cells were detected by GFP fluorescence (arrows). B, Swiss 3T3 fibroblasts in serum (10%) were microinjected with Rac1 V12 alone, Rac1 V12 + Crmp-2, Rac1 V12 + Crmp-2 T555A, and Crmp-2 alone, or they were uninjected. Cells were fixed and stained after 5 h with antivinculin and detected with Cy5-conjugated secondary antibody and TRITC-phalloidin. GFP was used as a marker of injected cells (arrows). C, N1E-115 neuroblastoma cells were transfected with Rac1 V12 alone, Rac1 V12 + Crmp-2, Rac1 V12 + Crmp-2 T555A, and Crmp-2 alone, or they were uninjected. Cells were fixed and stained after 5 h with antigF-actin and detected with Cy5-conjugated secondary antibody and TRITC-phalloidin. GFP was used as a marker of injected cells (arrows). D, N1E-115 cells were transfected with RhoA V14 alone or in combination with Crmp-2 or Crmp-2 T555A. Quantification of the percentage of transfected cells showing Rac phenotypes (black columns) was determined from two to three experiments. Error bars represent S.E.

DISCUSSION

In N1E-115 cells Rho and Cdc42/Rac have antagonistic effects on cell morphology. Rho (and ROK) activation cause neurite retraction and cell rounding, whereas Rac/Cdc42 activity promotes cell flattening and neurite outgrowth (1, 4, 18).
NIH3T3 fibroblasts also show a reciprocal balance between Rac and Rho activation (21). We have found that Crmp-2, in combination with dominant active RhoA V14, can generate Rac morphology, a switch in GTPase signaling. This result is unexpected; activation of Rac (and Cdc42) in response to Rho activation does not usually occur, although there is some activation of Rho in a hierarchy downstream of Rac in Swiss 3T3 cells (22). In the absence of dominant active RhoA V14, Crmp-2 alone had little effect, indicating this is a positive signaling event. The morphological changes generated by Crmp-2 with RhoA V14 were blocked by co-expression of dominant negative Rac1 N17 in N1E-115 cells, but to a much lesser extent by Cdc42 N17, indicating that Rac is activated independently of Cdc42.

Conversely, in N1E-115 cells already expressing dominant active Rac1 V12, microinjection of Crmp-2 caused peripheral collapse and formation of microspikes, which could be blocked by co-injection of RhoA N19 and Cdc42 N17, respectively. The formation of Rac morphology was inhibited by co-expression of Crmp-2 with Rac1 V12. These effects are consistent with inhibition of Rac-GTP and subsequent activation of Rho (and Cdc42). Crmp-2 thus effects a reversible switch between Rho and Rac signaling pathways, which could contribute to the dynamics of growth cone guidance. Sema 3A-induced growth cone collapse in sensory neurones is Rac-dependent (8, 9). Our results suggest that Crmp-2, a component of the Sema 3A pathway (12), can initiate collapse downstream of Rac activation.

The activity of Crmp-2 in promoting Rac inhibition appears to be regulated by phosphorylation by ROK, because mutation of Crmp-2 at threonine 555, or co-expression of kinase-inactive ROK with Crmp-2, prevented the RacV12-dependent Crmp-2 effects. In contrast, RhoA V14/Crmp-2 generation of a Rac phenotype was not inhibited by the T555A mutation. Phosphorylation of Crmp-2 T555 by ROK thus provides a dynamic regulatory mechanism. In axonal outgrowth in which Rac/Cdc42 signaling may predominate, growth cone collapse could be initiated by Crmp-2 activated locally (in response to guidance signals) by ROK. Inhibition of active Rac could lead to amplification of Rho-dependent collapse, an activity rapidly terminated by dephosphorylation, reactivating the Rac signaling, which can occur in response to active Rho and Crmp-2. Immature dendrites are extremely dynamic, changing their structure over seconds (23). It may be relevant that threonine 555 in Crmp-2 is also one of two consensus sites for cGMP-dependent protein kinase phosphorylation. The signaling of guidance factors can be switched from a repulsive to an attractive cue by altering local concentrations of cAMP/cGMP (24). Sema 3A acts as a chemoattractant in cortical apical dendrites but not axons, a response mediated by the asymmetrical localization of soluble guanylate cyclase, elevating local cGMP levels (10) with the potential to activate cGMP-dependent protein kinase phosphorylation. The time course of Rac1 V12/Crmp-2 effects in N1E-115 cells does not preclude the involvement of an autocrine pathway, although in preliminary experiments conditioned media from cells expressing Crmp-2 for 24 h did not affect morphology of Rac1 V12- or RhoA V14-expressing cells.

Crmp-2 is one of a family of Crmp proteins widely expressed in the developing nervous system (25) and persisting in adult brain (26) that could function (possibly as heteromers) in response to other signaling cues. The threonine 555 phosphorylation site is not conserved in the other Crmp proteins, providing some specificity in regulation of Crmp-2/Crmp heteromers. Sema 3A binds neuropilin and its co-receptor plexin A1 (28). Recently, activated Rac has been shown to bind directly to plexin B1 (29–31). Clustering of these receptors (in the absence of Rac binding) was sufficient to activate Rho signaling (31); however, the Rac dependence of the subsequent Rho morphology could implicate a Crmp-like component. Interestingly, myelin-induced growth cone collapse is inhibited by dominant active Rho (32) (rather than dominant negative Rac N17 as with Sema 3A (8)), which also caused extensive outgrowth of motor neuron axons on a myelin substrate (32).

In DRG neurones Crmp-2 is phosphorylated by ROK in response to LPA but not Sema 3A, and mutation of Crmp-2 at Thr–555 inhibited LPA-induced collapse (14), pointing to a role for Crmp-2 (phosphorylated by ROK) in the LPA collapse. However, this inhibition was only partial (14), which could result from a balance between Rac- and Rho-driven morphological effects regulated by Crmp-2. Ephrin-A5-induced collapse of retinal growth cones, by a Rho/ROK-dependent pathway (33), was also only partially inhibited by Rho and ROK inhibitors. In the Rac-dependent Sema 3A pathway in DRG neurones, phosphorylation of Crmp-2 by ROK was not detected, but it may be phosphorylated by other kinases (14) and/or complexed with other proteins. The effect of the Crmp-2 T555A mutant in Sema 3A-induced growth cone collapse was not described. The dynamics of Sema 3A-induced collapse can produce transient changes in phosphorylation status, with the phosphorylation of cofilin by LIM kinase (activated downstream of ROK or PAK), which is necessary for growth cone collapse (34).

The mechanism by which Crmp-2 alters Rho family GTPase activity is unclear, but Crmp is related to dihydroxyprymidine (11, 35) and has some sequence similarity to guanine aminohydrolase (36), although no enzyme activity has so far been identified (12). Its effects in Swiss 3T3 fibroblasts argue in favor of a direct effect of Crmp-2 on Rho/ROK or alternatively that other components of Crmp-2 signaling pathways are not restricted to neuronal cell types. COS cells transfected with neuropilin neuropilin-1/plexin co-receptors show Sema 3A-induced collapse (28), and neuropilin-1, a receptor for the angiogenesis factor vascular endothelia growth factor, as well as Sema 3A, is also expressed in endothelial and tumor cells (37). It is possible that Crmp-2 directly inhibits ROK through its interaction with activated ROK or Rho-GTP.2 Co-expression of Crmp-2 with Rac1 V12, but not RhoA V14, displaces Crmp-2 from the membrane.3 In the Rac-dependent Sema 3A pathway in neurones, endogenous Crmp-2 may become relocated and/or complexed with other proteins and act upstream of Rho. Guanine exchange factors such as Trio, a dual specificity Rho/Rac guanine exchange factor involved in axonal guidance (38), or GTPase-activating protein may also participate in modulation of Rho and Rac activity. Chimaerin (with which Crmp-2 can interact) itself affects growth cone dynamics (17). Crmp-2, in combination with either active Rho or Rac GTPases, and pivoitally regulated by ROK, can switch GTPase signaling, which has implications for the dynamics of neuronal growth cone guidance.

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REFERENCES
1. Kozma, R., Sarner, S., Ahmed, S., and Lim, L. (1997) Mol. Cell. Biol. 17, 1203–1211
2. Kozma, R., Ahmed, S., Best, A., and Lim, L. (1995) Mol. Cell. Biol. 15, 1942–1952
3. Manier, F., Lee, T.-H., Koh, C.-G., Zhao, Z.-S., Chen, X.-Q., Tan, L., Tan, I., Leung, T., and Lim, L. (1998) Mol. Cell. 1, 183–192
4. Tigg, G., Fischer, D. J., Nebok, A., Marshall, F., Dyer, D. L., and Miledi, R. (1996) J. Neurochem. 66, 549–558
5. Chen, X.-Q., Tan, I., Leung, T., and Lim, L. (1999) J. Biol. Chem. 274, 1901–19095
6. Bitto, H., Furuyashiki, T., Ishihara, H., Shibasaki, Y., Ohashi, K., Minami, K., Maekawa, M., Isihaki, T., and Narumiya, S. (2000) Neuron 28, 431–441
7. Tessier-Lavigne, M., and Goodman, C. S. (1996) Science 274, 1123–1133
8. Jin, Z., and Strittmater, S. M. (1997) J. Neurosci. 17, 6256–6263
9. Vastrick, I., Kielkott, B. J., Walsh, P. S., Ridley, A., and Doherty, P. (1999) Curr.
Biol. 9, 991–997
10. Polleux, F., Morrow, T., and Ghosh, A. (2000) Nature 404, 567–573
11. Minturn, J. E., Fryer, H. J. L., Geschwind, D. H., and Hockfield, S. (1995) J. Neurosci. 15, 6767–6766
12. Goshima, Y., Nakamura, F., Strittmater, P., and Strittmater, S. M. (1995) Nature 376, 509–514
13. Li, W., Herman, R. K., and Shaw, J. E. (1992) Genetics 132, 675–689
14. Goshima, Y., Nakamura, F., Strittmater, P., and Strittmater, S. M. (1995) Nature 376, 509–514
15. Li, W., Herman, R. K., and Shaw, J. E. (1992) Genetics 132, 675–689
16. Arimura, N., Inagaki, N., Chihara, K., Menager, C., Nakamura, N., Amano, M., Iwamatsu, A., Goshima, Y., and Kaibuchi K. (2000) J. Biol. Chem. 275, 23973–23980
17. Hall, C., Michael, G. J., Cann, N., Ferrari, G., Teo, M., Jacobs, T., Monfries, C., and Lim, L. (2001) J. Neurosci. 21, 5191–5202
18. Manser, E., Huang, H.-Y., Loo, T.-H., Chen, X.-Q., Dong, J.-M., Leung, T., and Lim, L. (1997) Mol. Cell. Biol. 17, 1129–1143
19. Kozma, R., Ahmed, S., Best, A., and Lim, L. (1996) Mol. Cell. Biol. 16, 5069–5080
20. van Leeuwen, F. N., Kain, H. E. T., van der Kammen, R. A., Michaels, F., Kranenburg, O. W., and Collard, J. G. (1997) J. Cell Biol. 139, 797–807
21. Ridley, A. J., and Hall, A. (1992) Cell 70, 389–399
22. Ridley, A. J., Patterson, H. F., Johnson, C. L., Diekman, D., and Hall, A. (1992) Cell 70, 401–410
23. Sander, E. E., ten Klooster, J. P., van Delft, S., van der Kammen, R. A., and Collard, J. G. (1999) J. Cell Biol. 147, 1009–1022
24. Nobes, C. D., and Hall, A. (1995) Cell 81, 53–62
25. Wang, L.-H., and Strittmater, S. M. (1996) J. Neurosci. 16, 6197–6207
26. Kamata, T., Subleski, M., Hara, Y., Yuhki, N., Kung, H., Copeland, N. G., Jenkins, N. A., Yoshimura, T., Modi, W., and Copeland, T. D. (1998) Mol. Brain. Res. 54, 219–236
27. Wang, L.-H., and Strittmater, S. M. (1997) J. Neurochem. 69, 2261–2269
28. Takahashi, T., Fournier, A., Nakamura, F., Wang, L.-H., Murakami, Y., Kalb, R. G., Fujisawa, H., and Strittmater, S. (1999) Cell 99, 59–69
29. Rohm, B., Rahim, B., Kleiber, B., Hevatta, L., and Puschel, A. W. (2000) FEBS Lett. 46, 68–72
30. Vikis, H. G., Li, W., He, Z., and Guan, K.-L. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 12457–12462
31. Driessens, M. H. E., Hu, H., Nobes, C. D., Self, A., Jordens, I., Goodman, C. S., and Hall, A. (2001) Curr. Biol. 11, 339–344
32. Kuhn, T. B., Brown, M. D., Wilcox, C. L., Raper, J. A., and Bamburg, J. R. (1999) J. Neurosci. 9, 1865–1875
33. Wahl, S., Barth, H., Cloos, T., Aktories, K., and Mueller, B. K. (2000) J. Cell Biol. 149, 263–270
34. Aizawa, H., Wakatsuki, S., Ishii, A., Moriyama, K., Sasaki, Y., Ohashi, K., Sekine-Aizawa, Y., Sekihara-Fujisawa, A., Mizuno, K., Goshima, Y., and Yahara, I. (2001) Nat. Neurosci. 4, 367–373
35. Hamajima, N., Matsuda, K., Sakata, S., Tamaki, N., Sasaki, M., and Nonaka, M. (1996) Gene 180, 157–163
36. Yuan, G., Bin, J. C., McKay, D. J., and Snyder, F. F. (1999) J. Biol. Chem. 274, 8175–8180
37. Sokol, S., Takashima, S., Miao, H. Q., Neufeld, G., and Klagsbrun, M. (1998) Cell 92, 735–745
38. Newsome, T. P., Schmidt, S., Dietzl, G., Keleman, K., Asling, B., Debant, A., and Dickson, B. J. (2000) Cell 101, 283–294