representing different vegetative compatibility groups were found to support replication of resurrected L-dsRNA (Fig. 3A) suggests the possibility that transformation vectors similar to pXH9 could be used to engineer hypovirulence in field isolates that represent the range of vegetative compatibility groups present in a specific ecosystem. Moreover, because sexual compatibility in *C. parasitica* is determined by a single mating-type locus with two alleles (*29*), it is also likely that the integrated viral cDNA copy would spread through the virulent strain population by nuclear inheritance as the result of mating, irrespective of the barriers normally imposed by the vegetative compatibility system. Subsequent resurrection of cytoplasmic L-dsRNA from the inherited viral cDNA could then result in expanded vegetative dissemination. Because the surviving root systems of blight-infested American chestnut trees continue to produce sprouts throughout the natural range (*1*), it is conceivable that the release of improved, genetically engineered hypovirulent *C. parasitica* strains could lead to the restoration of this valuable forest species.

REFERENCES AND NOTES

1. S. L. Anagnostakis, *Science* **215**, 466 (1982).
2. M. K. Roane, G. J. Griffin, J. R. Elkins, in *Chestnut Blight, Other Endothia Diseases and the Genus Endothia* (APS Press, St. Paul, MN, 1986), pp. 1–53.
3. G. J. Griffin, *Hortic. Rev.* **8**, 291 (1986).
4. W. L. MacDonald and D. W. Fulbright, *Plant Dis.* **75**, 656 (1991).
5. J. Grente, *C.R. Hebd. Seances Acad. Agric. Fr.* **51**, 1033 (1965).
6. J. Grente and S. Berthelay-Sauret, in *Proceedings of the American Chestnut Symposium*, W. L. MacDonald, F. C. Cech, J. Luchok, C. Smith, Eds. (West Virginia Univ. Press, Morgantown, WV, 1978), pp. 30–34.
7. N. K. Van Alfen, R. A. Jaynes, S. L. Anagnostakis, P. R. Day, *Science* **189**, 890 (1975).
8. P. R. Day, J. R. Dodds, J. E. Elliston, R. A. Jaynes, S. L. Anagnostakis, *Phytopathology* **67**, 1393 (1977).
9. G. H. Choi, R. Shapira, D. L. Nuss, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1167 (1991).
10. G. H. Choi, D. M. Pawlyk, D. L. Nuss, *Virology* **183**, 747 (1991).
11. R. Shapira, G. H. Choi, D. L. Nuss, *EMBO J.* **10**, 731 (1991).
12. R. Shapira and D. L. Nuss, *J. Biol. Chem.* **266**, 19419 (1991).
13. E. V. Koonin, G. H. Choi, D. L. Nuss, R. Shapira, J. C. Carrington, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10647 (1991).
14. K. W. Buck, in *Fungal Virology*, K. W. Buck, Ed. (CRC Press, Boca Raton, FL, 1986), pp. 1–84.
15. R. B. Wickner, *FASEB J.* **3**, 2257 (1989).
16. M. El-Sherbeini, D. J. Tipper, D. J. Mitchell, K. A. Bostian, *Mol. Cell. Biol.* **4**, 2818 (1984).
17. S. Hiremath, B. L'Hostis, S. A. Ghabrial, R. E. Rhoads, *Nucleic Acids Res.* **14**, 9877 (1986).
18. R. K. Saiki *et al.*, *Science* **239**, 487 (1988).
19. G. H. Choi and D. L. Nuss, *EMBO J.* **11**, 473 (1992). Plasmid pCPXHY1 is a derivative of pAXHY2 in which the ORF A coding region was replaced by several unique cloning sites that included a Stu I restriction site that was subsequently used in the construction of pXH9.
20. D. Cullen, S. A. Leong, L. J. Wilson, D. J. Henner, *Gene* **57**, 21 (1987).
21. B. I. Hillman, R. Shapira, D. L. Nuss, *Phytopathology* **80**, 950 (1990).
22. G. H. Choi and D. L. Nuss, data not shown.
23. R. Shapira, G. H. Choi, B. I. Hillman, D. L. Nuss, *EMBO J.* **10**, 741 (1991).
24. S. L. Anagnostakis, *Exp. Mycol.* **1**, 306 (1977).
25. J. E. Elliston, in *Proceedings of the American Chestnut Symposium*, W. L. MacDonald, F. C. Cech, J. Luchok, C. Smith, Eds. (West Virginia Univ. Press, Morgantown, WV, 1978), pp. 95–100.
26. R. A. Jaynes and J. F. Elliston, *Phytopathology* **70**, 453 (1980).
27. S. L. Anagnostakis, B. Hau, J. Kranz, *Plant Dis.* **70**, 536 (1986).
28. S. L. Anagnostakis, *Genetics* **102**, 25 (1982).
29. ———, in *The Ecology and Physiology of the Fungal Mycelium*, D. H. Jennings and A. D. M. Ryaner, Eds. (Cambridge Univ. Press, Cambridge, 1984), pp. 499–507.
30. S. L. Anagnostakis, *Mycologia* **75**, 777 (1983).
31. B. I. Hillman, Y. Tian, P. J. Bedker, M. P. Brown, *J. Gen. Virol.* **73**, 681 (1992).

32. The authors thank P. Bedker, Rutgers University, for assistance with virulence assays and statistical analysis. Strains EP146 and NB58-19 were gifts from S. Anagnostakis, Connecticut Agricultural Experiment Station, and B. Hillman, Rutgers University, respectively.

24 March 1992; accepted 29 May 1992

Identification of a Protein That Binds to the SH3 Region of Abl and Is Similar to Bcr and GAP-rho

Piera Cicchetti, Bruce J. Mayer, Gerald Thiel,* David Baltimore†

A Src homology 3 (SH3) region is a sequence of approximately 50 amino acids found in many nonreceptor tyrosine kinases and other proteins. Deletion of the SH3 region from the protein encoded by the c-*abl* proto-oncogene activates the protein's transforming capacity, thereby suggesting the participation of the SH3 region in the negative regulation of transformation. A complementary DNA was isolated that encoded a protein, 3BP-1, to which the SH3 region of Abl bound with high specificity and to which SH3 regions from other proteins bound differentially. The sequence of the 3BP-1 protein is similar to that of a COOH-terminal segment of Bcr and to guanosine triphosphatase–activating protein (GAP)–rho, which suggests that it might have GAP activity for Ras-related proteins. The 3BP-1 protein may therefore be a mediator of SH3 function in transformation inhibition and may link tyrosine kinases to Ras-related proteins.

The c-*abl* proto-oncogene is one of many genes that encode nonreceptor tyrosine kinases that contain SH3 regions (*1*). These regions also occur in a wide variety of other proteins, such as phospholipase C-γ and the cytoskeletal proteins, myosin1, spectrin, and ABP-1, an actin binding protein from yeast (*1*). SH3 regions might interact with the actin cytoskeleton and mediate their functions through protein-to-protein interactions (*2*). However, we did not detect any direct binding of the SH3 region of Abl to filamentous actin (F-actin) (*3*). Deletion or mutation of the SH3 region in both Src and Abl activates their transforming abilities, which indicates that this region has a negative regulatory function in transformation (*4*). One possible mode of action of the SH3 region might be to bind to another protein that mediates a negative effect on transformation. We therefore searched for proteins that bind to the Abl SH3 region.

We used the pGEX bacterial expression vector to create a fusion protein that contained the glutathione-S-transferase (GST) of pGEX and the 55–amino acid SH3 region of Abl (*5*). This fusion protein was biotinylated (*6*) and used to probe a λgt11 cDNA expression library made from the mouse pre-B cell line 22D6. Six positive clones out of approximately 7×10^6 were identified (*7*), and five were plaque-purified, yielding cDNA fragments that represented two independent clones. To verify that the β-galactosidase fusion proteins from the recombinant λgt11 vectors were responsible for the reactivity, we produced lysogens from these clones (*8*). These lysogens were induced with isopropylthio-β-D-galactoside (IPTG), and the induced and uninduced proteins were fractionated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose, and probed with biotinylated SH3-GST fusion peptides (*9*). The GST–Abl SH3 probe recognized a protein of approximately 130 kD from the induced lysate of clone 1 but not from its uninduced lysate and a protein of approximately 150 kD from the induced lysate of clone II but not from its uninduced lysate (Fig. 1). A GST fusion protein probe that contained the SH3 region of murine Src also recognized the 150-kD protein of clone II but did not recognize the 130-kD protein of clone I (Fig. 1). A biotinylated control GST probe reacted only with background proteins of the induced and uninduced lysates. The IPTG-induced 130-kD and 150-kD β-galactosidase fusion proteins of clones I and II

The Rockefeller University, New York, NY 10021.

*Present address: Institut fur Genetik, Universitat zu Koln, D-5000 Koln 1, Germany.
†To whom correspondence should be addressed.

were recognized by an antibody to β-galactosidase (*3*, *10*), which confirms that they are β-galactosidase fusion proteins of the recombinant λgt11 vectors. Thus, we have identified two distinct clones: clone I, which encodes a protein recognized by the Abl SH3 probe, and clone II, which encodes a protein recognized by both the Abl and Src SH3 probes. Because the Abl SH3 probe selectively bound to these proteins and no others in extensive expression library screening, the reactivity of Abl SH3 for them appears to have a very high selectivity and avidity.

We further characterized clone II, the product of which we refer to as 3BP-1. To obtain sequence information, we subcloned the 1362-bp λgt11 insert that encoded 3BP-1 into the Eco RI site of the pBluescript vector (pBS) (*11*) and sequenced it (*12*). An open reading frame of 339 amino acids was identified that started at the 5′ end of the insert, which indicates that the clone is a partial one encoding the COOH-terminal region of 3BP-1 (Fig. 2A). A computer-assisted sequence search with the GenBank database revealed that the sequence of 3BP-1 is similar to both that of the COOH-terminal region of the human Bcr polypeptide, the product of the breakpoint cluster region gene of the Philadelphia chromosome translocation that participates in chronic myelogenous leukemia (*13*), and that of the neuronal protein n-chimaerin (*14*) (Fig. 2B). All three proteins showed similarity to the available partial sequence of GAP-rho (*15*), a guanosine triphosphatase–activating protein (GAP) for rho, a Ras-related protein that appears to participate in cytoskeletal organization (*16*). The region of similarity starts at the extreme NH_2-terminus of the 3BP-1 clone and extends for approximately 128 amino acids, having 33.6% identity in this region to Bcr, 37% identity to n-chimaerin, and 71% identity over a 17–amino acid region of the GAP-rho peptide. These domains of Bcr and n-chimaerin (with additional NH_2-terminal flanking sequences) have GAP activity (*15*), which suggests that the 3BP-1 protein might also have GAP activity.

The binding site for the Abl SH3 domain on 3BP-1 was determined by ligation of various fragments of the 3BP-1 cDNA (*17*) (Fig. 3A) in-frame to the GST of pGEX vectors and by their expression after transformation into *Escherichia coli*. Transformants were induced with IPTG, and the electrophoretically separated proteins were probed with the biotinylated GST–Abl SH3 domain fusion protein. We normalized the amount of GST fusion protein for all samples by probing with an antibody to GST (*3*). The full-length 3BP-1 clone was bound by the probe (Fig. 3B), but the NH_2-terminal 204 amino acids showed no binding. Cleavage at nucleotide 750 led to a minimally active fragment, which suggests that this fragment contains part of the binding site. Other constructs showed that strong binding could be achieved by the fusion protein that contained amino acids 248 to 275 of 3BP-1. This 28–amino acid binding sequence is located 120 amino acids COOH-terminal to the region of simi-

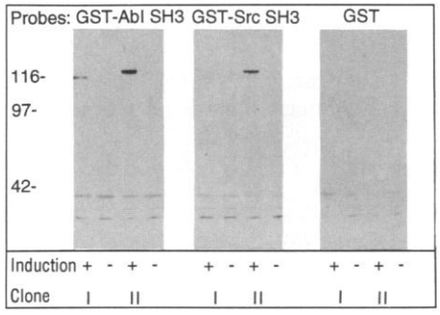

Fig. 1. Binding of SH3 domains to β-galactosidase fusion proteins from selected λgt11 clone lysates (clones I and II). Left, probed with the biotinylated Abl SH3 domain fusion protein probe; middle, probed with the biotinylated Src SH3 domain fusion protein probe; right, probed with the control GST protein. Molecular size markers are indicated to the left in kilodaltons.

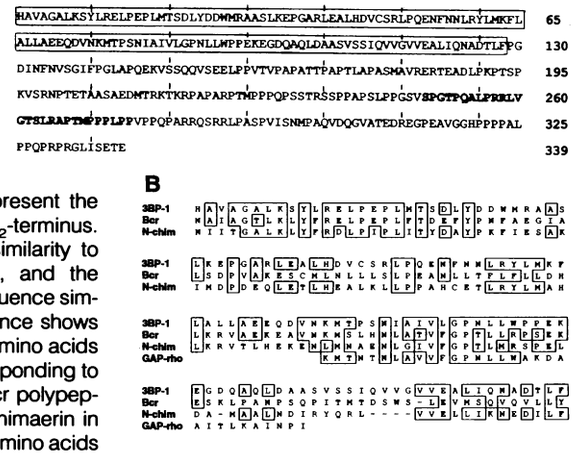

Fig. 2. Amino acid sequence of 3BP-1 and comparison to Bcr, n-chimaerin, and GAP-rho (*31*). (**A**) Sequence of the partial clone of 3BP-1. Boxing shows area of similarity to the COOH-terminus of Bcr; bold shows the Abl SH3 binding domain. Lines mark every ten amino acids from the NH_2-terminus; numbers on the side represent the number of amino acids from the NH_2-terminus. (**B**) 3BP-1 amino acid sequence similarity to human Bcr, n-chimaerin (N-chim), and the GAP-rho peptide. Boxing shows sequence similarity. The 3BP-1 amino acid sequence shows 33.6% identity with a region of 128 amino acids in the COOH-terminus of Bcr, corresponding to amino acids 1078 to 1203 of the Bcr polypeptide. 3BP-1 has 37% identity to n-chimaerin in this same region, corresponding to amino acids 177 to 303 of the human n-chimaerin protein. 3BP-1 also shares 71% identity with the GAP-rho peptide over a 17–amino acid region. Dashes indicate gaps in the sequence for alignment.

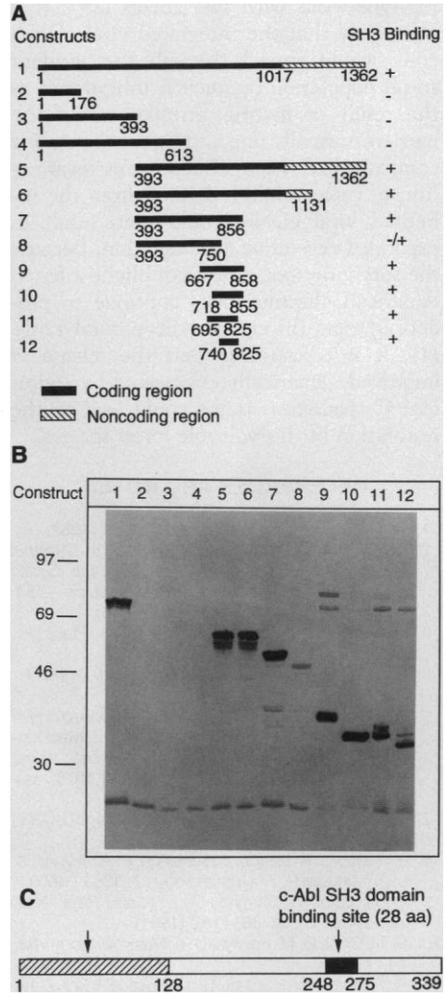

Fig. 3. Mapping of the SH3 domain binding site on 3BP-1. (**A**) 3BP-1 cDNA fragments fused in-frame to the GST gene in pGEX. Construct 1 represents the full-length 3BP-1 cDNA insert, and constructs 2 through 12 represent various fragments of 3BP-1. The numbers under the bars represent the nucleotides from the 5′ end of the full-length insert. (**B**) Binding of Abl SH3 to 3BP-1 fragments. Proteins from induced lysates from bacteria that expressed recombinant pGEX vectors encoding the various GST–3BP-1 fragment fusion proteins were probed with the biotinylated GST–Abl SH3 fusion protein. Numbers on left represent molecular size standards in kilodaltons. (**C**) Location of the Abl SH3 binding site region of 3BP-1. The arrow on the left shows the region homologous to the COOH-terminus of Bcr, to n-chimaerin, and to GAP-rho [128 amino acids (aa)]. Numbers are as in Fig. 2A.

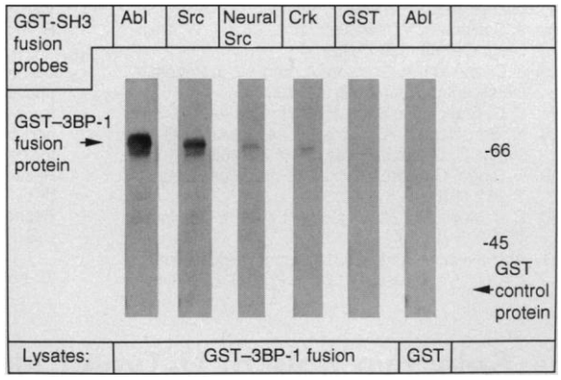

Fig. 4. Differential binding of 3BP-1 to various SH3 regions. Cell lysate proteins from an induced bacterial culture expressing the recombinant GST–Abl SH3 binding site region of 3BP-1 were fractionated by SDS-PAGE, transferred to nitrocellulose, and probed with the biotinylated GST–fusion protein probes containing the Abl, Src, neural Src, and Crk SH3 regions, and the control GST. An induced lysate from bacteria that expressed a nonrecombinant pGEX vector was probed with the biotinylated Abl SH3 protein. Numbers on the right represent molecular size in kilodaltons.

larity with Bcr and GAP-rho (Fig. 3C); this region of 3BP-1 is not similar to other protein sequences in GenBank.

To evaluate the relative affinity of binding of various SH3 regions to 3BP-1, we used the pGEX vector to make fusion proteins that contained the SH3 regions of both Crk and neural Src (9). The latter protein has six more amino acids in the SH3 region than Src (*18*). These probes and Src, Abl, and GST control probes were biotinylated (6) and normalized for protein and biotin content (*19*). Proteins from an induced bacterial cell lysate that contained the GST–3BP-1 fusion protein (construct 5 in Fig. 3A) were subjected to SDS-PAGE and transferred to nitrocellulose. At probe concentrations of about 30 nM, the 3BP-1 fusion protein bound most strongly to the Abl SH3 domain probe and slightly less strongly to the Src SH3 domain probe (Fig. 4). However, the 3BP-1 fusion protein bound very weakly to the neural Src SH3 and Crk SH3 probes and did not bind at all to the control GST probe. The Abl SH3 probe did not bind to any protein in the induced GST control lysate. Thus, 3BP-1 binds differentially to different SH3 domains and provides a molecular probe that can distinguish among SH3 domains. The lack of reactivity of Crk SH3 with 3BP-1 suggests that different SH3 domains are not equivalent and presumably may interact with different targets. The fact that the neural Src insert disrupts the binding of Src SH3 to 3BP-1 suggests that neural Src may escape regulation by SH3. Thus, in neurons Src may be regulated differently than in other cells.

The SH3 region is a module of protein structure that is found in many proteins. Most of these proteins participate in growth control or are cytoskeletal proteins (*20*). The SH3 region might function by either internal interaction within proteins or by interaction with other molecules. The cloning of a cDNA segment that encodes a protein that selectively binds with high affinity to particular SH3 domains suggests that the SH3 domain functions through a specific protein-to-protein interaction. The rarity of the cloned segment in the cDNA expression library suggests that the binding protein is of low abundance, and measurements of mRNA abundance have confirmed this (*21*). The second partial clone isolated from the cDNA library (clone 1, Fig. 1) is unrelated in sequence to 3BP-1 with the exception of a five–amino acid identity broken by a four–amino acid gap in the SH3 domain binding region of 3BP-1 (*3*).

3BP-1 may take part in normal Abl function by binding to the Abl SH3 domain. Because 3BP-1 is similar to GAP-rho and other proteins with GAP activity, it could function as an intermediary between SH3 domains and the signal transduction pathway mediated by the Ras-related guanosine triphosphate (GTP) binding proteins. In the Abl and Src nonreceptor protein tyrosine kinases, mutation or deletion of the SH3 region can activate a strong oncogenic transforming potential, which suggests that it has an anti-oncogenic function in normal proteins (*4*) and that their lack of oncogenic activity could result from binding to 3BP-1. The binding of 3BP-1 to the Abl SH3 could influence protein tyrosine kinase activity and the SH2 region of Abl, both of which are needed for the protein to transform (*22*). The 3BP-1 protein could also counter the transforming potential of these kinases through its implied GAP activity, which could downregulate the activity of a Ras-related protein that might be in the transformation pathway. The similarity of the 3BP-1 protein sequence to that of GAP-rho raises the possibility that 3BP-1 could be a GAP for rho, thus potentially linking SH3 domain function to transformation inhibition, GAP activity, and cytoskeletal organization.

The linkage of protein tyrosine kinases, SH3 domains, and Bcr or GAP-related proteins has earlier precedents. The p85α subunit of phosphatidylinositol-3-kinase, an enzyme implicated in the signal transduction pathway of growth factor tyrosine kinases, and the related p85β protein both contain not only an SH3 domain but also a region that is related in sequence to the region of similarity shared by 3BP-1 and the COOH-terminus of Bcr (*23*). The Ras-GAP–associated protein p190 (*24*) and 3BP-1 have sequence similarity to the same COOH-terminal region of Bcr (*25*), which further implicates a family of Bcr-related proteins in the Ras signaling pathway. Properties of the yeast BEM genes, whose products take part in bud formation and cell polarity, further support the association of SH3 domains with Bcr-related proteins. BEM1 contains two SH3 domains (*26*), whereas BEM2 and BEM3 both have regions similar to the COOH-terminus of Bcr (*23*). Finally, *Sem-5*, a *Caenorhabditis elegans* gene, has been identified; it encodes a protein composed almost entirely of SH2 and SH3 domains. The Sem-5 protein product, along with Let-23, a receptor tyrosine kinase, and Let-60, a Ras protein, participates in signal transduction during vulval cell development (*27*). These various studies imply a functional association of SH3 domains, GAP-related proteins, and Ras-related GTP binding proteins. Our results provide direct evidence of a physical link between SH3 regions and a protein that may have GAP activity as a result of its similarity to GAP-rho and the GAP-active COOH-terminus of Bcr. The binding of 3BP-1 to the SH3 regions of Abl and Src suggests the possible direct association of nonreceptor tyrosine kinases with the Ras-related GTP-binding protein signal transduction pathway.

REFERENCES AND NOTES

1. C. A. Koch, D. Anderson, M. F. Moran, C. Ellis, T. Pawson, *Science* **252**, 668 (1991).
2. D. G. Drubin, J. Mulholland, Z. Zhu, D. Botstein, *Nature* **343**, 288 (1990).
3. P. Cicchetti, B. J. Mayer, G. Thiel, D. Baltimore, unpublished results.
4. J. Hirai and H. E. Varmus, *Mol. Cell. Biol.* **10**, 1307 (1990); P. Jackson and D. Baltimore, *EMBO J.* **8**, 449 (1989); J-Y. Kato *et al.*, *Mol. Cell. Biol.* **6**, 4155 (1986); W. M. Potts, A. B. Reynolds, T. J. Lansing, J. T. Parsons, *Oncogene Res.* **3**, 343 (1988); W. M. Franz, P. Berger, J. Y. J. Wang, *EMBO J.* **8**, 137 (1989); C. Seidel-Dugan, B. E. Meyer, S. M. Thomas, J. S. Brugge, *Mol. Cell. Biol.* **12**, 1835 (1992).
5. The Abl SH3 probe contains amino acids 84 through 138 of murine type IV c-*abl* (*28*) fused in-frame at the Bam HI site of pGEX-2T.
6. Biotinylations were performed as described [B. Mayer, P. K. Jackson, D. Baltimore, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 627 (1991)].
7. The expression library was screened as described (*29*) with the following modifications: after nitrocellulose filters were incubated on the plaque-containing Luria broth (LB) plates for 4 hours at 37°C, filters were blocked for 30 min in a TBST [50 mM tris (pH 8.0), 150 mM NaCl, and Tween (0.1%)] and gelatin (0.2%) (G) solution at room temperature (RT) with constant shaking (CS). Because incubation with streptavidin alka-

line phosphatase at this point caused high background, a sequential block with avidin and then biotin was performed first as follows: avidin (1 μM) (Pierce) was added to the TBST-G and incubated for 1 hour. Filters were then washed four times with TBST and then incubated with TBST-G and biotin (0.1 mM to 1 mM) (Sigma) for 40 min at RT with CS. Filters were washed as above and incubated with the biotinylated peptide probe (1 μg/ml) in TBST-G for 4 hours at 4°C with CS. Filters were washed as above except at 4°C then incubated for 1 hour with streptavidin alkaline phosphatase (Boehringer Mannheim) at a dilution of 1:5000 in TBST-G at 4°C with CS, washed as before, and then washed once with alkaline phosphatase buffer [100 mM tris (pH 9.5), 100 mM NaCl, and 5 mM MgCl], and developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Promega), as described by the manufacturer.

8. Lysogens were prepared as described (*29*) except that the preparation of extracts was stopped after the lysozyme was added to thawed cell suspensions.
9. The SH3 domains of murine Src, neural Src, and the 5′ SH3 of murine Crk were prepared by reverse transcription and polymerase chain reaction (PCR) amplification of total RNA from 3T3 cells or mouse forebrain (for neural Src). Primers were synthesized with the 5′ Bam HI and 3′ Eco RI sites for insertion in-frame with the GST pGEX-2T. The Src SH3 probe contained amino acids 88 to 141 (amino acids 88 to 147 of neural Src) and did not differ from the published sequence (*18*). The Crk SH3 contained amino acids that corresponded to amino acids 372 through 428 of v-*crk* (*30*) and differs from the chicken sequence at positions corresponding to amino acids 384, 385, 396, 411, and 492, where the mouse gene encodes Glu, Glu, Arg, Ser, and Glu, respectively. These probes and a control GST probe were biotinylated (*6*).
10. Biotinylated anti–β-galactosidase antibody was purchased from Pierce.
11. The pBS vector was purchased from Stratagene.
12. Sequencing reactions were carried out with the Sequenase kit (U.S. Biochemical) according to the manufacturer's protocols.
13. E. Shtivelman, B. Lifshitz, R. P. Gale, E. Canaani, *Nature* **315**, 550 (1985).
14. C. Hall *et al.*, *J. Mol. Biol.* **211**, 11 (1990).
15. D. Diekmann *et al.*, *Nature* **351**, 400 (1991).
16. H. F. Paterson *et al.*, *J. Cell Biol.* 111, 1001 (1990).
17. Constructs 1 through 9 represent various restriction enzyme fragments of 3BP-1 subcloned from pBS in-frame with the GST of pGEX (data not shown). Constructs 10 through 12 represent 3BP-1 fragments in pGEX derived from synthetic oligonucleotide–directed PCR of the 3BP-1 cDNA. PCR fragments were constructed with synthetic oligonucleotide primers directed against internal sites in construct 9 as shown (Fig. 3A). Oligos contained 24 bp of identical sequence for hybridization and either entire Bam HI or Eco RI sites with five extra base pairs at the 5′ end for stability and recutting efficiency. The 5′ oligos were in-frame with the GST of pGEX.
18. R. Martinez, B. Mathey-Prevot, A. Bernards, D. Baltimore, *Science* **237**, 411 (1987).
19. Protein concentration of the fusion protein probes was determined with the Bio-Rad protein assay kit for standardization of the GST fusion protein probes. These proteins were further assayed by SDS-PAGE and staining with Coomassie blue. To compare the biotinylation of the protein probes, we transferred proteins in an identical gel to nitrocellulose and assayed the biotin content by binding to streptavidin alkaline phosphatase; the color was developed as described (*7*).
20. L. C. Cantley *et al.*, *Cell* **64**, 281 (1991).
21. W. S. Pear, P. Cicchetti, D. Baltimore, unpublished results.
22. R. Prywes, J. G. Foulkes, N. Rosenberg, D. Baltimore, *Cell* **34**, 569 (1983); R. Prywes, J. Hoag, N. Rosenberg, D. Baltimore, *J. Virol.* **54**, 123 (1985).
23. M. J. Fry, *Curr. Biol.* **2**, 78 (1992).
24. C. Ellis, M. Moran, F. McCormick, T. Pawson, *Nature* **343**, 377 (1990).
25. J. Settleman, V. Narasimhan, L. Foster, R. Weinberg, *Cell* **69**, 539 (1992).
26. J. Chenevert, K. Corrado, A. Bender, J. Pringle, I. Herskowitz, *Nature* **356**, 77 (1992).
27. S. G. Clark, M. J. Stern, H. R. Horvitz, *ibid.*, p. 340.
28. Y. Ben Neriah, A. Bernards, M. Paskind, G. Q. Daley, D. Baltimore, *Cell* **44**, 577 (1986).
29. R. Singh, G. Clerc, J. H. LeBowitz, *Biotechniques* **7**, 252 (1989).
30. B. J. Mayer, M. Hamaguchi, H. Hanafusa, *Nature* **332**, 272 (1988).
31. Abbreviations for the amino acid residues are: 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.
32. The λgt11 library was the kind gift of A. Winoto. We thank P. K. Jackson for helpful discussions and the c-*abl* SH3-GST fusion probe, R. Darnell for mouse brain mRNA, A. Ray for helpful advice and the Y1089 bacterial strain, and E. Schaeffer for bacterial strain Y1090. Supported by National Research Service award training grant A107233 (P.C.), B. Duke Glenn Fellowship (P.C.), National Research Service award CA 08875 (B.J.M.), and U.S. Public Health Service grant CA 51462 (D.B.).

22 April 1992; accepted 16 June 1992

Selective Role of N-Type Calcium Channels in Neuronal Migration

Hitoshi Komuro* and Pasko Rakic

Analysis of neuronal migration in mouse cerebellar slice preparations by a laser scanning confocal microscope revealed that postmitotic granule cells initiate their migration only after the expression of N-type calcium channels on their plasmalemmal surface. Furthermore, selective blockade of these channels by addition of ω-conotoxin to the incubation medium curtailed cell movement. In contrast, inhibitors of L- and T-type calcium channels, as well as those of sodium and potassium channels, had no effect on the rate of granule cell migration. These results suggest that N-type calcium channels, which have been predominantly associated with neurotransmitter release in adult brain, also play a transient but specific developmental role in directed migration of immature neurons before the establishment of their synaptic circuits.

The majority of neurons in the developing central nervous system migrate from the site of their last cell division to their distant final positions (*1*). Proper acquisition of this position, attained through the process of active migration, ultimately affects a neuron's morphology, synaptic connectivity, and function (*2*). However, the cellular and molecular mechanisms of cell migration are not well understood. So far it has been established that in the cerebellar cortex, postmitotic granule cells migrate away from the germinal external granular layer toward the internal granular layer along the elongated fibers of Bergmann glial cells (*3*), and several cell adhesion molecules, which may play a role in this movement, have been isolated (*4*). Much less attention has been paid to the cell membrane properties and ionic concentration during cell movement. Only recently has it been recognized that migrating neurons in the developing central and peripheral nervous system express voltage-sensitive ion channels before reaching their final destinations (*5*). Here, we present evidence that the directed migration of postmitotic granule cells in the developing cerebellum requires functional ω-conotoxin–sensitive (N-type) Ca^{2+} channels.

Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510.

*To whom correspondence should be addressed.

We analyzed the mode of granule cell migration in slice preparations obtained from postnatal 10-day-old mouse cerebella labeled with a lipophilic carbocyanine dye (DiI) using a laser scanning confocal microscope. This method of analysis was chosen in preference to cultures of dissociated cells because it allows the visualization of both the soma and leading processes of migrating granule cells in situ without disturbing the ambient microenvironment (Fig. 1). The labeling of a large number of postmitotic granule cells with DiI allows precise localization of their position over time (*6*). Our measurements of the length of the migratory pathway during the first 6 hours in slice preparations indicate that the dynamics and rate of granule cell migration in vitro of 10 to 17 μm per hour are comparable to those measured in vivo (Fig. 2) (*6*). It was also possible to follow the movement of individual neurons in the slice preparation by collecting images of identified migrating granule cells every 1 to 10 min over a period of several hours. The rate of migration observed in the first 2 hours, 16.7 ± 5.0 μm per hour (mean ± SD, $n = 21$), was similar to the rate obtained from the methods described in (*6*) and is consistent with the measurements performed on cocultures of dissociated cerebellar granule and Bergmann glial cells (*7*). It also corresponds well with autoradiographic data obtained from studies of cells labeled with [^{3}H]thymidine