A New Member of the Amphiphysin Family Connecting Endocytosis and Signal Transduction Pathways*

(Received for publication, February 5, 1997, and in revised form, April 9, 1997)

Corinne Leprince‡§, Francisco Romero**, Didier Cussac**, Beatrice Vayssiere‡, Roland Berger‡‡, Armand Tavitian‡, and Jacques H. Camonis‡

From ‡INSERM U248, Institut Curie, 26 rue d’Ulm, 75231 Paris Cedex 05, France, §INSERM U363, Institut Cochin de Génétique Moléculaire, **INSERM U266-URA D1500 CNRS, Université Rene Descartes, and ‡INSERM U901, Institut de Génétique Moléculaire, Paris, France

Src homology 3 (SH3) domains are conserved modules which participate in protein interaction by recognizing proline-rich motifs on target molecules. To identify new SH3-containing proteins, we performed a two-hybrid screen with a proline-rich region of human SOS-1. One of the specific SOS-1 interacting clones that were isolated from a mouse brain cDNA library defines a new protein that was named amphiphysin 2 because of its homology to the previously reported amphiphysin. Amphiphysin 2 is expressed in a number of mouse tissues through multiple RNA transcripts. Here, we report the amino acid sequence of a brain form of amphiphysin 2 (BRAMP2) encoded by a 2.5-kilobase mRNA. BRAMP2 associates in vitro with elements of the endocytosis machinery such as α-adaptin and dynamin. On a biosensor surface, the BRAMP2/dynamin interaction appeared to be direct and partly dependent on a proline-rich sequence of dynamin. Association with dynamin was also observed in PC12 cells after cell stimulation with nerve growth factor, suggesting that amphiphysin 2 may be connected to receptor-dependent signaling pathways. This hypothesis is strengthened by the ability of BRAMP2 to interact with the p21ras exchange factor SOS, in vitro, as a possible point of interconnection between the endocytic and signaling pathways.

A limited set of protein modules mediates molecular interactions and underlies the diversity of intracellular signaling pathways. Among these modules, Src homology 3 (SH3)1 domains were first identified as non-catalytic regions of 55–70 AA, present in signaling and cytoskeletal proteins (1). A common feature of SH3 domains is their ability to bind proline-rich sequences on target molecules. A SH3 domain is composed of a hydrophobic binding pocket with finely positioned aromatic residues and charged loops outside of this pocket that determine the specificity of the interaction with proline-rich motifs. Phage display or peptide library screenings with individual SH3 domains demonstrated the absence of a strict exclusivity for SH3 and proline-rich partners (2, 3). This feature may be the basis for the identification of new SH3-containing proteins.

Human SOS-1 C terminus contains a multitude of intermingled proline-rich motifs. It was used as a “pseudo-degenerated” bait in search for new SH3 domains in a two-hybrid screen. Several SH3 domains were isolated, and one of them allowed us to identify a new protein, homologous to, but different from, amphiphysin (4), that was named amphiphysin 2. Amphiphysin is a strictly neuronal protein that plays a crucial role in the endocytosis of synaptic vesicles (5). A current hypothesis suggests that a ubiquitous homologue involved in general endocytosis should exist. We show here that this hypothesis is correct and incomplete. Amphiphysin 2 is expressed under several forms: a 2.0-kb transcript which is expressed almost ubiquitously and a 2.5-kb transcript that is brain-specific. The latter form was further characterized in vitro and in vivo. We show that the brain form of amphiphysin 2 (BRAMP2) retains the same interactive abilities as amphiphysin itself, i.e. it binds dynamin and α-adaptin in vitro. In vivo, PC12 amphiphysin 2 co-immunoprecipitates with dynamin when cells have been stimulated with NGF. Thus, the interaction of PC12 amphiphysin 2 with key players of the endocytosis machinery is dependent on the stimulation of a signaling pathway initiated by NGF binding to its membrane receptor. The interconnection between endocytosis and membrane receptor signaling is further supported by the ability of brain amphiphysin 2 to interact with SOS in vitro as well as within the yeast two-hybrid system. This result brings evidence for an additional level of molecular interaction between endocytosis and signal transduction pathways, supporting the idea that both pathways may be mutually regulated.

EXPERIMENTAL PROCEDURES

Two-hybrid Screen and Full-length cDNA Cloning

Briefly, a fusion between the GAL4-binding domain and the C-terminal portion of human SOS-1 (hSOS-1, AA 1131–1333 into the pGBT10 vector) (6) was used as bait to screen a Balb/c mouse brain cDNA library constructed in the pGAD1318 vectors (7, 8). The cDNA inserts from His’/LacZ’ specific clones were sequenced with an automatic sequencer (Applied Biosystems Inc., model 373A). One of them was hybridized to a mouse brain agt10 cDNA library (CLONTECH, Palo Alto, CA). The cDNA inserts were subcloned into pBluescriptSK– for automatic sequencing.

Northern Blot Analysis

Hybridizations were performed sequentially with three random-primed 32P-labeled probes corresponding to bp 1283–1581, bp 1824–

* This work was supported in part by grants from Association pour la Recherche contre le Cancer (ARC), Ligue Nationale contre le Cancer (Comité de Paris), and Groupement de Recherche et d’Etudes sur les Génomes. The costs of publication of this article were defrayed in part solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EBI Data Bank with accession number(s) U86405.

† To whom correspondence should be addressed: INSERM U248, Institut Curie, 26 rue d’Ulm, 75231 Paris Cedex 05, France. Tel.: 33-1-42-34-66-43; Fax: 33-1-42-34-66-50; E-mail: leprince@curie.fr.

‡ Supported by a postdoctoral European Community fellowship.

1 The abbreviations used are: SH, Src homology; AA, amino acid; GST, glutathione S-transferase; mAb, monoclonal antibody; MBP, maltose-binding protein; NGF, nerve growth factor; PAGE, polyacrylamide gel electrophoresis; SPR, surface plasmon resonance; bp, base pair(s); kb, kilobase(s); Chaps, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid.
2374 of the full-length BRAMP2, and β-actin, using mouse multi-tissue RNA blots from CLONTECH, according to the manufacturer's instructions.

**Recombinant Proteins, Purified Proteins, and Peptide**

A central fragment of BRAMP2 cDNA corresponding to AA 176–511 (bp 784–1784) was subcloned into pGEX4T1 (Pharmacia Biotech, Uppsala, Sweden) and pMalC2 (New England Biolabs, Beverly, MA) to produce a glutathione S-transferase (GST) and a maltose-binding protein (MBP) fusion protein, respectively. The full coding sequence of BRAMP2 (bp 250–2506) was subcloned into pGEX4T1. After production in *Escherichia coli*, GST fusion proteins were purified on glutathione-Sepharose beads, and MBP fusion proteins were purified on amylose resin. Rabbit Ab were produced against GST-BRAMP2 (AA 178–511) and affinity-purified over a HiTrap NHS-activated column (Pharmacia) to which MBP-BRAMP2 (AA 178–511) and affinity-purified over a HiTrap NHS-activated column (Pharmacia) to which MBP-BRAMP2 (AA 178–511) had been covalently bound, before use in biochemical experiments. Rat brain dynamin was purified according to Gout et al. (9). A dynamin I peptide, GPPQVPSRPNR, was synthesized in a solid-phase method on an Applied Biosystems apparatus and purified by reverse phase high performance liquid chromatography. The anti-dynamin monoclonal antibody (mAb) recognizing dynamin I and II was purchased from Transpath (Boulder, CO).

**In Vitro Interactions**

With [35S]Labeled Products—Human SOS-1 and SOS-2 subcloned into pBluescriptSK– were used as DNA templates in a transcription/translation reaction with reticulocyte lysates and T7 RNA polymerase. Luciferase DNA provided by the manufacturer was used as control in a translation reaction with reticulocyte lysates and T7 RNA polymerase. Bound Ab were detected by peroxidase-labeled anti-mouse Ab and the ECL system (Amersham, Aylesbury, UK).

**Immunoprecipitations**

Serum-deprived PC12 cells were incubated with 50 ng/ml NGF (Sigma) for 10 min at 37 °C, before lysis in 10 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40. After centrifugation at 10,000 × g for 30 min, the supernatant was precleared on protein A-Sepharose beads (Pharmacia) and incubated with anti-BRAMP2 Ab previously bound to protein A beads. After washing in lysis buffer, proteins bound to beads were separated by electrophoresis and transferred to nitrocellulose before immunoblot analysis, as mentioned above.

**Fluorescence in Situ Hybridization Analysis**

Fluorescence in situ hybridization to metaphase chromosomes prepared from a normal human male was carried out according to a usual technique, using R-banding (11). The probe was a BRAMP2 DNA fragment from agt10 screening (bp 305–2530).

**Surface Plasmon Resonance (SPR)**

SPR experiments were carried out on a Biosensor Biacore apparatus (Pharmacia Biosensor). Dynamin or GST-BRAMP2 in 10 mM potassium acetate buffer, pH 4.0 and pH 5.25, respectively, were covalently bound to the dextran matrix of a CM5 sensor chip, according to a method previously described (12). Binding experiments were performed at 25 °C with a flow rate of 20 or 30 μl/min, in HBS running buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 1 mM dithiothreitol, 0.005% surfactant P20). Control experiments were performed with a blank cell allowing us to measure the resonance units due to injected proteins that were subtracted from the specific binding values. After each binding experiment, the sensor chip was regenerated with 10 μl of 3 M guanidinium chloride. Estimation of kinetic parameters was done by repetitive injections of a range of protein concentrations over different densities of immobilized partner. Data analysis was performed with the interactive software BIA evaluation version 2.0.

**RESULTS**

**Primary Structure of BRAMP2**—To identify new SH3-containing molecules, we performed a two-hybrid screen with a proline-rich region of the SOS-1 and a mouse brain cDNA library. One of the specific SOS-1 interacting clones was subsequently used to screen a mouse brain agt10 cDNA library, and positive overlapping inserts were sequenced. The 2.5-kb full-length sequence, BRAMP2, contains an open reading frame of 1765 bp (bp 250–2014, data not shown) encoding a protein of 588 AA.

The first methionine following an in-frame stop codon is within a good Kozak consensus sequence. Analysis of the predicted AA sequence revealed an SH3 domain in the C-terminal part of the molecule (AA 520 to 588) and two proline-rich motifs (AA 297–306 and AA 340–346) that could be seen as internal SH3-binding sites. Comparison of BRAMP2 with data base sequences demonstrated significant homology to human amphiphysin (4) (Fig. 1), with a 42% identity at the AA level. The BRAMP2 DNA fragment allowed us to localize the corresponding gene in human 2q21 (data not shown), a locus different from 7p13-p14 defined for amphiphysin. Based on its sequence...
similarity to amphiphysin, we named the product of this gene, amphiphysin 2, another member of the “amphiphysin family.” Data base analysis also revealed that BRAMP2 is similar to recently reported sequences, murine SH3P9 (13) and human BIN1 (14), with portions of almost complete identity in the N- and C-terminal ends of the molecules. This suggests that BRAMP2, SH3P9, and BIN1 are three splicing isoforms of the same genomic sequence. With a 108-AA central insert absent in the latter two molecules, BRAMP2 is structurally closer to amphiphysin.

**BRAMP2, a Long Isoform of Amphiphysin 2 Has a Brain-restricted Expression**—A mouse multi-tissue RNA blot was hybridized with a first BRAMP2 probe (bp 1824–2374) possibly recognizing multiple amphiphysin 2 isoforms and with a second probe (bp 1283–1581) corresponding to the 108-AA central insert. As shown in Fig. 2A, amphiphysin 2 mRNAs are expressed in a number of tissues tested: brain, muscle, liver, lung, spleen, and kidney, as 2.0- and 2.5-kb messengers. In contrast to amphiphysin, amphiphysin 2 expression does not appear to be restricted to neuronal cells. Nonetheless, the splicing isoform corresponding to the BRAMP2 cDNA shows a more restricted pattern of expression and is mostly expressed in brain as a 2.5-kb RNA (Fig. 2B). A control β-actin probe indicates that variations in the detection of messengers cannot be due to differences in RNA loading (Fig. 2C). As detected by Western blotting, the expression of pRK5-BRAMP2 in NIH-3T3 transfected cells produces a doublet of polypeptides migrating at 88/96 kDa, an apparent molecular mass different from the theoretical 64.5 kDa (Fig. 2D). This difference is probably due to post-translational modifications that may also account for the protein doublet since no particular cleavage site has been localized in the sequence. Such a doublet can also be detected in a PC12 cell extract as well as in a mouse brain extract, reinforcing the idea that this amphiphysin 2 isoform is expressed in neuronal cell types. Anti-BRAMP2 Ab were able to recognize other polypeptides in NIH-3T3 cells, probably because the portion of BRAMP2 used for Ab production is common to multiple isoforms. These 57- and 67-kDa polypeptides could represent different splicing isoforms of amphiphysin 2 such as BIN1, which was reported to be a 70-kDa molecule (14).

**BRAMP2 Interacts with SOS, Dynamin, and α-Adaptin**—The BRAMP2 cDNA was first isolated in a two-hybrid screen with the proline-rich region of SOS-1. To confirm the yeast genetic data, we tested in vitro binding of GST-BRAMP2 to 35S-labeled SOS1 or SOS2 or control luciferase was incubated with purified GST (central panel) or GST-BRAMP2 (right panel). Samples were run on SDS-PAGE in parallel with an aliquot of the 35S-labeled starting material (left panel) and autoradiographed. B, 3T3 cell extracts were incubated with GST or GST-BRAMP2. Samples were run on SDS-PAGE in parallel with an aliquot of the starting cell lysate. After transfer to nitrocellulose, protein blots were incubated either with anti-dynamin (left panel) or with anti-α-adaptin (right panel). In the central panel, PC12 cells were either unstimulated or stimulated shortly with NGF before lysis in 1.0% Chaps. Immunoprecipitations were performed with anti-BRAMP2 Ab or a control rabbit Ig. Samples were run on SDS-PAGE, in parallel with an aliquot of unstimulated or stimulated PC12 cell lysates, transferred to nitrocellulose, and blotted with an anti-dynamin mAb.
immunoblot. As shown in Fig. 3B (left panel), anti-dynamin mAb was able to specifically recognize the 100-kDa dynamin in GST-BRAMP2 samples. Identical results were obtained with PC12 cell extracts (data not shown). Similarly, a 110/115-kDa doublet of α-adaptins was specifically retained by GST-BRAMP2 beads (Fig. 3B, right panel) and detected by anti-α-adaptin mAb. For both immunodetections, the specificity of the interaction was confirmed by using Sepharose beads loaded with equivalent amounts of GST as negative control. The in vitro interaction between BRAMP2 and dynamin was strengthened by coimmunoprecipitation experiments. After lysis of PC12 cells in mild conditions (1.0% Chaps), an anti-BRAMP2 immunoprecipitate was shown to contain the 100-kDa dynamin when cells had previously been stimulated with NGF. In the same lysis conditions, amphiphysin 2 immunoprecipitated from unstimulated PC12 cells was not associated with dynamin (Fig. 3B, central panel). Taken together, these results strongly link the BRAMP2 form of amphiphysin 2 to the endocytic pathway, as was previously reported for amphiphysin.

Analysis of BRAMP2/Dynamin Interaction—To further study BRAMP2/dynamin interaction, we have used a SPR assay with a BIAcore Biosensor. This method allowed us to detect molecular interactions in real time and to estimate their kinetic parameters. Fig. 4A shows the direct binding of increasing concentrations of GST-BRAMP2 to immobilized dynamin (from 0 to 300 s), followed by a dissociation phase (300 to 600 s). Control GST did not give any specific binding on the dynamin sensor (data not shown). The association rate for GST-BRAMP2, $k_a = 1.5 \times 10^4$ M$^{-1}$s$^{-1}$, was calculated from the slope of a plot of $k_a$, against GST-BRAMP2 concentration (Fig. 4B). The dissociation rate in buffer flow was near 0 and could not be studied by dynamin injection because of the oligomerization of the molecule. To prevent this problem, the dissociation rate was measured after binding of 0.5 $\mu$M dynamin to a GST-BRAMP2-coated membrane by injection of 1 $\mu$M GST-BRAMP2 (Fig. 4C). Repetitive experiments gave two dissociation rate constant values on the order of $k_{d1} = 1.6 \times 10^{-1}$ s$^{-1}$ and $k_{d2} = 3.5 \times 10^{-3}$ s$^{-1}$. However, when low concentrations of dynamin were used, the faster dissociation rate was not observed, suggesting that it could represent dynamin aggregation on the sensor. The equilibrium dissociation constant was thus calculated with $k_{d2}$ as $k_{d2} = k_{d1}/k_{d2} \sim 240$ nm. As shown in Fig. 4D, BRAMP2/dynamin interaction could be inhibited partially by the GPPQVPSRPNR peptide from dynamin, involving this particular proline-rich motif and the SH3 domain of BRAMP2 in the association between both molecules.

**DISCUSSION**

We have defined a new SH3-containing molecule, highly homologous to amphiphysin. It is encoded by a gene located in human chromosome 2q21, a locus different from the 7p13-14 identified for amphiphysin. Based on its similarity to amphiphysin, the product of this gene was named amphiphysin 2. Unlike amphiphysin, it has a broad expression in several mouse tissues and may be seen as a ubiquitous amphiphysin homologue. In patients with stiff-man syndrome associated with breast cancer (15), it is a potential target outside the brain for anti-amphiphysin autoantibodies. From one mouse tissue to another, the expression of amphiphysin 2 seems to be dependant on multiple transcripts, generated either by different sites of initiation/polyadenylation or by alternative splicing. One of these transcripts encodes the BRAMP2 sequence that we report herein. Shorter transcripts should be related to the previously reported SH3P9 (13) and BIN1 (14) sequences which are identical to BRAMP2 in their N- and C-terminal ends. BRAMP2, SH3P9, and BIN1 are probably three splicing variants of the same genomic sequence that may give rise to functionally diverse molecules. For example, BIN1 was reported to have a short nuclear localization signal absent in the other isoforms and a Myc-binding domain maintained in the other isoforms (14). BRAMP2 has a long central insert absent in SH3P9 and BIN1 that makes it structurally closer to amphiphysin and correlates with a brain-restricted expression. It remains to be determined whether the same brain cell types express amphiphysin and this form of amphiphysin 2.

We have shown that BRAMP2 associates in vitro with key elements of the endocytic pathway: α-adaptin and dynamin from 3T3 as well as from PC12 cells. The interaction with dynamin is direct and likely to rely on the SH3 domain of amphiphysin 2 on one side and on a proline-rich sequence of dynamin on the other side. This conclusion is based on molecular interactions studies on a BIAcore biosensor where a proline-rich peptide of dynamin I corresponding to one of the previously described Grb2-binding sites is partially inhibitory (9, 16). This suggests that BRAMP2 interacts at least in part with this proline-rich sequence. The affinity of BRAMP2/dynamin interaction on the biosensor membrane was estimated in the 240 nm range, revealing an affinity similar to other SH3 domain/protein ligand interactions (17) and much higher than what have been described with proline-rich peptide ligands.

**Fig. 4. Interaction of BRAMP2 with dynamin studied by surface plasmon resonance.** A, increasing concentrations of GST-BRAMP2 (40, 80, 120, 160, 240, 320, and 400 nM) were injected over immobilized dynamin. The arrows indicate the beginning and the end of injection. B, the $k_a$ value was obtained from the slope of $k_a$, calculated by using the BIAcore software versus concentration of injected GST-BRAMP2. C, real dissociation value ($k_{d2}$) was determined by injection of a high concentration of GST-BRAMP2 (1 $\mu$M) after injection of a high concentration of dynamin (0.5 $\mu$M) over immobilized GST-BRAMP2. D, specificity of BRAMP2/dynamin interaction was determined by the decrease of GST-BRAMP2 (0.4 $\mu$M) binding preincubated with a proline-rich peptide (PRP, 500 $\mu$M) from dynamin.
The association of brain amphiphysin 2 with dynamin was also detected in vivo after stimulation of PC12 cells with NGF. This result shows that interaction of amphiphysin 2 with a protein of the endocytosis machinery is dependent on a signaling event coming from a cell surface receptor. A number of previous reports demonstrated that the organization of the clathrin-dependent endocytic machinery is tightly controlled by the activation of the surface receptors that are going to be endocytosed, as part of signal attenuation. First, endocytosis is dependent on the tyrosine kinase activity associated with the receptor (19). Second, ligand binding stimulates the interaction of activated receptors with the AP2 complex on one hand (20) and with dynamin through signal transduction molecules on the other hand (21). Grb2 is an important element in this cascade that was reported to interact with dynamin upon ligand binding and stimulate dynamin GTPase activity, possibly by promoting dynamin self-association (9, 21, 22). It is conceivable that upon receptor stimulation, amphiphysin 2 joins the multimolecular complex located at the cytoplasmic end of the receptor. The possible competition between amphiphysin 2 and Grb2 for dynamin binding can be reconciled in a sequential scheme involving phosphorylation- or nucleotide binding-dependent changes of conformation. Inside the multimolecular complex depicted above, elements of the endocytic pathway are intimately associated with elements of the signaling pathway. Cumulative evidence suggests that both pathways regulate each other, at multiple levels (23). Even though we need to confirm it in vivo in mammalian cells, our ability to detect an interaction between the brain form of amphiphysin 2 and the p21ras exchange factor SOS, brings further support to the interconnection between both pathways, at the site of receptor stimulation. We are currently testing whether a ternary complex of amphiphysin 2/SOS/dynamin exists in vivo and defines a new point of functional interaction, regulating endocytic function as well as signaling function.

REFERENCES
1. Pawson, T. (1995) Nature 373, 573–580
2. Yu, H., Chen, J. K., Feng, S., Dalgaro, D. C., Brauer, A. W., and Schreiber, S. L. (1994) Cell 76, 933–945
3. Lim, W. A., Richards, F. M., and Fex, R. O. (1994) Nature 372, 375–379
4. Lichter, B., Veh, R. W., Meyer, H. E., and Kiliarn, M. W. (1992) EMBO J. 11, 2531–2539
5. David, C., McPherson, P. S., Mundigl, O., and De Camilli, P. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 331–335
6. Chardin, P., Camonis, J. H., Gale, N. W., Aelst, L. V., Schlessinger, J., Wigler, M. H., and Bar-Sagi, D. (1993) Science 260, 1338–1343
7. Felletter H. E., Hannon G. J., Ruddle C. J., and Beach D. (1994) Nucleic Acids Res. 22, 1502–1503
8. Janoueix-Lerosey, I., Jelivet, F., Camonis, J., Marche, P., and Goud, B. (1995) J. Biol. Chem. 270, 14801–14808
9. Gout, I., Dhand, R., Hiles, I. D., Fry, M. J., Panayotou, G., Das, P., Truong, O., Totty, N. F., Huanan, J., Booker, G. W., Campbell, I. D., and Waterfield, M. D. (1993) Cell 75, 25–36
10. Zaleman, G., Closson, V., Camonis, J., Honore, N., Rousseau-Merck, M. F., Tavitian, A., and Odofson, B. (1996) J. Biol. Chem. 271, 30366–30374
11. Cherif, D., Julien, C., Delattre, O., Derre, J., Lathrop, G. M., and Berger, R. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 6639–6643
12. Panayotou, G., Gish, G., End, P., Truong, O., Gout, I., Dhand, R., Fry, M. J., Hiles, I., Pawson, T., and Waterfield, M. D. (1993) Mol. Cell. Biol. 13, 3567–3576
13. Sparks, A. B., Hoffman, N. G., McConnell, S. J., Fowlkes, D. M., and Kay, B. K. (1996) Nature Biotech. 14, 741–744
14. Sakamuro, D., Elliott, K. J., Wechsler-Reya, R., and Prendergast, G. C. (1996) Nature Genet. 14, 69–77
15. De Camilli, P., Thomas, A., Cofiell, R., Foll, F., Lichte, B., Piccolo, G., Meinck, H. M., Austen, M., Fassettia, G., Bottazzo, G., Bates, D., Cartlidge, N., Solimena, M., and Kiliman, M. W. (1993) J. Exp. Med. 178, 2219–2223
16. Scaife, R., Gout, I., Waterfield, M. D., and Margolis, R. L. (1994) EMBO J. 13, 2574–2580
17. Lee, C. H., Leung, B., Lemmon, M. A., Zheng, J., Cowburn, D., Kuriyan, J., and Saksela, K. (1995) EMBO J. 14, 5096–5105
18. Cussac, D., Frech, M., and Chardin, P. (1994) EMBO J. 13, 4011–4021
19. Sorokin, A., and Waters, C. (1993) BioEssays 15, 375–382
20. Sorokin, A., and Carpenter, G. (1993) Science 252, 612–615
21. Wang, Z., and Moran, M. F. (1996) Science 272, 1935–1939
22. Warnock, D. E., Terlecky, L. J., and Schmid, S. L. (1995) EMBO J. 14, 1322–1328
23. Vieira, A. V., Lamaze, C., and Schmid, S. L. (1996) Science 274, 2086–2089