13. K. T. O'Neil and W. F. DeGrado, *Science* **250**, 646 (1990).
14. Although the basal activities of the MAPKK(ΔN1) and MAPKK(ΔN2) mutants were less than that of wild-type MAPKK, the activities of these mutants after stimulation in vitro with v-Mos were similar to that of wild-type enzyme, which indicates that residues 1 to 51 are not required for MAPKK activity.
15. M. Veron, E. Radzio-Andzelm, I. Tsigelny, L. F. Ten Eyck, S. S. Taylor, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 10618 (1993).
16. D. M. Payne *et al.*, *EMBO J.* **10**, 885 (1991).
17. K. A. Resing, A. S. Hermann, N. G. Ahn, unpublished data.
18. C. A. Lange-Carter, C. M. Pleiman, A. M. Gardner, K. J. Blumer, G. L. Johnson, *Science* **260**, 315 (1993).
19. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
20. MAPKK was subcloned into a derivative of pMB1, in which expression was driven by the MSV-LTR [D. G. Blair, W. L. McClements, M. K. Oskarsson, P. J. Fischinger, G. F. Vande Woude, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 3504 (1980)]. The plasmid pMB1 was modified to introduce convenient restriction sites, yielding pMM9. To subclone untagged wild-type or mutant MAPKK into pMM9, the 1.5-kb Bam HI to Hind III fragment from pKH-1 was ligated with pMM9 that had been digested with Bam HI and Hind III. To subclone HA-tagged MAPKK into pMM9, the 1.6-kb Xba I to Hind III fragment from pMCL-MAPKK (*33*) was ligated with pMM9 that had been digested with Spe I and Hind III.
21. S. Rong *et al.*, *Mol. Cell. Biol.* **12**, 5152 (1992).
22. D. G. Blair *et al.*, *Cell* **46**, 785 (1986).
23. An equivalent amount of exogenously expressed MAPKK was observed in cell lines transfected with the HA-tagged constructs, as determined by protein immunoblotting with antibody 12CA5.
24. B. Derijard *et al.*, *Cell* **76**, 1025 (1994); J. M. Kyriakis *et al.*, *Nature* **369**, 156 (1994).
25. E. Nishida and Y. Gotoh, *Int. Rev. Cytol.* **138**, 211 (1992).
26. M. H. Verlhac, J. Z. Kubiak, H. J. Clarke, B. Maro, *Development* **120**, 1017 (1994).
27. N. Yew, M. Strobel, G. F. Vande Woude, *Curr. Opin. Genet. Dev.* **3**, 19 (1993).
28. S. Cowley, H. Paterson, P. Kemp, C. T. Marshall, *Cell* **77**, 841 (1994).
29. The bacterial expression construct pKH-1 encodes wild-type human MAPKK1 as a fusion protein with an NH_2-terminal hexahistidine tag (S. J. Mansour *et al.*, *J. Biochem.*, in press). Point mutations were introduced to generate the amino acid substitutions K97M, S218E, S222D, and S218E-S222D (*19*). To generate MAPKK(ΔN3), pKH-1 was digested with Stu I, and two fragments of 0.4 kb and 3.9 kb were recovered and ligated in the proper orientation, which resulted in the deletion of a 60–base pair (bp) fragment from the coding sequence. The ΔN3 deletion was combined with the various point mutations by replacement of a 0.9-kb Eco RI fragment in the ΔN3 construct with corresponding fragments from constructs containing the point mutations. Wild-type and mutant MAPKKs were purified by Ni^{2+}–nitrilotriacetic acid and DEAE chromatography as described (S. J. Mansour *et al.*, *J. Biochem.*, in press).
30. D. J. Robbins *et al.*, *J. Biol. Chem.* **268**, 5097 (1993).
31. K. Fukasawa *et al.*, *Cell Growth Differ.*, in press.
32. R. S. Paules, R. Buccione, R. C. Moschel, G. F. Vande Woude, J. J. Eppig, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 5395 (1989).
33. Expression of genes in pCEP4-Lerner, modified from pCEP4 (Invitrogen), is driven by the CMV promoter and appends a nine–amino acid influenza HA tag at the NH_2-terminus of the expressed protein [I. Wilson *et al.*, *Cell* **37**, 767 (1984)]. The pCEP4-Lerner was modified to pMCL by introduction of convenient restriction sites, and a 1.5-kb Bam HI to Hind III fragment from pKH-1 (encoding wild-type MAPKK without the hexahistidine tag) was recovered and subcloned into pMCL digested with Bam HI and Hind III. The same approach was used to generate constructs encoding mutant HA-tagged MAPKK. We transfected the 293 cell line by electroporation with 15 μg of pMCL-MAPKK plasmid DNA. The efficiency of transfection was >80%. For CAT assays, cells were transfected with the pMCL-MAPKK plasmid (15 μg) and with the reporter plasmid pTE3ΔS-N (7.5 μg), which contains the CAT gene under control of 10 tandem repeats of the AP-1 site from SV40. Fresh medium was added to cells 24 hours after transfection. Twenty-four hours later, cell extracts were recovered and assays were done according to guidelines in the CAT Enzyme Assay System kit (Promega).
34. We thank L. Somparyac for assistance with soft agar assays; M. Kissinger and M. Oskarsson for assistance with cell culture; K. Resing for mass spectrometric analysis; M. Cobb, S.-C. Xu, and T. Geppert for ERK2 and the pCEP4-Lerner plasmid; M. Karin and A. Aronhein for the $pTE_3\Delta S$-N construct; M. Winey for 12CA5 antibodies; and A. Pardi, L. Sompayrac, and S. Berger for critical review of the manuscript. Supported by NIH grant GM48521 and the Searle Scholars Program (N.G.A.) and by the National Cancer Institute under contract N01-CO-74101 (G.F.V.W.).

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Calcineurin Inhibition of Dynamin I GTPase Activity Coupled to Nerve Terminal Depolarization

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Dynamin I is a nerve terminal phosphoprotein with intrinsic guanosine triphosphatase (GTPase) activity that is required for endocytosis. Upon depolarization and synaptic vesicle recycling, dynamin I undergoes a rapid dephosphorylation. Dynamin I was found to be a specific high-affinity substrate for calcineurin in vitro. At low concentrations, calcineurin dephosphorylated dynamin I that had been phosphorylated by protein kinase C. The dephosphorylation inhibited dynamin I GTPase activity in vitro and after depolarization of nerve terminals. The effect in nerve terminals was prevented by the calcineurin inhibitor cyclosporin A. This suggests that in nerve terminals, calcineurin serves as a Ca^{2+}-sensitive switch for depolarization-evoked synaptic vesicle recycling.

Dynamin I (previously called dephosphin or p96) was discovered as a phosphoprotein in nerve terminals (*1*, *2*) and as a microtubule-binding protein with GTPase activity (*3*). Dynamin I is a family of four neural isoforms, dynamin II is a family of four isoforms expressed in most other tissues (*4*, *5*), and dynamin III is an apparently testis-specific form (previously called dynamin-2) (*6*, *7*). The GTPase activity of dynamin I is stimulated in vitro by microtubules (*8*, *9*), phospholipids (*10*), SH3 domain–containing proteins (*11*), and protein kinase C–mediated phosphorylation (*12*). Dynamin I is a good in vitro substrate of protein kinase C (Michaelis constant, K_m, of 0.35 μM) (*7*), and in resting nerve terminals, it is phosphorylated by this protein kinase (*13*). However, when synaptic vesicle recycling is stimulated by depolarization, dynamin I is dephosphorylated (*2*, *12*, *14*). Because mutations of the GTP-binding domain of dynamin I expressed in mammalian cells, or of the related dynamin family in the *Drosophila* mutant *shibire*, are defective in endocytosis (*15*–*18*), dynamin I and its phosphorylation control mechanisms are thus placed in the context of nerve terminal endocytosis (*12*). Endocytosis is a generalized intracellular pathway for the recycling of vesicle membrane from the plasma membrane, in which recycling vesicles are transiently surrounded by a protein coat of clathrin and adaptins, then invag-

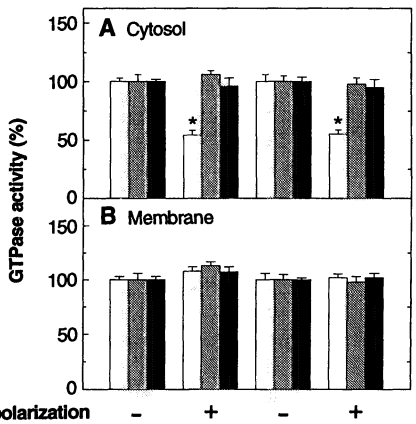

Fig. 1. Regulation of dynamin I GTPase activity in nerve terminals. Dynamin I was immunoprecipitated from isolated nerve terminals with specific antibodies and was assayed for GTPase activity before and after depolarization for 5 s with 41 mM K^+ in the presence of extracellular 0.1 mM Ca^{2+} (blank bar), 1 mM EGTA (striped bar), or 1 μM cyclosporin A (filled bar). (**A**) Dynamin I GTPase activity from the cytosolic fraction. (**B**) Dynamin I GTPase activity from the peripheral membrane fraction. Results are means of two experiments performed in quadruplicate. The asterisks indicate $P < 0.01$. For methods, see (*22*).

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inated into the cell and finally pinched off (*19*, *20*). Synaptic vesicle recycling is a specialized neuronal form of receptor-mediated endocytosis with many features in common but which uses neural-specific forms of proteins such as clathrin and dynamin (*4*, *20*). Depolarization initiates synaptic vesicle recycling, but the recycling does not require the continued presence of intracellular Ca^{2+} and is complete within 1 min (*21*). The aim of this study was to examine the mechanism of depolarization-elicited endocytosis by determining the regulation of dynamin I GTPase in nerve terminals.

We immunoprecipitated dynamin I with a specific antibody (*22*) from the cytosol and solubilized membrane fractions of isolated nerve endings (synaptosomes) before and after depolarization. Dynamin I immunoprecipitated from resting nerve terminal cytosol retained intrinsic GTPase activity (Fig. 1A). When the nerve terminals were depolarized for 5 s with 41 mM KCl, the GTPase activity of the immunoprecipitated dynamin I was decreased (Fig. 1A). Depolarization-induced inhibition of dynamin I GTPase activity was also observed when the synaptosomes were repolarized and depolarized a second time, and it was dependent on extracellular Ca^{2+} because the effect was abolished in the presence of EGTA (Fig. 1A). Dynamin I GTPase activity in immunoprecipitates from synaptosomal membranes was not altered by depolarization (Fig. 1B). This suggests that cytosolic dynamin I GTPase activity is probably regulated by Ca^{2+} influx. To determine if calcineurin, the Ca^{2+}- and calmodulin-dependent phosphatase IIB, might be involved, we included the calcineurin inhibitor cyclosporin A (*23*) in the incubation of synaptosomes. In the presence of cyclosporin A (1 μM), the inhibition of dynamin I GTPase activity by depolarization was blocked, suggesting that calcineurin mediates the inhibition of dynamin I GTPase activity, presumably by Ca^{2+}-activated dephosphorylation of dynamin I in the nerve

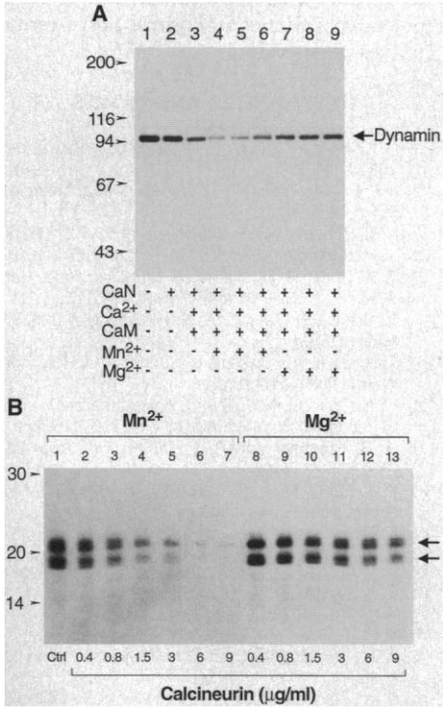

Fig. 2. Dynamin I dephosphorylation by calcineurin. (**A**) Activation of calcineurin dephosphorylation of dynamin I by cations and calmodulin. Purified phospho–dynamin I was incubated for 15 min with calcineurin as well as the different activators indicated, and dephosphorylation was measured by autoradiography. The concentrations used were as follows: 18 nM calcineurin (CaN); 200 nM calmodulin (CaM); 200 μM Ca^{2+}; 1, 2, and 5 mM Mn^{2+} in lanes 4, 5, and 6, respectively; and 1, 2, and 5 mM Mg^{2+} in lanes 7, 8, and 9, respectively. Results are representative of two to three independent experiments in triplicate. Molecular sizes are indicated in kilodaltons. (**B**) Phosphopeptide map of dynamin I after cleavage with *Staphylococcus aureus* V8 protease. The two phosphopeptides 21 and 19 kD from dynamin I are indicated with arrows on the right. Calcineurin was stimulated in the presence of Ca^{2+} and calmodulin plus either Mn^{2+} (1 mM) or Mg^{2+} (1 mM). Ctrl, control. For methods, see (*24*).

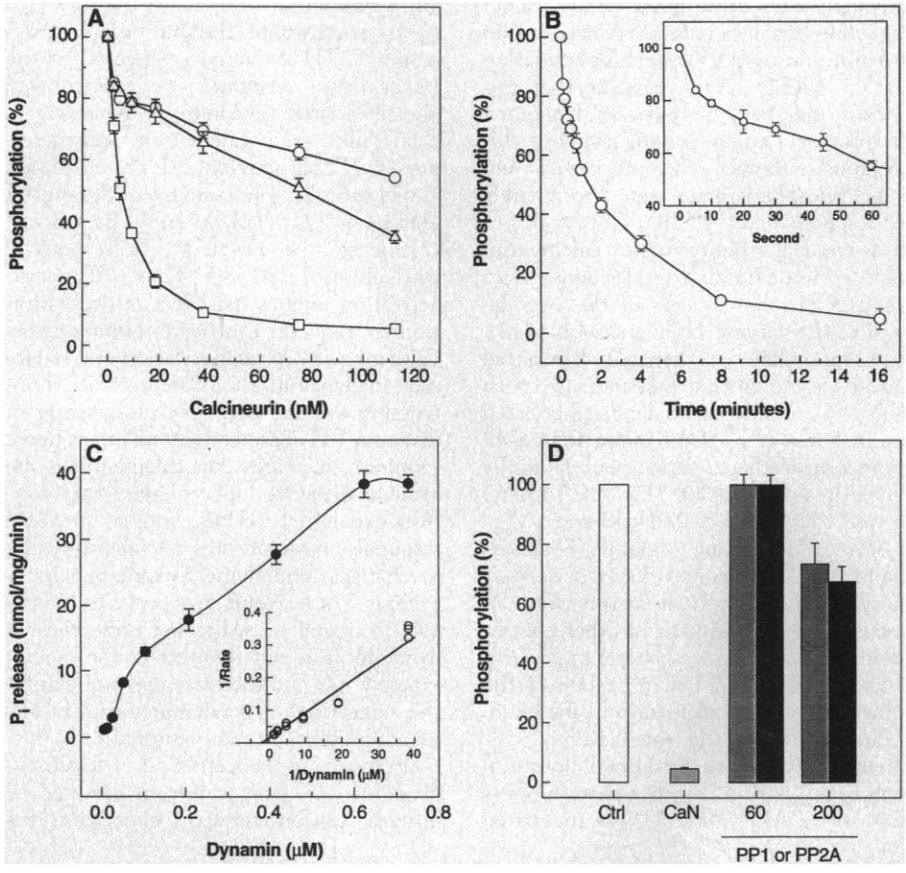

Fig. 3. Characterization of dynamin I dephosphorylation by calcineurin. (**A**) Concentration-dependent dephosphorylation of dynamin I by calcineurin. Purified phospho–dynamin I was incubated with different concentrations of calcineurin for 15 min, and the dephosphorylation was expressed as percentage of total phosphorylation. All conditions included Ca^{2+} and CaM, with further addition of Mg^{2+} (△), Mn^{2+} (□), or no addition (○). Results were from one of three similar experiments determined in triplicate. The concentrations used were Mg^{2+}, 1 mM; Mn^{2+}, 1 mM; Ca^{2+}, 200 μM; and CaM, 200 nM. (**B**) Time course of calcineurin dephosphorylation of dynamin I. Purified phospho–dynamin I time periods in the presence of Ca^{2+}, calmodulin, and Mn^{2+}. Results are from a typical single experiment determined in triplicate. (**C**) Kinetic analysis of dynamin I dephosphorylation by calcineurin. Various concentrations of purified phospho–dynamin I were dephosphorylated by calcineurin (9 nM). Kinetic parameters (K_m and V_{max}) were derived from the double reciprocal plot (insert). P_i, inorganic phosphate. (**D**) Specificity of dynamin I dephosphorylation. Purified phospho–dynamin I was used as a substrate for different protein phosphatases. The concentration of purified calcineurin was 9 nM (or 21 units, where 1 unit is equivalent to dephosphorylation of 1 pmol/min of PNPP). The amounts of partially purified phosphatase 1 (PP1, crosshatch bar) and phosphatase 2A (PP2A, solid bar) used are expressed as relative units. One unit is equivalent to the dephosphorylation of 1 pmol/min of ^{32}P-labeled glycogen phosphorylase. The results were from one of two experiments determined in triplicate. Ctrl, control. For methods, see (*25*).

terminals. Thus, protein kinase C phosphorylation and calcineurin dephosphorylation may underlie the effects of membrane excitation on cytosolic dynamin I GTPase activity.

Interactions between dynamin I and calcineurin were further analyzed in vitro (*24*, *25*) to determine the underlying mechanism. Dynamin I was purified from rat brain, stoichiometrically phosphorylated by protein kinase C, and repurified to remove protein kinase C (*24*). Calcineurin dephosphorylated dynamin I in a Ca^{2+}- and calmodulin-dependent manner (Fig. 2A). Dephosphorylation was optimal with addition of Mn^{2+}, a characteristic of calcineurin activation (*26*). When calcineurin was fully activated, 50% dephosphorylation (ED_{50}) was achieved at a calcineurin concentration of 5 nM and over 95% dephosphorylation at 75 nM (Fig. 3A), suggesting that calcineurin may be a high-affinity dynamin I phosphatase. Phosphopeptide mapping with V8 protease showed that both the 21- and 19-kD phosphopeptides were coordinately dephosphorylated (Fig. 2B). Thus, both isoforms are dephosphorylated by calcineurin, because these phosphopeptides derive from the COOH-terminal tails of the two dynamin I size variants of 96 and 94 kD (*12*). Dephosphorylation of dynamin I occurred within 5 s, the shortest time measured with this assay, and it was 50% dephosphorylated within 1 min (Fig. 3B). Kinetic analysis of calcineurin-mediated dephosphorylation by double reciprocal plot (Fig. 3C, insert) showed a K_m of 0.5 ± 0.01 μM and a V_{max} of 66 ± 1.3 nmol mg^{-1} min^{-1}. Thus, dynamin I has a moderate velocity of dephosphorylation, but a high affinity for calcineurin, three times that of other characterized calcineurin substrates (*26*–*28*). Therefore, dynamin I may be one of the primary targets for calcineurin in the brain.

To determine the specificity of calcineurin action, we examined potential dephosphorylation by other protein phosphatases (*25*). Dynamin I was incubated with calcineurin, phosphatase 1 (PP1), or phosphatase 2A (PP2A) (Fig. 3D). Whereas calcineurin [at concentrations equivalent to 21 units (1 unit equals 1 pmol/min for the substrate PNPP)] almost fully dephosphorylated 10 pmol of dynamin I, substantially high concentrations of either phosphatase PP1 or PP2A (60 or 200 units for the substrate glycogen phosphorylase) had little effect. The maximal extent of dephosphorylation by PP1 or PP2A was 30%, indicating the specificity of calcineurin. This is consistent with our previous report that dephosphorylation of dynamin I in nerve terminals is not sensitive to the low concentrations of okadaic acid (<1 μM) that inhibit PP1 and PP2A (*14*).

To reconstitute the inhibition of dynamin I GTPase activity observed in the intact nerve terminals, we co-incubated phosphorylated dynamin I with Ca^{2+}, calmodulin, and calcineurin and determined GTPase activity (*29*). Protein kinase C phosphorylation stimulated dynamin I GTPase activity (*12*), but in the presence of calcineurin the elevated GTPase activity was inhibited (Fig. 4). Thus, calcineurin activation mimics the effect of depolarization on dynamin I in nerve terminals, mediating both dephosphorylation and reduction in dynamin I GTPase activity. This, together with the effect of cyclosporin A on dynamin I GTPase activity in intact nerve terminals, indicates that calcineurin is the primary dynamin I phosphatase that controls dynamin I GTPase activity in nerve terminals and implicates calcineurin in the mechanisms underlying synaptic vesicle recycling. These events may prove to be specific to neural cells, because non-neuronal dynamin II is not a substrate for protein kinase C (*4*) and therefore may not exhibit the same Ca^{2+}-dependent regulation during endocytosis in non-neuronal cells.

In resting or recovering nerve terminals, dynamin I GTPase activity is elevated by phosphorylation and upon stimulation it is reduced. Therefore, we propose that Ca^{2+} is the trigger for synaptic vesicle recycling by means of activation of calcineurin and rapid dynamin I dephosphorylation. After depolarization, the recycling is then independent of intracellular Ca^{2+} and could then be terminated by rephosphorylation of dynamin I by protein kinase C. In support of this, dynamin I phosphorylation after depolarization occurred with a similar time course to the completion of vesicle recycling (*13*, *21*). The functional consequence of calcineurin-mediated dynamin I dephosphorylation was an inhibition of its elevated GTPase activity, which is directly required for endocytosis (*17*, *18*). Although it is not clear exactly how dynamin I GTPase activity operates in the regulation of vesicle recycling, this study suggests that dynamin I phosphorylation by protein kinase C and dephosphorylation by calcineurin serve as molecular switches to control nerve terminal vesicle recycling.

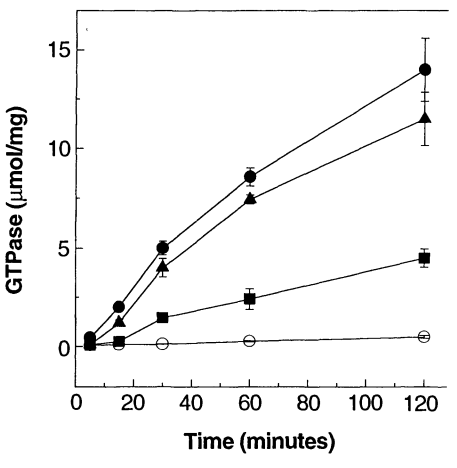

Fig. 4. Calcineurin dephosphorylation inhibits phospho–dynamin I GTPase activity. Purified dynamin I or phospho–dynamin I GTPase activity was measured in the presence or absence of calcineurin under the following conditions: ●, phospho-dynamin; ▲, phospho-dynamin + CaN-Mg^{2+}; ■, phospho-dynamin + CaN-Mn^{2+}; and ○, dynamin. Results were from one of two similar experiments determined in quadruplicate. For methods, see (*29*).

REFERENCES AND NOTES

1. B. K. Krueger, J. Forn, P. Greengard, *J. Biol. Chem.* **252**, 2764 (1977).
2. P. J. Robinson and P. R. Dunkley, *J. Neurochem.* **41**, 909 (1983).
3. H. S. Shpetner and R. B. Vallee, *Cell* **59**, 421 (1989).
4. J.-M. Sontag *et al.*, *J. Biol. Chem.* **269**, 4547 (1994).
5. T. A. Cook, R. Urrutia, M. A. McNiven, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 644 (1994).
6. T. Nakata, R. Takemura, N. Hirokawa, *J. Cell Sci.* **105**, 1 (1993).
7. J.-P. Liu, K. A. Powell, T. C. Südhof, P. J. Robinson, *J. Biol. Chem.*, in press.
8. R. A. Obar, C. A. Collins, J. A. Hammarback, H. S. Shpetner, R. B. Vallee, *Nature* **347**, 256 (1990).
9. H. S. Shpetner and R. B. Vallee, *ibid.* **355**, 733 (1992).
10. P. L. Tuma, M. C. Stachniak, C. A. Collins, *J. Biol. Chem.* **268**, 17240 (1993).
11. I. Gout *et al.*, *Cell* **75**, 25 (1993).
12. P. J. Robinson *et al.*, *Nature* **365**, 163 (1993).
13. P. J. Robinson, *J. Biol. Chem.* **267**, 21637 (1992).
14. A. T. R. Sim, P. R. Dunkley, P. E. Jarvie, J. A. P. Rostas, *Neurosci. Lett.* **126**, 203 (1991).
15. M. S. Chen *et al.*, *Nature* **351**, 583 (1991).
16. A. M. Van der Bliek and E. M. Meyerowitz, *ibid.*, p. 411.
17. A. M. Van der Bliek *et al.*, *J. Cell Biol.* **122**, 553 (1993).
18. J. S. Herskovits, C. C. Burgess, R. A. Obar, R. B. Vallee, *ibid.*, p. 565.
19. S. L. Schmid, *BioEssays* **14**, 589 (1992).
20. U. Pley and P. Parham, *Crit. Rev. Biochem. Mol. Biol.* **28**, 431 (1993).
21. T. A. Ryan *et al.*, *Neuron* **11**, 713 (1993).
22. Intact nerve terminals were incubated at 37°C, depolarized by addition of KCl (final concentration 41 mM), repolarized by centrifugation (1 min, 1000*g*), and then resuspended in buffer containing 4.7 mM KCl as described (*12*). Cytosolic and peripheral membrane fractions were prepared (*7*) and immunoprecipitations of dynamin I conducted with specific polyclonal antibodies (*12*). Determination of immunoprecipitated dynamin I GTPase activity was performed by incubation of the immunoprecipitated dynamin I in 40 μl containing 10 mM tris-HCl (pH 7.4), 2 mM Mg^{2+}, and 10 mM NaCl at 37°C with [γ-^{32}P]GTP (0.9 mM GTP and 2 μCi of [γ-^{32}P]GTP) for 15 min. The GTP hydrolysis rate was determined as described (*12*).
23. J. Liu *et al.*, *Cell* **66**, 807 (1991).
24. Dynamin I was purified from rat brain (*7*) and 100 μg was stoichiometrically phosphorylated by purified

protein kinase C and [γ-^{32}P]ATP and repurified with S-Sepharose cation-exchange chromatography (*12*). The repurified phospho–dynamin I (0.25 μg) was incubated with calcineurin in a 40-μl mixture containing 10 mM tris buffer, pH 7.4, and various cofactors for 15 min at 30°C and terminated by addition of SDS stop solution (*7*). Dephosphorylation was then analyzed by SDS–polyacrylamide gel electrophoresis and autoradiography. Phosphopeptide mapping was as described (*12*). Purified calcineurin (Upstate Biotechnology Incorporated, New York) had a specific activity of 1 μmol mg^{-1} min^{-1} [ρ-nitrophenol phosphate (PNPP)] and was homogeneous on Coomassie staining of polyacrylamide gels.

25. Purified dynamin I was phosphorylated by protein kinase C and repurified. Phospho–dynamin I (0.75 μg) was incubated with various concentrations of calcineurin plus Ca^{2+} and calmodulin (CaM) in the absence or presence of Mn^{2+} or Mg^{2+} for 15 min, or as otherwise indicated, in 40 μl of 10 mM tris buffer (pH 7.4) at 30°C, and incubations were terminated with 8 μl of phosphoric acid (375 mM). The reaction mixture (30 μl) was then spotted onto P81 phosphocellulose paper, and the dynamin I and radiolabeled inorganic phosphate ($^{32}P_i$) released were separated by extensive washing of the paper with 75 mM phosphoric acid. The paper was then dried, cut, and counted in a β-counter. Dephosphorylation is expressed as $^{32}P_i$ released or as a percentage of total phosphorylation. The K_m and V_{max} were calculated from the inset double reciprocal plot with the PC program Enzfit (Elsevier). For dephosphorylation specificity, purified phospho–dynamin I (1.0 μg) was incubated with or without calcineurin (9 nM, 21 U) or excess partially purified PP1 and PP2A. Dephosphorylation conditions were as before except that 0.1% β-mercaptoethanol was present for optimal PP1 and PP2A activity. PP1 and PP2A were partially purified from rat liver by ethanol extraction and DEAE-sephadex and polylysine-Sepharose chromatography.

26. T. S. Ingebritsen and P. Cohen, *Eur. J. Biochem.* **132**, 255 (1983).
27. M. M. King *et al.*, *J. Biol. Chem.* **259**, 8080 (1984).
28. Y. C. Liu and D. R. Storm, *ibid.* **264**, 12800 (1989).
29. Purified dynamin I (0.5 μg) or phospho–dynamin I (0.25 μg) was incubated in 40 μl of 10 mM tris (pH 7.4) containing 10 mM NaCl without or with 18 nM calcineurin, 200 μM Ca^{2+}, and 200 nM CaM in the presence of 2 mM Mg^{2+} or 1 mM Mn^{2+} as indicated in Fig. 4 for 15 min. The reaction was then terminated with addition of 2 mM EGTA, and GTPase assay was initiated with the addition of [γ-^{32}P]GTP (0.9 mM GTP and 2 μCi of [γ-^{32}P]GTP) for the further time periods indicated. GTP hydrolysis was determined as described (*12*).
30. Supported by the National Health and Medical Research Council of Australia.

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