Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 340 (2006) 62–68
BBRC
www.elsevier.com/locate/ybbrc

Ras regulates neuronal polarity via the PI3-kinase/Akt/GSK-3β/CRMP-2 pathway

Takeshi Yoshimura, Nariko Arimura, Yoji Kawano, Saeko Kawabata, Shujie Wang, Kozo Kaibuchi *

Department of Cell Pharmacology, Graduate School of Medicine, Nagoya University, 65 Tsurumai, Showa-ku, Nagoya, Aichi 466-8550, Japan

Received 18 November 2005
Available online 6 December 2005

Abstract

The establishment of a polarized morphology is an essential event in the differentiation of neurons into a single axon and dendrites. We previously showed that glycogen synthase kinase-3β (GSK-3β) is critical for specifying axon/dendrite fate by the regulation of the phosphorylation of collapsin response mediator protein-2 (CRMP-2). Here, we found that the overexpression of the small GTPase Ras induced the formation of multiple axons in cultured hippocampal neurons, whereas the ectopic expression of the dominant negative form of Ras inhibited the formation of axons. Inhibition of phosphatidylinositol-3-kinase (PI3-kinase) or extracellular signal-related kinase (ERK) kinase (MEK) suppressed the Ras-induced formation of multiple axons. The expression of the constitutively active form of PI3-kinase or Akt (also called protein kinase B) induced the formation of multiple axons. The overexpression of Ras prevented the phosphorylation of CRMP-2 by GSK-3β. Taken together, these results suggest that Ras plays critical roles in establishing neuronal polarity upstream of the PI3-kinase/Akt/GSK-3β/CRMP-2 pathway and mitogen-activated protein kinase cascade.

Keywords: Akt; Axon; CRMP-2; GSK-3β; MAPK; Neuronal polarity; PI3-kinase; Ras

Neurons are highly polarized cells comprised of two structurally and functionally distinct parts, an axon and dendrites [1]. Neuronal polarity is essential for unidirectional signal flow from somata/dendrites to axons in neurons. Cultured hippocampal neurons are commonly used for studying the establishment of neuronal polarization [2]. In cultured hippocampal neurons, neurons extend several immature neurites during the first 12–24 h after plating (stages 1 and 2). Then one of the neurites begins to extend rapidly to form an axon, resulting in the morphological polarization of the neuron (stage 3), and the remaining neurites develop into dendrites (stage 4). By 7 days in vitro (DIV), the neurons become highly polarized, and the axon and dendrites continue to mature and subsequently develop (stage 5).

Several groups, including ours, reported that local activation of PI3-kinase and accumulation of the lipid product of PI3-kinase, phosphatidylinositol-3,4,5-triphosphate (PIP_3), at the tip of one of immature neurites, are important for axon specification [3,4]. PI3-kinase inhibitors, such as LY294002, delay the transition from stage 1 to stage 3 neurons, affecting both axon formation and elongation [3,4]. Local contact of immature neurites with the extracellular matrix (ECM), such as laminin, induces rapid production of PIP_3 at the tip of the neurite through the action of PI3-kinase, and PIP_3 is involved in axon specification, possibly by stimulating elongation of an immature neurite [4]. The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a lipid and protein phosphatase that functions opposite to PI3-kinase by dephosphorylating PIP_3 [5]. The overexpression of PTEN inhibits axon formation, whereas knockdown of PTEN by siRNA induces the formation of multiple axons [6]. Constitutively active myristoylated Akt leads to the formation of multiple axons [6]. GSK-3β relays

* Corresponding author. Fax: +81 52 744 2083.
E-mail address: kaibuchi@med.nagoya-u.ac.jp (K. Kaibuchi).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.11.147

signals from PTEN and Akt, thus playing critical roles in neuronal polarity [6]. We previously showed that GSK-3β specifies axon/dendrite fate through the phosphorylation of CRMP-2 [7]. CRMP-2 is enriched in the growing axon of hippocampal neurons and is critical for specifying axon/dendrite fate, possibly by promoting neurite elongation via microtubule assembly [8,9]. The binding activity of CRMP-2 to tubulin is decreased by the phosphorylation of CRMP-2 by GSK-3β [7,10]. Thus, the PI3-kinase/Akt/GSK-3β/CRMP-2 pathway is essential for axon specification in neurons. However, the upstream signaling of PI3-kinase on neuronal polarity remains largely unknown.

Ras proteins (H-Ras, K-Ras, and N-Ras) are small GTPases that regulate cell growth and differentiation [11]. Ras cycles between GTP-bound and GDP-bound forms and operates as a molecular switch in signal transduction pathways. Active GTP-bound Ras interacts with several effector proteins, and among the best characterized are PI3-kinase and Raf kinase [12–15]. In *Dictyostelium*, localized Ras signaling at the leading edge regulates directional cell movement through the activation of PI3-kinase, indicating that Ras regulates cell polarity [16]. However, it remains unknown whether Ras is involved in neuronal polarity.

In this study, we found that Ras was important for axon specification through PI3-kinase in hippocampal neurons. Ras regulated the phosphorylation of CRMP-2 via GSK-3β. These findings suggest that Ras plays critical roles in establishing neuronal polarity via the PI3-kinase/Akt/GSK-3β/CRMP-2 pathway.

Materials and methods

Materials and chemicals. cDNA encoding human CRMP-2 was obtained using the methods of Arimura et al. [17]. pCAGGS vector was provided by Dr. M. Nakafuku (Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA). The following antibodies were used: anti-CRMP-2 polyclonal antibody raised against MBP-CRMP-2, anti-phospho-CRMP-2 (anti-pT514) [7], polyclonal anti-c-Myc (A-14, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and monoclonal Tau-1 (Chemicon, Temecula, CA, USA) antibodies. PI3-kinase inhibitor (wortmannin) was purchased from Wako (Osaka, Japan), and MEK inhibitor (PD98059) was obtained from Calbiochem (San Diego, CA, USA).

Plasmid constructs. The cDNA fragments encoding H-Ras wild-type (WT), H-Ras V12 (constitutively active mutant), and H-Ras N17 (dominant negative mutant) were each subcloned into pEF-BOS-myc vector. pCAG-Myr-p110α-IP (constitutively active PI3-kinase) was provided by Dr. S. Yamanaka (Kyoto University, Kyoto, Japan). pcDNA3-Akt1 WT, pcDNA3-Akt1 myrΔPH (constitutively active mutant), and pcDNA3-Akt1 3A (K197A/T308A/S473A; dominant negative mutant) were provided by Dr. Y. Gotoh (University of Tokyo, Tokyo, Japan). pCGN-HA-GSK-3β WT was provided by Dr. A. Kikuchi (Hiroshima University, Hiroshima, Japan).

Culture of COS7 cells for immunoblot analysis. COS7 cells were seeded on a 60-mm dish in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and cultured overnight at 37 °C in an air–5% CO_2 atmosphere at constant humidity. Transfections were carried out using Lipofectamine reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After transfections, cells were grown in DMEM with 10% FBS for 1 day and then in DMEM for 1 day. Cells were treated with 10% (w/v) trichloroacetic acid. The resulting precipitates were subjected to SDS–PAGE and immunoblot analysis.

Culture of hippocampal neurons. Culture of hippocampal neurons prepared from E18 rat embryos using papain was performed as described by Inagaki et al. [8]. Neurons were seeded on coverslips with poly-D-lysine (Sigma, St. Louis, MO, USA) in Neurobasal medium (Invitrogen) with B-27 supplement (Invitrogen) and 1 mM glutamine. To analyze the morphology, neurons were transfected using a calcium phosphate method before plating [4,7]. Neurons were fixed at DIV6 with 3.7% formaldehyde in PBS for 10 min at room temperature, followed by treatment for 10 min with 0.05% Triton X-100 on ice and 10% NGS in PBS for 1 h at room temperature. Neurons were then immunostained with anti-myc and Tau-1 antibodies, and observed with a confocal laser microscope (LSM510 Carl Zeiss, Oberkochen, Germany) built around an Axiovert 100 M (Carl Zeiss). The percentage of multiple axons was scored as the percentage of neurons with the second longest neurite whose length was longer than a half-length of the longest neurite and immunostained by Tau-1.

Results and discussion

Ras is involved in axon specification

To examine whether Ras regulates neuronal polarity, hippocampal neurons were transfected with H-Ras WT, V12, or N17. Neurons were fixed at DIV6 to visualize secondary axons [7]. The control neuron expressing Myc-GST had one axon stained by Tau-1. Ectopic H-Ras was diffusely overexpressed in neurons (Fig. 1). The ectopic expression of H-Ras WT and the dominant active form of H-Ras (V12) induced the formation of multiple Tau-1-positive neurites (i.e., axons), whereas that of the dominant negative form of H-Ras (N17) did not (Figs. 1 and 2). It should be noted that some of the neurons transfected with the dominant negative form of H-Ras (N17) bore no axon (Figs. 1 and 2). These results suggest that Ras can regulate axon specification.

Next, we examined whether Ras regulates axon specification through the PI3-kinase pathway and the Raf/MEK/ERK pathway (MAPK cascade). Hippocampal neurons were cultured in the presence of PI3-kinase inhibitor (200 nM wortmannin) or MEK inhibitor (50 μM PD98059) for 5 days before fixation. In the control neurons expressing Myc-GST, PI3-kinase inhibitor increased the percentage of neurons that had no axon, as previously reported (Fig. 2) [3,4]. MEK inhibitor seemed to have no apparent effects on axon formation in the control cells under this condition (Fig. 2). It was reported that MEK inhibitor (PD98059) does not alter the growth rate of axons and immature neurites in cultured hippocampal neurons [18]. Under the same conditions, inhibition of PI3-kinase suppressed the H-Ras-induced formation of multiple axons (Fig. 2), and a similar result was obtained using MEK inhibitor. These results suggest that Ras plays a crucial role in specifying neuronal polarity through the PI3-kinase pathway and MAPK cascade.

Ras regulates CRMP-2 via GSK-3β

We recently showed that GSK-3β phosphorylates CRMP-2 at Thr-514, thus inactivating the CRMP-2 activity, and participates in neuronal polarization through

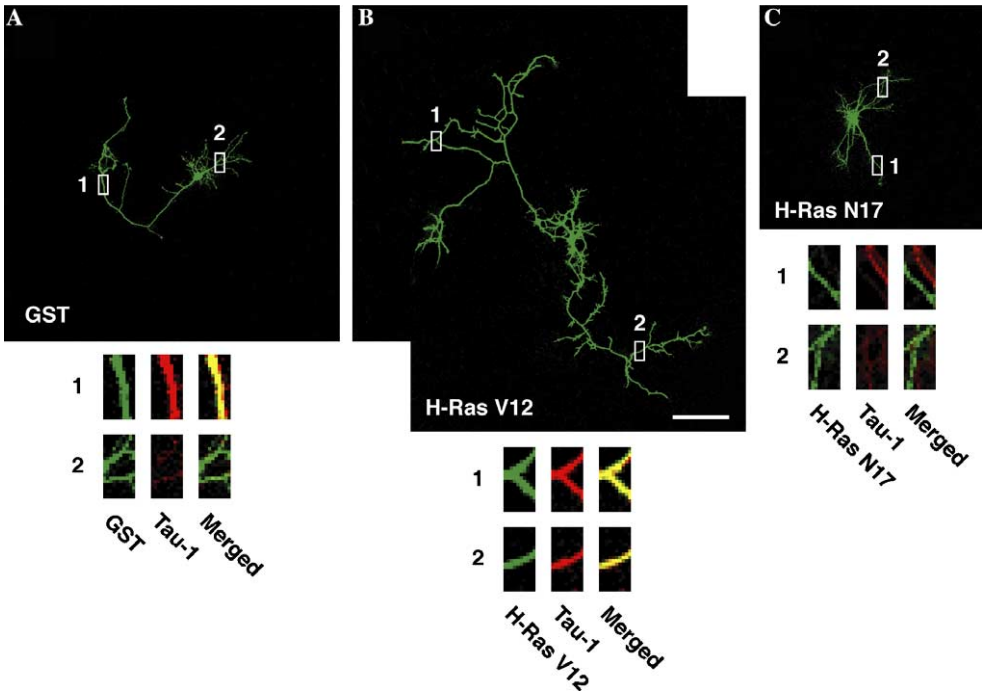

Fig. 1. H-Ras V12 induced the formation of multiple axons, whereas H-Ras N17 inhibited the formation of axons. Hippocampal neurons were transfected with Myc-GST (A), Myc-H-Ras V12 (B), or Myc-H-Ras N17 (C). Neurons were fixed at DIV6 and then immunostained with anti-Myc and axonal marker Tau-1 antibodies. The enlarged images of the neurites (1 and 2) are shown. The neuron transfected with Myc-GST (A) had one Tau-1-positive neurite (1). The neuron transfected with Myc-H-Ras V12 (B) had multiple Tau-1-positive neurites (1 and 2), whereas the neuron transfected with Myc-H-Ras N17 (C) had no Tau-1 positive neurites. Scale bar represents 100 μm.

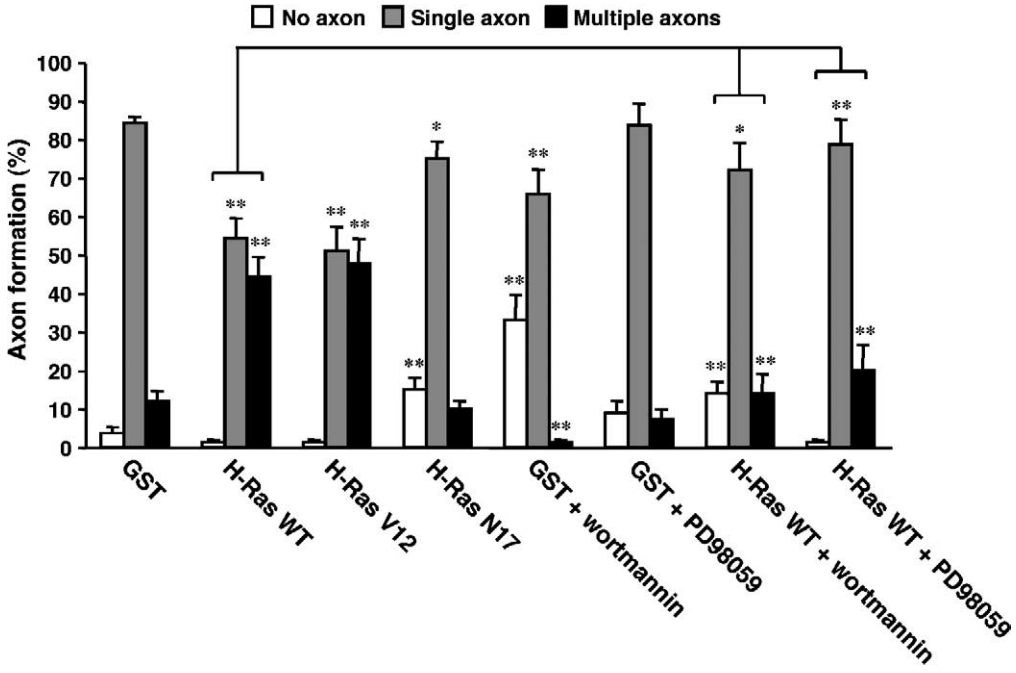

Fig. 2. Inhibition of PI3-kinase or MEK suppressed the H-Ras-induced formation of multiple axons. The percentage of cells that had no axon, a single axon, and multiple axons were estimated in DIV6 neurons transfected with the indicated plasmids. Fifty cells for each plasmid were estimated by immunostaining with anti-Myc and Tau-1 antibodies. Data are means ± SD of triplicate determinations. Asterisks indicate the difference from the value of GST or H-Ras WT (Student's t test; $^{*}P < 0.05$; $^{**}P < 0.01$).

CRMP-2 [7]. In addition, neurotrophins, Neurotrophin-3 and brain-derived neurotrophic factor, decrease the phosphorylation levels of CRMP-2 at Thr-514 and increase nonphosphorylated active CRMP-2 via the PI3-kinase/Akt/GSK-3β pathway to promote axon outgrowth [7]. In dorsal root ganglion neurons, localized inactivation of

GSK-3β at the growth cone is required for rapid axon elongation induced by neurotrophin, such as nerve growth factor, via PI3-kinase and integrin-linked kinase (ILK) [19]. The Ras activity and activation of both the PI3-kinase/Akt and the Raf/MEK pathways are required for neurotrophin-induced axon growth in sympathetic neurons of the superior cervical ganglion and cultured embryonic sensory neurons [20,21].

To investigate whether Ras regulates the phosphorylation of CRMP-2 via GSK-3β, COS7 cells were cotransfected with H-Ras WT and CRMP-2 WT with GSK-3β WT and subjected to immunoblot analysis (Fig. 3). The mobility shift of CRMP-2 (asterisk) was observed in the cells cotransfected with CRMP-2 WT and GSK-3β WT, as previously reported [7]. In runs with CRMP-2 WT and GSK-3β WT, anti-pT514 antibody recognized the upper bands, which corresponded to the phosphorylated CRMP-2 at Thr-514. In contrast, the immunoreactive band was decreased in the COS7 cells cotransfected with CRMP-2 WT, GSK-3β WT, and H-Ras WT. Consistent with the result using anti-pT514 antibody, the mobility shift of CRMP-2 (asterisk) was decreased in these cells. A similar result was obtained in runs with the dominant active form of H-Ras (V12). The number of COS7 cells decreased after transfection with CRMP-2 WT, GSK-3β WT, and the dominant negative form of H-Ras (N17; data not shown). Because the Ras activity is necessary for survival of certain cells, the ectopic expression of the dominant negative form of Ras (N17) may induce cell death [22–24]. Taken together, these results suggest that Ras can regulate the phosphorylation of CRMP-2 by acting through the PI3-kinase/Akt/GSK-3β pathway.

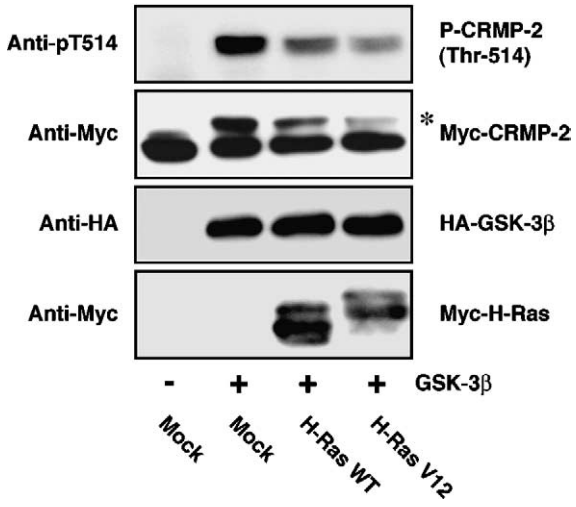

Fig. 3. H-Ras regulated the phosphorylation of CRMP-2 via GSK-3β. COS7 cells were cotransfected with Myc-CRMP-2 WT and HA-GSK-3β WT with Myc-H-Ras WT or V12. Samples were subjected to immunoblot analysis with anti-pT514, anti-Myc, and anti-HA antibodies. The mobility shift of CRMP-2 (asterisk) was caused by the phosphorylation of CRMP-2 at Thr-514.

PI3-kinase and Akt regulate neuronal polarity

To further confirm that PI3-kinase and Akt regulate neuronal polarity, hippocampal neurons were

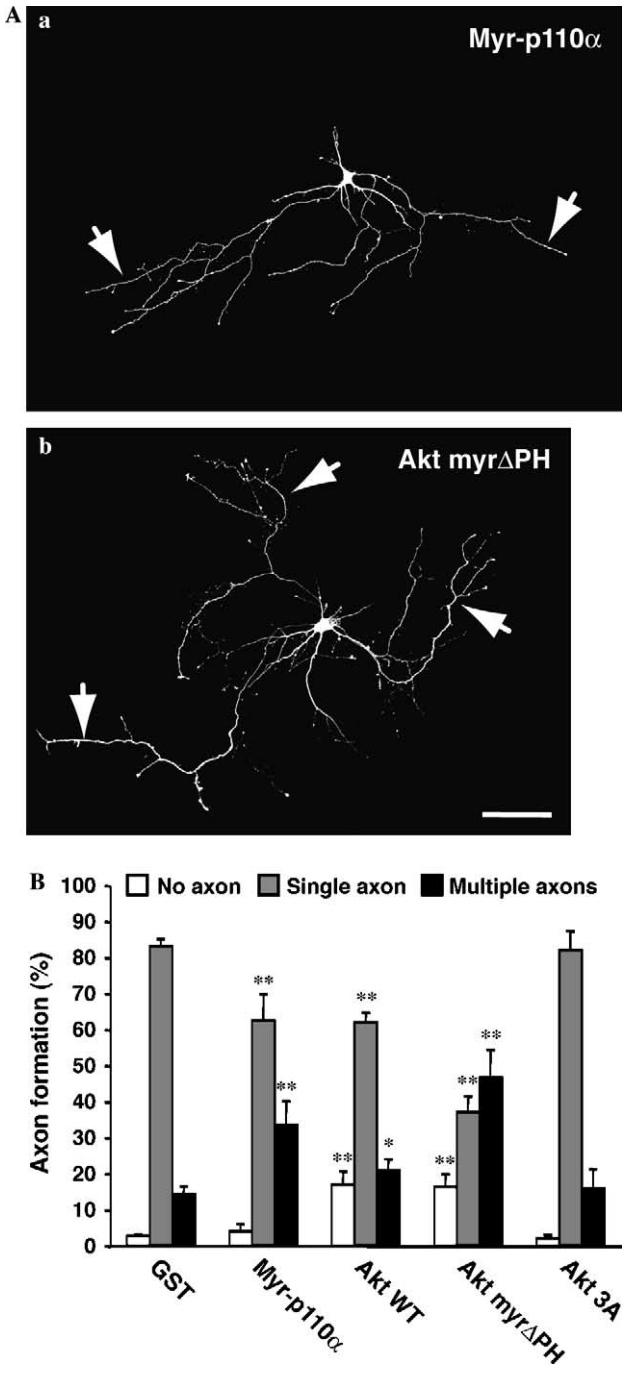

Fig. 4. PI3-kinase and Akt regulate axon formation. (A) Hippocampal neurons were transfected with Myr-p110α (a) or Akt myrΔPH (b) with Myc-GST. Neurons were fixed at DIV6 and then immunostained with anti-Myc and axonal marker Tau-1 antibodies. Arrows indicate Tau-1-positive neurites. Scale bar represents 100 μm. (B) The percentage of cells that had no axon, a single axon, and multiple axons was estimated in DIV6 neurons transfected with the indicated plasmids. Fifty cells for each plasmid were estimated by immunostaining with anti-Myc and Tau-1 antibodies. Data are means ± SD of triplicate determinations. Asterisks indicate the difference from the value of GST (Student's t test; $*P < 0.05$; $**P < 0.01$).

cotransfected with constitutively active PI3-kinase (Myr-p110α), Akt WT, constitutively active Akt (Akt myrΔPH), or dominant negative Akt (Akt 3A) and Myc-GST, and then were fixed at DIV6. The ectopic expression of constitutively active PI3-kinase (Myr-p110α), Akt WT, and constitutively active Akt (Akt myrΔPH) induced the formation of multiple axons (Fig. 4). Some of the neurons transfected with Akt WT and constitutively active Akt (Akt myrΔPH) bore no Tau-1-positive neurite. The excessive activity of Akt may impair normal axon formation. The ectopic expression of Akt WT and constitutively active Akt (Akt myrΔPH) induced abnormal polarization, whereas dominant negative Akt (Akt 3A) had no obvious effect (Fig. 4). Because the activity of Akt is essential for survival of various types of cells, the ectopic expression of dominant negative Akt (Akt 3A) may induce apoptosis of neurons [25,26]. These results, together with the previous observations, indicate that both PI3-kinase and Akt are necessary in neuronal polarity.

Polarity proteins regulate axon specification

In this study, several pieces of evidence indicate that Ras is involved in neuronal polarity. First, the overexpression of H-Ras or the ectopic expression of constitutively active H-Ras induced multiple axons in cultured hippocampal neurons, whereas the expression of the dominant negative H-Ras impaired the formation of the primary axon. Second, the inhibitors of PI3-kinase and MEK prevented the H-Ras-induced multiple axon formation. Third, constitutively active H-Ras inhibited the GSK-3β-induced CRMP-2 phosphorylation. Fourth, the ectopic expression of constitutively active PI3-kinase or Akt induced multiple axons. Thus, Ras appears to play a crucial role in neuronal polarization through the PI3-kinase/Akt/GSK-3β/CRMP-2 pathway and MAPK cascade.

Fig. 5 depicts a model schema of the regulation of axon formation [27]. Nonphosphorylated active CRMP-2 interacts with tubulin heterodimers and promotes microtubule assembly for axon formation. Active CRMP-2 also regulates the endocytosis of specific adhesion molecules including L1 through the interaction with Numb and the reorganization of actin filaments acting through Sra-1 [28,29]. To lower the microtubule stability, GSK-3β phosphorylates MAP1B, the adenomatous polyposis coli gene product (APC), and CRMP-2 [30–32]. However, the PI3-kinase activity is also required for proper localization of the Par complex (including Par3 and Par6), atypical protein kinase C (aPKC), and Cdc42 at the tips of the growing axons, all of which are necessary for neuronal polarization [3,33]. Cdc42-GTP binds to Par6 and determines the localization of the Par complex. Par3 directly interacts with STEF/Tiam1 (the guanine nucleotide exchange factors for Rac1), and the Par3/Par6 complex mediates the signal from Cdc42 to Rac1 for axon specification [34]. Although it remains unclear how ERK contributes to neuronal polarity, it may promote axon formation through the activation of certain transcription factors such as cAMP-response element-binding protein [35].

What are the upstream signals of Ras? Because neurons develop polarity in culture without any directional gradients of extracellular cues, an internal polarization program

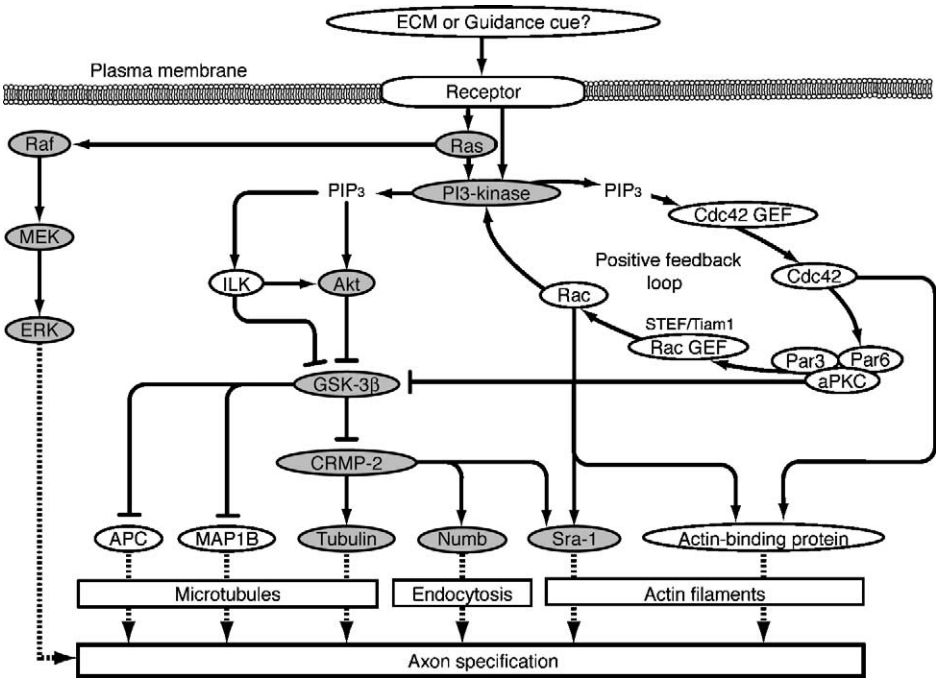

Fig. 5. Model schema of the regulation of axon formation. In one of the immature neurites (the future axon), Ras activates the MAPK cascade and PI3-kinase, and accumulated PIP_3 activates the Akt/GSK-3β/CRMP-2 pathway and the positive feedback loop. Ras regulates not only transcriptions but also cytoskeletons and endocytosis to specify axon/dendrite fate.

appears to exist in neurons [1]. However, it has been shown that extracellular substrate molecules such as laminin can govern whether an immature neurite becomes an axon [4,36]. Thus, signaling cascades accelerated by laminin through integrin can initiate the growth of the immature neurite and subsequent axon specification, and certain extracellular cues may determine axon/dendrite fate during physiological development. It is possible that Ras is activated at the tip of the immature neurite when it contacts ECM such as laminin. In addition to the major Ras forms (H-, K-, and N-Ras), R-Ras, another member of the Ras subfamily, may be involved in axon specification because R-Ras is necessary for the integrin-mediated neurite outgrowth on laminin [37]. R-Ras is thought to activate PI3-kinase rather than the MAPK cascade [38]. Rap1B, another member of the Ras subfamily, is localized at the tips of axons preceding accumulation of the Par complex, and Rap1B activation induces the multiple axon-like neurites and accumulation of the Par complex in each axon [33]. However, Rap1 functions downstream of PI3-kinase and activates the MAPK cascade rather than PI3-kinase [39]. Further studies are required to examine whether Ras is involved in ECM-induced axon specification, and if so, which member of the Ras subfamily is activated.

Acknowledgments

We thank Drs. S. Yamanaka (Kyoto University, Kyoto, Japan), Y. Gotoh (University of Tokyo, Tokyo, Japan), and A. Kikuchi (Hiroshima University, Hiroshima, Japan) for their kind gifts of materials; Drs. M. Amano, S. Taya, T. Nishimura, Mr. A. Hattori, and Mr. T. Hirai for helpful discussion; Miss K. Yamada for preparing materials and technical assistance; and Mrs. T. Ishii for secretarial assistance. This research was supported in part by Grants-in-Aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT); Grant-in-Aid for Creative Scientific Research from MEXT, The 21st Century Centre of Excellence (COE) Program from MEXT; and the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO).

References

[1] A.M. Craig, G. Banker, Neuronal polarity, Annu. Rev. Neurosci. 17 (1994) 267–310.
[2] C.G. Dotti, C.A. Sullivan, G.A. Banker, The establishment of polarity by hippocampal neurons in culture, J. Neurosci. 8 (1988) 1454–1468.
[3] S.H. Shi, L.Y. Jan, Y.N. Jan, Hippocampal neuronal polarity specified by spatially localized mPar3/mPar6 and PI 3-kinase activity, Cell 112 (2003) 63–75.
[4] C. Menager, N. Arimura, Y. Fukata, K. Kaibuchi, PIP_3 is involved in neuronal polarization and axon formation, J. Neurochem. 89 (2004) 109–118.
[5] T. Maehama, J.E. Dixon, The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate, J. Biol. Chem. 273 (1998) 13375–13378.
[6] H. Jiang, W. Guo, X. Liang, Y. Rao, Both the establishment and the maintenance of neuronal polarity require active mechanisms: critical roles of GSK-3β and its upstream regulators, Cell 120 (2005) 123–135.
[7] T. Yoshimura, Y. Kawano, N. Arimura, S. Kawabata, A. Kikuchi, K. Kaibuchi, GSK-3β regulates phosphorylation of CRMP-2 and neuronal polarity, Cell 120 (2005) 137–149.
[8] N. Inagaki, K. Chihara, N. Arimura, C. Menager, Y. Kawano, N. Matsuo, T. Nishimura, M. Amano, K. Kaibuchi, CRMP-2 induces axons in cultured hippocampal neurons, Nat. Neurosci. 4 (2001) 781–782.
[9] Y. Fukata, T.J. Itoh, T. Kimura, C. Menager, T. Nishimura, T. Shiromizu, H. Watanabe, N. Inagaki, A. Iwamatsu, H. Hotani, K. Kaibuchi, CRMP-2 binds to tubulin heterodimers to promote microtubule assembly, Nat. Cell Biol. 4 (2002) 583–591.
[10] N. Arimura, C. Menager, Y. Kawano, T. Yoshimura, S. Kawabata, A. Hattori, Y. Fukara, M. Amano, Y. Goshima, M. Inagaki, N. Morone, J. Usukura, K. Kaibuchi, Phosphorylation by Rho kinase regulates CRMP-2 activity in growth cones, Mol. Cell. Biol. 25 (2005) 9973–9984.
[11] J.F. Hancock, Ras proteins: different signals from different locations, Nat. Rev. Mol. Cell Biol. 4 (2003) 373–384.
[12] P. Rodriguez-Viciana, P.H. Warne, R. Dhand, B. Vanhaesebroeck, I. Gout, M.J. Fry, M.D. Waterfield, J. Downward, Phosphatidylinositol-3-OH kinase as a direct target of Ras, Nature 370 (1994) 527–532.
[13] P. Rodriguez-Viciana, P.H. Warne, B. Vanhaesebroeck, M.D. Waterfield, J. Downward, Activation of phosphoinositide 3-kinase by interaction with Ras and by point mutation, EMBO J. 15 (1996) 2442–2451.
[14] B. Dickson, F. Sprenger, D. Morrison, E. Hafen, Raf functions downstream of Ras1 in the Sevenless signal transduction pathway, Nature 360 (1992) 600–603.
[15] A.B. Vojtek, S.M. Hollenberg, J.A. Cooper, Mammalian Ras interacts directly with the serine/threonine kinase Raf, Cell 74 (1993) 205–214.
[16] A.T. Sasaki, C. Chun, K. Takeda, R.A. Firtel, Localized Ras signaling at the leading edge regulates PI3K, cell polarity, and directional cell movement, J. Cell Biol. 167 (2004) 505–518.
[17] N. Arimura, N. Inagaki, K. Chihara, C. Menager, N. Nakamura, M. Amano, A. Iwamatsu, Y. Goshima, K. Kaibuchi, Phosphorylation of collapsin response mediator protein-2 by Rho-kinase: evidence for two separate signaling pathways for growth cone collapse, J. Biol. Chem. 275 (2000) 23973–23980.
[18] L. Karasewski, A. Ferreira, MAPK signal transduction pathway mediates agrin effects on neurite elongation in cultured hippocampal neurons, J. Neurobiol. 55 (2003) 14–24.
[19] F.Q. Zhou, J. Zhou, S. Dedhar, Y.H. Wu, W.D. Snider, NGF-induced axon growth is mediated by localized inactivation of GSK-3β and functions of the microtubule plus end binding protein APC, Neuron 42 (2004) 897–912.
[20] J.K. Atwal, B. Massie, F.D. Miller, D.R. Kaplan, The TrkB-Shc site signals neuronal survival and local axon growth via MEK and P13-kinase, Neuron 27 (2000) 265–277.
[21] A. Markus, J. Zhong, W.D. Snider, Raf and Akt mediate distinct aspects of sensory axon growth, Neuron 35 (2002) 65–76.
[22] G.D. Borasio, A. Markus, A. Wittinghofer, Y.A. Barde, R. Heumann, Involvement of Ras p21 in neurotrophin-induced response of sensory, but not sympathetic neurons, J. Cell Biol. 121 (1993) 665–672.
[23] C.D. Nobes, A.M. Tolkovsky, Neutralizing anti-p21ras Fabs suppress rat sympathetic neuron survival induced by NGF, LIF, CNTF and cAMP, Eur. J. Neurosci. 7 (1995) 344–350.
[24] L.A. Feig, R.J. Buchsbaum, Cell signaling: life or death decisions of Ras proteins, Curr. Biol. 12 (2002) R259–R261.
[25] H. Dudek, S.R. Datta, T.F. Franke, M.J. Birnbaum, R. Yao, G.M. Cooper, R.A. Segal, D.R. Kaplan, M.E. Greenberg, Regulation of neuronal survival by the serine-threonine protein kinase Akt, Science 275 (1997) 661–665.
[26] K. Namikawa, M. Honma, K. Abe, M. Takeda, K. Mansur, T. Obata, A. Miwa, H. Okado, H. Kiyama, Akt/protein kinase B

prevents injury-induced motoneuron death and accelerates axonal regeneration, J. Neurosci. 20 (2000) 2875–2886.
[27] N. Arimura, K. Kaibuchi, Key regulators in neuronal polarity, Neuron (2005) in press.
[28] T. Nishimura, Y. Fukata, K. Kato, T. Yamaguchi, Y. Matsuura, H. Kamiguchi, K. Kaibuchi, CRMP-2 regulates polarized Numb-mediated endocytosis for axon growth, Nat. Cell Biol. 5 (2003) 819–826.
[29] Y. Kawano, T. Yoshimura, D. Tsuboi, S. Kawabata, T. Kaneko-Kawano, H. Shirataki, T. Takenawa, K. Kaibuchi, CRMP-2 is involved in Kinesin-1–dependent transport of the Sra-1/WAVE1 complex and axon formation, Mol. Cell. Biol. 25 (2005) 9920–9935.
[30] R.G. Goold, R. Owen, P.R. Gordon-Weeks, Glycogen synthase kinase 3β phosphorylation of microtubule-associated protein 1B regulates the stability of microtubules in growth cones, J. Cell Sci. 112 (1999) 3373–3384.
[31] N. Trivedi, P. Marsh, R.G. Goold, A. Wood-Kaczmar, P.R. Gordon-Weeks, Glycogen synthase kinase-3β phosphorylation of MAP1B at Ser1260 and Thr1265 is spatially restricted to growing axons, J. Cell Sci. 118 (2005) 993–1005.
[32] J. Zumbrunn, K. Kinoshita, A.A. Hyman, I.S. Nathke, Binding of the adenomatous polyposis coli protein to microtubules increases microtubule stability and is regulated by GSK3β phosphorylation, Curr. Biol. 11 (2001) 44–49.
[33] J.C. Schwamborn, A.W. Puschel, The sequential activity of the GTPases Rap1B and Cdc42 determines neuronal polarity, Nat. Neurosci. 7 (2004) 923–929.
[34] T. Nishimura, T. Yamaguchi, K. Kato, M. Yoshizawa, Y. Nabeshima, S. Ohno, M. Hoshino, K. Kaibuchi, PAR-6-PAR-3 mediates Cdc42-induced Rac activation through the Rac GEFs STEF/Tiam1, Nat. Cell Biol. 7 (2005) 270–277.
[35] A. Markus, T.D. Patel, W.D. Snider, Neurotrophic factors and axonal growth, Curr. Opin. Neurobiol. 12 (2002) 523–531.
[36] T. Esch, V. Lemmon, G. Banker, Local presentation of substrate molecules directs axon specification by cultured hippocampal neurons, J. Neurosci. 19 (1999) 6417–6426.
[37] J.K. Ivins, P.D. Yurchenco, A.D. Lander, Regulation of neurite outgrowth by integrin activation, J. Neurosci. 20 (2000) 6551–6560.
[38] B.M. Marte, P. Rodriguez-Viciana, S. Wennstrom, P.H. Warne, J. Downward, R-Ras can activate the phosphoinositide 3-kinase but not the MAP kinase arm of the Ras effector pathways, Curr. Biol. 7 (1997) 63–70.
[39] R.D. York, D.C. Molliver, S.S. Grewal, P.E. Stenberg, E.W. McCleskey, P.J. Stork, Role of phosphoinositide 3-kinase and endocytosis in nerve growth factor-induced extracellular signal-regulated kinase activation via Ras and Rap1, Mol. Cell. Biol. 20 (2000) 8069–8083.