The activity of PI3K is necessary for polarized cell motility. To guide extending axons, environmental cues polarize the growth cone via asymmetric generation of Ca\(^{2+}\) signals and subsequent intracellular mechanical events, including membrane trafficking and cytoskeletal reorganization. However, it remains unclear how PI3K is involved in such events for axon guidance. Here, we demonstrate that PI3K plays a permissive role in growth cone turning by facilitating microtubule (MT)-dependent membrane transport. Using embryonic chick dorsal root ganglion neurons in culture, attractive axon turning was induced by Ca\(^{2+}\) elevations on one side of the growth cone by photolyzing caged Ca\(^{2+}\) or caged inositol 1,4,5-trisphosphate. We show that PI3K activity was required downstream of Ca\(^{2+}\) signals for growth cone turning. Attractive Ca\(^{2+}\) signals, generated with caged Ca\(^{2+}\) or caged inositol 1,4,5-trisphosphate, triggered asymmetric transport of membrane vesicles from the center to the periphery of growth cones in a MT-dependent manner. This centrifugal vesicle transport was abolished by PI3K inhibitors, suggesting that PI3K is involved in growth cone attraction at the level of membrane trafficking. Consistent with this observation, immunocytochemistry showed that PI3K inhibitors reduced MTs in the growth cone peripheral domain. Time-lapse imaging of EB1 on the plus-end of MTs revealed that MT advance into the growth cone peripheral domain was dependent on PI3K activity: inhibition of the PI3K signaling pathway attenuated MT advance, whereas exogenous phosphatidylinositol 3,4,5-trisphosphate (PIP3), the product of PI3K-catalyzed reactions, promoted MT advance. This study demonstrates the importance of PI3K-dependent membrane trafficking in chemotactic cell migration.

The correct wiring of the nervous system relies critically on the navigation of developing axons to their destinations. The growth cone, the tip of elongating axons, recognizes extracellular guidance cues to form proper neuronal connections (1, 2): graded distribution of guidance cues causes growth cone turning toward the higher concentration (attraction) or lower concentration (repulsion) of the cues. The graded distribution also elicits asymmetric increases in cytosolic Ca\(^{2+}\) concentrations across the growth cone, with higher Ca\(^{2+}\) concentrations on the side of the growth cone facing the higher concentra-

trations of the guidance cues (3–6). Such asymmetric Ca\(^{2+}\) signals mediate attractive or repulsive turning depending on the source of Ca\(^{2+}\). Two types of Ca\(^{2+}\) release from the endoplasmic reticulum, Ca\(^{2+}\)-induced Ca\(^{2+}\) release (CICR)\(^2\) through ryanodine receptors and inositol 1,4,5-trisphosphate (IP\(_3\))-induced Ca\(^{2+}\) release (IICR) through IP\(_3\) receptors, are sufficient to trigger growth cone attraction (6, 7). For example, CICR mediates netrin-1-induced attraction, and IICR mediates NGF- or BDNF-induced attraction (6, 8, 9). On the other hand, Ca\(^{2+}\) influx from the extracellular space through transient receptor potential channels or cyclic nucleotide-gated channels has been implicated in growth cone repulsion (10–14). In this way, Ca\(^{2+}\) serves as a critical messenger that polarizes the growth cone for guided migration.

PI3K, an enzyme that catalyzes the production of phosphatidylinositol 3,4,5-trisphosphate (PIP\(_3\)) from phosphatidylinositol 4,5-bisphosphate (PIP\(_{2}\)), is involved in various cellular functions during embryonic development, including proliferation, cell migration, and axon guidance (15–18). It has been reported that PI3K activity is required for growth cone attractive turning responses to guidance cues such as NGF, BDNF, and netrin-1 (6, 9, 18). The involvement of PI3K in repulsive turning depends on the type of guidance cues: PI3K is required for repulsion by myelin-associated glycoprotein but not by semaphorin 3A (18, 19). Despite the importance of PI3K, how it controls growth cone responses to guidance signals remains elusive. NGF and BDNF are able to elicit Ca\(^{2+}\) signals in growth cones in the absence of PI3K activity (6, 9), suggesting that PI3K acts downstream of Ca\(^{2+}\) signals in attractive axon guidance. Our previous study identified a mechanism by which Ca\(^{2+}\) signals cause attractive turning: growth cones turn by transporting membrane vesicles toward the new direction (20). Here, we examined whether PI3K is involved in such membrane trafficking and show that PI3K controls microtubule (MT) dynamics, thereby facilitating vesicle transport in growth cones during attractive turning.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Dorsal root ganglion (DRG) neurons from embryonic day 9 chicks were dissociated as described previously (21). The dissociated cells were plated on a glass-based...
dish coated with L1-Fc chimeric proteins that consisted of the whole extracellular domain of L1 and the Fc region of human immunoglobulin G (21). Cultures were maintained in Leibovitz L-15 medium (Invitrogen) supplemented with N2 (Invitrogen), 750 μg/ml bovine serum albumin (Invitrogen), and 20 ng/ml NGF (Promega) in a humidified atmosphere of 100% air at 37 °C.

Loading of Caged Compounds—The acetoxymethyl ester of the caged Ca$^{2+}$ compound o-nitrophényl-EGTA (2 μM; Invitrogen) was loaded into DRG neurons as described previously (7). The caged IP$_3$ compound d-nitrophenyl 1,4,5-triphosphate P$_i$P$_3$-1-(2-nitrophényl)ethyl ester (200 μM; Invitrogen) was introduced into DRG neurons by trituration loading (6). Alexa Fluor-conjugated dextran (100 μM; Invitrogen) was loaded simultaneously to allow for determination of neurons with positive loading.

Growth Cone Turning Assay—Growth cone turning induced by focal laser-induced photolysis (FLIP) of caged compounds was assessed as described previously (6, 7). In some experiments, the following reagents were applied to the culture medium at least 20 min before time-lapse imaging: 10 μM LY294002, 60 nM wortmannin, or 10 nM nocodazole was applied to the culture medium at least 20 min before time-lapse imaging for 30 min. The cells were incubated with anti-β-tubulin monoclonal antibody (1:1000 dilution in cytoskeleton buffer; Chemicon, Billerica, MA) overnight at 4 °C. Primary antibody binding was visualized with Alexa Fluor 594-conjugated secondary antibody (10 μg/ml; Invitrogen). Differential interference contrast (DIC) and fluorescent images were acquired with a ×100 objective (UPlanSapo) and a CCD camera (ORCA-ERG with binning set at 1 × 1, Hamamatsu Photonics) on an inverted microscope (IX81). Distribution of MTs in the growth cone P-domain was quantified by measuring the number and length of MTs (supplemental Fig. 2). The C-domain/P-domain boundary and the area of the P-domain were determined on DIC images. Individual MTs were traced from the C-domain/P-domain boundary to their tips, and the length of all individual MTs was averaged in each growth cone. The total length was obtained by summing up the length of all individual MTs in a growth cone. The number and total length of MTs in each growth cone were divided by the area of the P-domain. These three parameters of MTs were normalized to the mean of control growth cones to even out the interexperiment variance. The combined data of three independent experiments (n = 15 growth cones for each experiment) are shown in Fig. 4 (B–D).

Transfection—DRG neurons were transfected with the following cDNA constructs using Nucleofector II (Lonza, Basel, Switzerland) according to the manufacturer’s protocols: enhanced GFP (EGFP; Clontech), EGFP- or mCherry-tagged EB1 (end-binding protein 1; a kind gift of Dr. K. Kaibuchi, Nagoya University, Aichi, Japan), EGFP-tagged pleckstrin homology (PH) domain of Akt (Akt-PH-EGFP; a kind gift of Dr. T. Balla, National Institutes of Health, Bethesda, MD), and DsRed-Mem (Clontech).

EB1 Imaging—Fluorescent images of growth cones expressing EGFP- or mCherry-tagged EB1 were acquired every 3 s with a ×100 objective (UPlanSapo) and a CCD camera (ORCA-ERG with binning set at 2 × 2) on an inverted microscope (IX81). To quantify MT dynamics, we counted the number of EB1 comets that passed across the C-domain/P-domain boundary and migrated into the P-domain. In each growth cone, the number of EB1 comets advancing into the P-domain was divided by the length of the boundary. We also measured the life time and speed of EB1 comets that had crossed the boundary. The life time was defined as the period that an EB1 comet spent from its crossing the boundary until its disappearance, and the values of all such comets were averaged in each growth cone. The speed of EB1 comets in each growth cone was defined as the mean speed of five randomly selected comets whose life time exceeded 6 s. The effects of the following drugs on MT dynamics were assessed by comparing these parameters in growth cones before and after a 10-min drug treatment: 10 μM LY294002, 60 nm wortmannin, 10 μM PIP$_3$ (Cayman Chemical, Ann Arbor, MI), and 10 μM PIP$_2$ (Cayman Chemical).

PIP$_3$ Imaging—Growth cones expressing both Akt-PH-EGFP and DsRed-Mem were visualized by total internal reflection fluorescence microscopy as described previously (23). Fluorescent images were acquired with a ×100 objective (PlanApo TIRFM, numerical aperture of 1.45, Olympus) and a
**RESULTS**

PI3K Is Required for Growth Cone Turning Induced by CICR and IICR—We have previously reported that, on an L1 substrate, photolysis of caged Ca\(^{2+}\) or caged IP\(_3\) on one side of the growth cone elicits localized CICR or IICR, respectively, and is sufficient to trigger growth cone attraction (6, 7, 25). Localized photolysis of caged compounds has been used extensively to replicate growth cone turning responses to physiological cues and to analyze molecular mechanisms underlying axon guidance (20, 23, 26, 27). The involvement of PI3K in axon turning was examined using embryonic chick DRG neurons that had been loaded with a caged Ca\(^{2+}\) compound, α-nitrophenyl-EGTA, or with a caged IP\(_3\) compound, d-myoinositol 1,4,5-triphosphate 5-(1-(2-nitrophenyl)ethyl) ester (Fig. 1). Repeated FLIP (3-s intervals) of either of these caged compounds on one side of the growth cone resulted in attractive turning. The CICR-induced attraction was abolished in the presence of LY294002 or wortmannin. Also, the IICR-induced attraction was blocked by LY294002. The inhibitory effect of wortmannin on IICR-induced attraction was reported in our previous study (6). We confirmed that the two PI3K inhibitors significantly decrease PIP\(_3\) density in the growth cone plasma membrane (supplemental Fig. 1). The involvement of PI3K in axon guidance was further examined using the PH domain of Akt, which blocks the PI3K signaling pathway (28, 29): transfection of Akt PH domain only on the side with CICR (Fig. 2, A and B, Control). UV irradiation in the absence of caged Ca\(^{2+}\) loading had no effect on vesicle transport (Fig. 2B, Blank). Pretreatment with PI3K inhibitors abolished CICR-elicited vesicle transport (Fig. 2B, +LY294002 and +Wortmannin), indicating that PI3K is required for vesicle transport during growth cone attraction.

**PI3K Is Required for CICR-elicited Centrifugal Vesicle Transport**—We next addressed the issue of how PI3K is involved in growth cone attraction downstream of Ca\(^{2+}\) signals. We have previously reported that CICR induces attractive turning via asymmetric centrifugal vesicle transport and subsequent exocytosis (20). Thus, the effect of PI3K inhibitors on CICR-elicited vesicle transport was examined (Fig. 2). Intra- and extracellular vesicles were visualized with FM1-43, a lipophilic fluorescent dye, while CICR was generated by photolyzing caged Ca\(^{2+}\) on one side of the growth cone. Consistent with our previous observation (20), CICR increased the number of FM1-43-labeled vesicles migrating centrifugally from the C- to P-domain only on the side with CICR (Fig. 2, A and B, Control). UV irradiation in the absence of caged Ca\(^{2+}\) loading had no effect on vesicle transport (Fig. 2B, Blank). Pretreatment with PI3K inhibitors abolished CICR-elicited vesicle transport (Fig. 2B, +LY294002 and +Wortmannin), indicating that PI3K is required for vesicle transport during growth cone attraction.

**IICR Elicits Centrifugal Vesicle Transport in a PI3K-dependent Manner**—So-called “Ca\(^{2+}\) nanodomains or microdomains,” spatially restricted Ca\(^{2+}\) elevations, exert distinct intracellular responses depending on the distance between the open Ca\(^{2+}\) channels and Ca\(^{2+}\)-sensitive effectors (30). There-
fore, although both CICR and IICR elicit growth cone attraction, their downstream mechanisms may be different. We tested whether IICR-elicited attraction depended on centrifugal vesicle transport and subsequent exocytosis in growth cones. Similar to the effect of CICR, IICR generated by photolyzing caged IP₃ facilitated centrifugal transport of FM1-43-labeled vesicles only on the side with Ca²⁺ signals (near side). Blue lines depict the growth cone outline. Digits represent seconds after the start of UV photolysis. Scale bars = 5 μm. B, frequency of centrifugal vesicle migration on the near and far sides of the growth cone before (Pre) and after (UV) the start of repetitive UV irradiation. Caged Ca²⁺ loading was omitted in Blank. Numbers in parentheses indicate the number of growth cones examined. The Bonferroni multiple comparison test was used to compare 1) the frequency between both sides of the growth cone and 2) the frequency before and after photolysis on each side. #, p < 0.05 (near side versus far side during UV photolysis); *, p < 0.05 (Pre versus UV on the near side).

FIGURE 2. CICR-elicited centrifugal vesicle transport depends on PI3K activity. A, fluorescent and DIC images of an FM1-43-loaded growth cone. Ca²⁺ signals were generated every 3 s by repetitive UV photolysis of caged Ca²⁺ at the area denoted by yellow circles. Arrowheads indicate FM1-43-labeled vesicles that migrated centrifugally on the side with Ca²⁺ signals (near side). Blue lines depict the growth cone outline. Digits represent seconds after the start of UV photolysis. Scale bars = 5 μm. B, frequency of centrifugal vesicle migration on the near and far sides of the growth cone before (Pre) and after (UV) the start of repetitive UV irradiation. Caged Ca²⁺ loading was omitted in Blank. Numbers in parentheses indicate the number of growth cones examined. The Bonferroni multiple comparison test was used to compare 1) the frequency between both sides of the growth cone and 2) the frequency before and after photolysis on each side. #, p < 0.05 (near side versus far side during UV photolysis); *, p < 0.05 (Pre versus UV on the near side).

CICR-elicited growth cone attraction requires tetanus toxin-sensitive exocytosis, we also examined the effect of tetanus toxin on IICR-elicited attraction. In the presence of tetanus toxin, IICR did not induce attractive turning (1.1 ± 3.6°, n = 16 growth cones). The effect of these two drugs on IICR-elicited attraction was analyzed statistically by comparing the turning angles of drug-treated growth cones with those of control growth cones (17.1 ± 3.8°, n = 16) (Fig. 1B, Control) using Dunnett’s multiple comparison test: p < 0.01 for nocodazole and p < 0.01 for tetanus toxin. Collectively, these results strongly suggest that CICR and IICR elicit growth cone attraction via a common mechanism: MT-dependent centrifugal vesicle transport and subsequent exocytosis.
We then examined the effect of PI3K inhibitors on IICR-elicited movement of FM1-43-labeled vesicles in growth cones and showed the requirement of PI3K for IICR-induced membrane trafficking (Fig. 3B, /H11001 LY294002 and /H11001 Wortmannin). Therefore, we concluded that PI3K is required for CICR- and IICR-elicited centrifugal vesicle transport, the critical mechanical event for growth cone attraction.

PI3K Controls MT Dynamics in the Growth Cone P-domain—Because Ca\(^{2+}\)-triggered vesicle transport depends on MT dynamics, we hypothesized that PI3K facilitates vesicle transport by controlling MT dynamics. MTs are concentrated in the axon shaft and C-domain and are sprayed out into the P-domain (31). In our study, the C-domain/P-domain boundary (shown in figures as dashed lines) was determined based on DIC images, and MTs distributed in the P-domain were analyzed (supplemental Fig. 2). In a control growth cone treated with 0.06% Me\(_2\)SO, many MTs advanced into the P-domain (Fig. 4A). On the contrary, fewer MTs were observed in the P-domain of growth cones treated with PI3K inhibitors. Quantitative analyses showed that the total length of MTs in the P-domain was significantly reduced by inhibition of PI3K (Fig. 4B). This reduction was attributable to the reduced number of MTs located in the P-domain because the length of individual MTs was unaffected (Fig. 4, C and D).

The role of PI3K in MT dynamics was further investigated in DRG neurons expressing EB1 tagged with fluorescent proteins. EB1 is a member of the plus-end tracking protein family that associates with the actively polymerizing plus-ends of MTs (32, 33). Therefore, EB1-EGFP has been used extensively to study MT dynamics in various cell types, including neurons (34–36). Importantly, EB1 binding to the plus-end of MTs is unaffected after inhibition of PI3K (22), indicating that EB1 imaging is a reliable method to assess MT dynamics even in the absence of PI3K activity. We examined the behavior of EB1 comets in growth cones before and after treatment with PI3K inhibitors or phosphoinositides. Although many EB1 comets showed centrifugal migration in an untreated growth cone, such comets tended to be less frequently observed in the same growth cone after treatment with PI3K inhibitors or phosphoinositides. Although many EB1 comets showed centrifugal migration in an untreated growth cone, such comets tended to be less frequently observed in the same growth cone after treatment with PI3K inhibitors (32, 33). Therefore, EB1 imaging is a reliable method to assess MT dynamics even in the absence of PI3K activity. We examined the behavior of EB1 comets in growth cones before and after treatment with PI3K inhibitors or phosphoinositides. Although many EB1 comets showed centrifugal migration in an untreated growth cone, such comets tended to be less frequently observed in the same growth cone after treatment with PI3K inhibitors (Fig. 5 and supplemental Movies 1 and 2). The number of EB1 comets crossing the C-domain/P-domain boundary toward the P-domain was normalized by the length of boundary, and this normalized number was used as an index of the frequency of MT advance into the P-domain. The frequency of boundary crossing was decreased by the PI3K inhibitors LY294002 and wortmannin (Fig. 6A) but not by control treatment with vehicle (Fig. 6A, DMSO). These results are consistent with the immunofluorescence data showing that the number of MTs in the P-domain was reduced after PI3K inhibit-
bition. Because PI3K catalyzes the conversion of PIP₂ to PIP₃, we examined the effect of these phospholipids. The addition of PIP₃ to the culture medium caused a significant increase in Akt-PH-EGFP recruitment to the cytoplasmic surface of the growth cone plasma membrane (supplemental Fig. 1), indicating that exogenous PIP₃ can mimic activation of intra-

**FIGURE 4.** PI3K regulates MT distribution in the P-domain of growth cones. A, immunofluorescent images showing MT distribution in growth cones treated with dimethyl sulfoxide (DMSO), LY294002, or wortmannin. Yellow dashed lines represent the C-domain/P-domain boundary. Scale bar = 5 μm. B–D, effect of PI3K inhibitors on the total length and number of MTs (B and C, respectively) and the length of individual MTs (D) in the P-domain of growth cones. The value of each growth cone was normalized to the mean of control growth cones. The graphs show pooled data of three independent experiments. Numbers in parentheses indicate the total number of growth cones examined. **, p < 0.01 versus the control (Dunnett’s multiple comparison test).

**FIGURE 5.** Visualization of the actively polymerizing plus-ends of MTs. Shown are time-lapse fluorescent images of an EB1-EGFP-expressing growth cone before (A) and after (B) treatment with LY294002. Digits represent time (minutes:seconds). LY294002 was applied to the medium at ~0 min. Scale bar = 5 μm. Magnified views of regions of interest (ROI) are shown.

Akt-PH-EGFP recruitment to the cytoplasmic surface of the growth cone plasma membrane (supplemental Fig. 1), indicating that exogenous PIP₃ can mimic activation of intra-
cellular PI3K signaling cascade. Conversely, PIP2 application attenuated Akt-PH-EGFP recruitment presumably because PIP2 decreased the amount of PIP3 through activation of PTEN (phosphatase and tensin homolog deleted on chromosome 10) (37, 38). PIP3 treatment of growth cones increased the frequency of MT advance, whereas vehicle (H2O) treatment had no detectable effect (Fig. 6A and supplemental Movie 3). In contrast, PIP2 slightly decreased the frequency of MT advance (Fig. 6A and supplemental Movie 4). We also quantified the life time and speed of EB1 comets that had crossed the C-domain/P-domain boundary (see “Experimental Procedures” for details). The PI3K inhibitors LY294002 and wortmannin shortened the life time and increased the speed of EB1 comets, although PIP2 had no detectable effect (Fig. 6B and C). Consistent with the effects of pharmacological agents, inhibition of the PI3K signaling pathway by the Akt PH domain also decreased the frequency of boundary crossing, life time, and speed of EB1 comets in the growth cones (Fig. 7 and supplemental Movies 5 and 6). Taken collectively, these results indicate that PI3K facilitates MT advance into the growth cone P-domain and thereby contributes to centrifugal membrane trafficking for axon turning.

DISCUSSION

Ca2+ mediates growth cone turning responses to various guidance cues, e.g. NGF and BDNF attract growth cones via
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generation of IICR (6, 9), and netrin-1 attracts growth cones via CICR (8). Here, we have demonstrated how PI3K is involved in attractive axon guidance downstream of CICR and IICR. We also showed that CICR and IICR share common downstream mechanisms for turning, in which membrane vesicles are transported centrifugally along MTs on the side with the Ca\textsuperscript{2+} signals. This type of membrane trafficking drives attractive turning toward guidance cues via asymmetric addition of membrane components and functional molecules to the growth cone surface (20). In this study, we have identified the critical role of PI3K in Ca\textsuperscript{2+}-triggered MT-dependent vesicle transport during growth cone attraction. This mechanism is likely to exist in axon guidance mediated by various chemoattractants. In contrast, the requirement of PI3K for repulsive turning depends on the type of guidance cues (18, 19). Recent studies have implicated PI3K in endocytosis that drives growth cone repulsion downstream of Ca\textsuperscript{2+} signals (23, 39). Although defining the precise role of PI3K in repulsive guidance requires future studies, including those that address whether the generation of repulsive Ca\textsuperscript{2+} signals depends on PI3K activity, this kinase may control membrane trafficking for bidirectional axon guidance.

Our study shows that the PI3K product PIP\textsubscript{3} promotes MT advance into the growth cone P-domain. PIP\textsubscript{3} activates Akt, which inactivates glycogen synthase kinase-3\(\beta\) (35). Control of MT dynamics by PI3K may be mediated by glycogen synthase kinase-3\(\beta\), which negatively regulates MT-binding proteins, including adenomatous polyposis coli and CRMP2 (collapsin-response mediator protein-2) (40, 41). It was reported that the PI3K/glycogen synthase kinase-3\(\beta\) pathway plays a role in neuronal polarization and axon elongation via regulating MT-binding proteins (22, 41). Also, functional perturbation of adenomatous polyposis coli on one side of the growth cone causes asymmetric growth cone expansion and its turning to the side with the increased area (42), suggesting the linkage between MT-dependent membrane trafficking and polarized migration. Besides the effect of PI3K on MT-binding proteins, PI3K could influence MT advance by altering the dynamics of F-actin. The position of MT plus-ends in the growth cone P-domain is determined by the balance between MT polymerization and retrograde F-actin flow because MTs are associated with F-actin and thereby pushed back continuously toward the C-domain (43). The retrograde F-actin flow is powered by myosin motors, in particular myosin II (44). PI3K inhibition causes myosin II activation (45) and would presumably accelerate the speed of retrograde F-actin flow. This might be an alternative mechanism that underlies the reduced frequency of MT advance into the P-domain after PI3K inhibition.

Mechanisms of directed migration in neuronal growth cones and non-neuronal cells share several common features. For example, localized Ca\textsuperscript{2+} signals at the leading edge of fibroblasts point to the direction of migration (46). Furthermore, MT dynamics and membrane trafficking have been implicated in directed cell migration (47–49). PI3K activity is required for efficient cell migration toward increasing concentrations of chemoattractants (50, 51), although more recent studies have suggested that PI3K is dispensable when chemoattractant gradients are steep (52, 53). During chemotaxis, PI3K activation is restricted to the region of the cell facing the source of chemoattractant, where it promotes F-actin assembly and plays an instructive role in leading edge formation and cell migration (51, 54, 55). Besides this mode of action of PI3K, our findings in neuronal growth cones imply that PI3K may also contribute to chemotactic migration of non-neuronal cells through facilitating MT-dependent membrane transport toward the cell front.

It remains unclear whether PI3K plays an instructive role in axon guidance because there is no direct evidence of spatially restricted activation of PI3K during growth cone turning. Our previous study showed that an extracellular gradient of NGF causes asymmetric IP\textsubscript{3} production across the growth cone most likely via asymmetric NGF binding to its high affinity receptor TrkA (6). Considering that PI3K can also be activated upon NGF binding to TrkA (56), it is possible that PI3K activity becomes asymmetric during NGF-induced growth cone attraction. Similarly, receptors for other attractive cues such as BDNF and Wnt-4 can activate the PI3K pathway (56–58). Therefore, localized PI3K activation on the side facing the source of attractive cues would polarize the growth cone by facilitating MT advance and centrifugal vesicle transport.

In summary, we have reported the precise role of PI3K in growth cone attractive turning: PI3K facilitates MT advance into the P-domain and therefore allows attractive Ca\textsuperscript{2+} signals to promote centrifugal vesicle transport. Our study contributes to a better understanding of the molecular mechanisms underlying axon guidance and suggests the mode of PI3K action in cell chemotaxis in general.

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REFERENCES

1. Tessier-Lavigne, M., and Goodman, C. S. (1996) Science 274, 1123–1133
2. Huber, A. B., Kolodkin, A. L., Ginty, D. D., and Cloutier, J. F. (2003) Annu. Rev. Neurosci. 26, 509–563
3. Henley, J., and Poo, M. M. (2004) Trends. Cell Biol. 14, 320–330
4. Henley, J. R., Huang, K. H., Wang, D., and Poo, M. M. (2004) Neuron 44, 909–916
5. Nishiyama, M., von Schimmelmann, M. J., Togashi, K., Findley, W. M., and Hong, K. (2008) Nat. Neurosci. 11, 762–771
6. Akiyama, H., Matsu-ura, T., Mikoshiba, K., and Kamiguchi, H. (2009) Sci. Signal. 2, ra34
7. Ooashi, N., Futatsugi, A., Yoshihara, F., Mikoshiba, K., and Kamiguchi, H. (2005) J. Cell Biol. 170, 1159–1167
8. Hong, K., Nishiyama, M., Henley, J., Tessier-Lavigne, M., and Poo, M. (2000) Nature 403, 93–98
9. Li, Y., Jia, Y. C., Cui, K., Li, N., Zheng, Z. Y., Wang, Y. Z., and Yuan, X. B. (2005) Nature 434, 894–898
10. Wang, G. X., and Poo, M. M. (2005) Nature 434, 898–904
11. Li, L., Hutchins, B. I., and Kalil, K. (2009) J. Neurosci. 29, 5873–5883
12. Togashi, K., von Schimmelmann, M. J., Nishiyama, M., Lin, C. S., Yoshida, N., Yun, B., Molday, R. S., Goshima, Y., and Hong, K. (2008) Neuron 58, 694–707
13. Shim, S., Goh, E. L., Ge, S., Sailor, K., Yuan, J. P., Roderick, H. L., Bookman, M. D., Worley, P. F., Song, H., and Ming, G. L. (2005) Nat. Neuro-
PI3K-dependent Membrane Trafficking for Growth Cone Guidance

sci. 8, 730–735

14. Wen, Z., Han, L., Bamburg, J. R., Shim, S., Ming, G. L., and Zheng, J. Q. (2007) J. Cell Biol. 178, 107–119

15. Cantley, L. C. (2002) Science 296, 1655–1657

16. Bi, L., Okabe, I., Bernard, D. J., Wynshaw-Boris, A., and Nussbaum, R. L. (1999) J. Biol. Chem. 274, 10963–10968

17. Chang, C., Adler, C. E., Krause, M., Clark, S. G., Gertler, F. B., Tessier-Lavigne, M., and Bargmann, C. I. (2006) Curr. Biol. 16, 854–862

18. Ming, G., Song, H., Berninger, B., Inagaki, N., Tessier-Lavigne, M., and Poo, M. (1999) Neuron 23, 139–148

19. Campbell, D. S., and Holt, C. E. (2001) Neuron 22, 1172–1180

20. Tojima, T., Akiyama, H., Itofusa, R., Li, Y., Katayama, H., Miyawaki, A., and Kamiguchi, H. (2007) J. Biol. Chem. 282, 29295–29304

21. Kamiguchi, H., and Yoshihara, F. (2001) J. Cell Biol. 152, 10963–10968

22. Zhou, F. Q., Zhou, J., Dedhar, S., Wu, Y. H., and Snider, W. D. (2004) Neuron 42, 1051–1064

23. Tojima, T., Itofusa, R., and Kamiguchi, H. (2010) J. Neurosci. 30, 9194–9203

24. Veale, E. L., Rees, K. A., Mathie, A., and Trapp, S. (2010) J. Biol. Chem. 285, 29295–29304

25. Tojima, T., Itofusa, R., and Kamiguchi, H. (2009) J. Neurosci. 29, 7886–7897

26. Yao, J., Sasaki, Y., Wen, Z., Bassell, G. J., and Zheng, J. Q. (2006) Nat. Neurosci. 9, 1265–1273

27. Robles, E., Huttonlocher, A., and Gomez, T. M. (2003) Neuron 38, 597–609

28. Vánai, P., and Balla, T. (1998) J. Cell Biol. 143, 501–510

29. Vánai, P., Bondeva, T., Tamás, P., Tóth, B., Buday, L., Hunyady, L., and Balla, T. (2005) J. Cell Sci. 118, 4879–4888

30. Augustine, G. J., Santamaria, F., and Tanaka, K. (2003) Neuron 40, 331–346

31. Dent, E. W., and Gertler, F. B. (2003) Neuron 40, 209–227

32. Mimori-Kiyosue, Y., Shiina, N., and Tsukita, S. (2000) Curr. Biol. 10, 865–868

33. Akhamanova, A., and Steinmetz, M. O. (2008) Nat. Rev. Mol. Cell Biol. 9, 309–322

34. Piehl, M., and Cassimeris, L. (2003) Mol. Biol. Cell 14, 916–925

35. Zheng, Y., Wildonger, J., Ye, B., Zhang, Y., Kita, A., Younger, S. H., Zimmerman, S., Jan, L. Y., and Jan, Y. N. (2008) Nat. Cell Biol. 10, 1172–1180

36. Kollins, K. M., Bell, R. L., Butts, M., and Withers, G. S. (2009) Neuronal News 4, 26

37. Campbell, R. B., Liu, F., and Ross, A. H. (2003) J. Biol. Chem. 278, 33617–33620

38. McConnachie, G., Pass, I., Walker, S. M., and Downes, C. P. (2003) Biochem. J. 371, 947–955

39. Hines, J. H., Abu-Rub, M., and Henley, J. R. (2010) Nat. Neurosci. 13, 829–837

40. Zumbrun, J., Kinoshita, K., Hyman, A. A., and Náthke, I. S. (2001) Curr. Biol. 11, 44–49

41. Yoshimura, T., Kawano, Y., Arimura, N., Kawabata, S., Kikuchi, A., and Kaibuchi, K. (2005) Cell 120, 137–149

42. Koester, M. P., Müller, O., and Pollerberg, G. E. (2007) J. Neurosci. 27, 12590–12600

43. Schaefer, A. W., Kabir, N., and Forscher, P. (2002) J. Cell Biol. 158, 139–152

44. Medeiros, N. A., Burnette, D. T., and Forscher, P. (2006) Nat. Cell Biol. 8, 215–226

45. Orlova, I., Silver, L., and Gallo, G. (2007) Dev. Neurobiol. 67, 1843–1851

46. Wei, C., Wang, X., Chen, M., Ouyang, K., Song, L. S., and Cheng, H. (2009) Nature 457, 901–905

47. Bretscher, M. S. (1996) Cell 87, 601–606

48. Bretscher, M. S., and Aguado-Velasco, C. (1998) Curr. Opin. Cell Biol. 10, 537–541

49. Dunn, G. A., Zicha, D., and Fraylich, P. E. (1997) J. Cell Sci. 110, 3091–3098

50. Funamoto, S., Milan, K., Meili, R., and Firtel, R. A. (2001) J. Cell Biol. 153, 795–810

51. von Philipsborn, A., and Bastmeyer, M. (2007) Int. Rev. Cytol. 263, 1–62

52. Takeda, K., Sasaki, A. T., Ha, H., Seung, H. A., and Firtel, R. A. (2007) J. Biol. Chem. 282, 11874–11884

53. Kölsch, V., Charest, P. G., and Firtel, R. A. (2008) J. Cell Sci. 121, 551–559

54. Stephens, L., Milne, L., and Hawkins, P. (2008) Curr. Biol. 18, R485–R494

55. Charest, P. G., and Firtel, R. A. (2006) Curr. Opin. Genet. Dev. 16, 339–347

56. Huang, E. J., and Reichardt, L. F. (2001) Annu. Rev. Neurosci. 24, 677–736

57. Force, T., Woulfe, K., Koch, W. J., and Kerkela, R. (2007) Sci. STKE, pe41

58. Wolf, A. M., Lyuksyutova, A. I., Fenstermaker, A. G., Shafer, B., Lo, C. G., and Zou, Y. (2008) J. Neurosci. 28, 3456–3467