The Subcellular Localizations of Atypical Synaptotagmins III and VI

SYNAPTOTAGMIN III IS ENRICHED IN SYNAPSES AND SYNAPTIC PLASMA MEMBRANES BUT NOT IN SYNAPTIC VESICLES*

(Received for publication, April 15, 1999)

Stefan Butz‡, Rafael Fernandez-Chacon‡§, Frank Schmitz‡, Reinhard Jahn‡, and Thomas C. Südhof‡||

From the ‡Center for Basic Neuroscience, Department of Molecular Genetics, and Howard Hughes Medical Institute, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235 and ||Abteilung Neurobiologie, Max-Planck-Institut für biophysikalische Chemie, 37075 Gottingen, Germany

Multiple synaptotagmins are expressed in brain, but only synaptotagmins I and II have known functions in fast, synchronous Ca2+-triggered neurotransmitter release. Synaptotagmin III was proposed to regulate other aspects of synaptic vesicle exocytosis, particularly its slow component. Such a function predicts that synaptotagmin III should be an obligatory synaptic vesicle protein, as would also be anticipated from its high homology to synaptotagmins I and II. To test this hypothesis, we studied the distribution, developmental expression, and localization of synaptotagmin III and its closest homolog, synaptotagmin VI. We find that synaptotagmins III and VI are present in all brain regions in heterogeneous distributions and that their levels increase during development in parallel with synaptogenesis. Furthermore, we show by immunocytochemistry that synaptotagmin III is concentrated in synapses, as expected. Surprisingly, however, we observed that synaptotagmin III is highly enriched in synaptic plasma membranes but not in synaptic vesicles. Synaptotagmin VI was also found to be relatively excluded from synaptic vesicles. Our data suggest that synaptotagmins III and VI perform roles in neurons that are not linked to synaptic vesicle exocytosis but to other Ca2+-related nerve terminal events, indicating that the functions of synaptotagmins are more diverse than originally thought.

Synaptotagmins represent a family of at least 12 proteins that are thought to function in membrane traffic (reviewed in Ref. 1). All synaptotagmins are composed of a short N-terminal sequence that is intraluminal and/or extracellular, a single transmembrane region, and a large cytoplasmic sequence that contains two C2-domains (1). In all synaptotagmins, the N-terminal sequence is translocated across the membrane during translation but lacks a cleaved signal peptide (2, 3), and the transmembrane regions contain multiple cysteine residues that may be palmitoylated (4, 5). The C2-domains, the most conserved feature of synaptotagmins (1), are separated from the transmembrane region by a variable connecting sequence. C2-domains are general Ca2+-binding motifs that function as Ca2+-binding modules in most synaptotagmins (reviewed in Ref. 6). In some synaptotagmins, however, the C2-domains contain sequence changes that probably abolish Ca2+-binding (e.g. synaptotagmins IV and XI; Refs. 3 and 7), suggesting that C2-domains can also perform Ca2+-independent functions.

Synaptotagmins I and II, the first molecularly characterized synaptotagmins (8, 9), are abundant synaptic vesicle proteins that are differentially expressed; synaptotagmin I is primarily present in rostral regions, and synaptotagmin II is primarily present in caudal brain regions (9, 10). Knockout experiments in mice revealed that synaptotagmin I is essential for fast Ca2+-triggered neurotransmitter release but is not required for exocytosis as such; slow Ca2+-triggered neurotransmitter release and Ca2+-independent release were still functional in the knockout animals (11). These results indicated that synaptotagmin I functions as a Ca2+-sensor for fast synaptic vesicle exocytosis in upper brain regions. Synaptotagmin II probably performs an equivalent role in lower brain regions because it is very homologous to synaptotagmin I and is also enriched in synaptic vesicles (9, 11).

Similar to synaptotagmins I and II, synaptotagmins III-XII are expressed primarily in brain (3, 12–17). However, the mRNAs of most of these other synaptotagmins are detectable at low levels outside of the brain (3, 12–17). Although the enrichment of synaptotagmins III-XII in brain and their homology to synaptotagmins I and II led to the notion that synaptotagmins III-XII may also reside on the synaptic vesicle, their localizations and potential functions have not been investigated in detail. Synaptotagmin III is particularly interesting because it is the most abundant synaptotagmin after synaptotagmins I and II, and because it exhibits Ca2+-binding characteristics that resemble the Ca2+-requirements of the slow component of Ca2+-dependent neurotransmitter release (18–20). These findings have led to the hypothesis that synaptotagmin III may mediate the slow component of neurotransmitter release (18). In support of this hypothesis, synaptotagmin III is enriched in synapses, detectable in purified synaptic vesicles, and heteromultimerizes with synaptotagmin I in vitro (10, 21). Furthermore, in pancreatic β-cells, synaptotagmin III is present on secretory granules and may mediate Ca2+-triggered exocytosis of these granules (22). If synaptotagmin III indeed functions as an exocytotic Ca2+-sensor similar to synaptotagmins I and II, it should also be on the secretory vesicles that undergo exocytosis. However, the localization of synaptotagmin III (or other synaptotagmins except for synaptotagmins I and II) was not investigated systematically and quantitatively, and its precise distribution is unknown.

In the current study, we have examined the subcellular localization of synaptotagmin III and its closest relative, synaptotagmin VI. Surprisingly, we find that synaptotagmins III and VI are not enriched in synaptic vesicles. Instead, synaptotagmins III and VI are present in all brain regions in heterogeneous distributions and that their levels increase during development in parallel with synaptogenesis. Furthermore, we show by immunocytochemistry that synaptotagmin III is concentrated in synapses, as expected. Surprisingly, however, we observed that synaptotagmin III is highly enriched in synaptic plasma membranes but not in synaptic vesicles. Synaptotagmin VI was also found to be relatively excluded from synaptic vesicles. Our data suggest that synaptotagmins III and VI perform roles in neurons that are not linked to synaptic vesicle exocytosis but to other Ca2+-related nerve terminal events, indicating that the functions of synaptotagmins are more diverse than originally thought.

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Supported by a fellowship from the Spanish Ministry of Education and Culture and by the Fulbright Commission.
§ To whom correspondence should be addressed. E-mail: Tsudho@mednet.swmed.edu.

This paper is available online at http://www.jbc.org
Synaptic Synaptotagmins Not on Synaptic Vesicles

18291

tagmin III is highly concentrated on synaptic plasma membranes, and synaptotagmin VI is widely present in membranes. Our data suggest that synaptotagmins may be functionally more diverse than previously envisioned.

EXPERIMENTAL PROCEDURES

Production and Affinity Purification of Antibodies—Specificity tests revealed that our previously raised antibodies against the C2-domains of synaptotagmins III and VI (L1681 and L1471, respectively) weakly cross-react with other synaptotagmins, presumably because their C2 domains are so similar. Therefore, we generated a new set of antibodies to the sequence that connects the transmembrane region with the C2-domain of synaptotagmins III and VI (residues 88–296 and 86–229 of synaptotagmins III and VI, respectively (3, 12)). Rabbits were immunized with glutathione S-transferase-fusion proteins (produced in the pGEX-KG vector (23)), and the resulting sera (S754 and S756 for synaptotagmins III and VI, respectively) were affinity-purified on immobilized maltose-binding protein fusion proteins containing the same sequences of synaptotagmins (in pMal-C2; New England Biolabs). The specificity of all antibodies was tested by probing extracts from COS cells transfected with individual synaptotagmins and by comparing their reactivities with brains from wild type and knockout mice that lack either synaptotagmin III or VI.

Immunocytochemistry—Cryostat sections (5–15 μm) from rat and mouse brains or bovine retina were probed with affinity-purified synaptotagmin antibodies followed by horseradish peroxidase-labeled or biotin-labeled secondary antibodies and development of the signal with the StreptABComplex/HRP kit (DAKO) or by metal enhancement essentially as described previously (24–26). The specificity of the signals was ascertained by two controls: 1) blocking of the signal with the recombinant protein used for antibody production, and 2) analysis of brains from knockout mice lacking synaptotagmin III.

Subcellular Fractionations—Rat brain homogenates were subfractionated into crude synaptic vesicles, mitochondria, myelin, and synaptic plasma membranes by the method of Jones and Matus (27). For this purpose, whole brains including cerebella, olfactory bulbs, and brain stem from 4–6-week-old rats were homogenized in ice-cold 0.32 M sucrose using a motorized glass-Teflon homogenizer. The homogenate was centrifuged at 800 × g for 10 min to remove nuclei and debris, and the resulting supernatant was re-centrifuged at 9,000 × g for 20 min to obtain synaptosomes and the synaptosomal supernatant. The synaptosomes in the pellet were washed once in 0.32 M sucrose, lysed hypotonically, and centrifuged at 250,000 × g for 20 min to isolate large synaptosomal membranes in the pellet, whereas the free synaptic vesicles remained in the supernatant under these conditions. The synaptosomal membranes were resuspended in dH2O, sucrose was added to 1.1 M, and the solution was placed at the bottom of a Beckman SW28 rotor tube. The sample was overlaid with 0.855 and 0.32 M sucrose solutions and centrifuged for 2.5 h at 19,000 rpm, resulting in the isolation of myelin (in the 0.32/0.855 M sucrose interface), synaptic plasma membranes (in the 0.855/1.1 M sucrose interface), and mitochondria (in the pellet). For the purification of synaptic vesicles, hypotonically lysed synaptosomes were used as a starting point for the isolation of crude synaptic vesicles that were further purified by velocity-density gradient centrifugation and controlled pore glass chromatography essentially as described previously (28, 29). For organelle immunolocalization, beads with immobilized monoclonal antibodies to synaptotagmin I (C141.1) or synapto- 
v
min II (C169.1) and control beads containing only immobilized glycine were prepared as described previously (30, 31). Rat brains were homogenized in 5 ml HEPES-NaOH, pH 7.4, 0.32 M sucrose, 0.1 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, 10 mg/ml leupeptin, 1 mg/ml pepstatin A, and 10 mg/ml aprotinin. The homogenates were centrifuged for 10 min at 7,800 × g to generate a postnuclear supernatant. Beads were incubated for 30 min at 4 °C under rotation with the postnuclear supernatant and washed four times, and bound proteins were analyzed by quantitative immunoblotting using 125I-labeled secondary antibodies.

Quantitative Immunoblotting—To quantitate the relative levels of proteins in various fractions for the purification of synaptic vesicles and subcellular fractionation or by immunobead precipitation, we reacted immunoblots of the various fractions with 125I-labeled secondary antibodies and measured the respective signals in a phosphorimager. All blots were probed simultaneously with antibodies to the protein under study and antibodies to synaptogyrin as an internal vesicle protein standard (32). The synaptogyrin signals were then normalized for the synaptogyrin signal by designating the ratio of the two signals observed in starting brain homogenates or postnuclear supernatants as 1.0. For the immunobead experiments, the signals obtained in pulldowns with glycine control beads were subtracted from the values from the immunobead pulldowns before the ratios were calculated.

RESULTS AND DISCUSSION

We raised multiple antibodies to synaptotagmins III and VI, affinity-purified the antibodies, and tested their specificity using COS cells transfected with various synaptotagmins (data not shown). Our initial antibodies were directed against the C2-domains of synaptotagmins III and VI. These antibodies, however, exhibited cross-reactivity with the more abundant synaptotagmins I and II, thereby limiting their usefulness. Therefore, we subsequently raised more specific antibodies using the sequence that connects the transmembrane region with the C2-domains and varies considerably between different synaptotagmins (7–10, 12–17) as an antigen. At least one of these antibodies for each synaptotagmin was specific for that particular isoform, based on the following evidence: 1) as shown for synaptotagmin III in Fig. 1, the antibodies specifically reacted with the cognate protein expressed in COS cells and recognized a single band (synaptotagmin III) or two closely co-migrating bands (synaptotagmin VI; see below) in total brain extract; and 2) immunoblots of total brain homogenates from wild type mice and from knockout mice lacking either synaptotagmin III or synaptotagmin VI were analyzed (Fig. 2). The generation and analysis of these knockout mice will be reported in a later, more detailed report. The mouse immunoblots confirmed that the synaptotagmin III antibodies reacted with a single band and that the synaptotagmin VI antibodies reacted with a set of two closely spaced bands in brain. In the knockouts that contain all other synaptotagmins, the reactivity was abolished (Fig. 2).

Using these antibodies, we examined the regional distribution of synaptotagmins I, III, and VI in rat brains (Fig. 3). As described previously (9), synaptotagmin I was expressed in much higher levels in upper brain regions than in hindbrain and spinal cord (which in turn synthesize higher levels of synaptotagmin II). CASK as a general synaptic marker was uniformly present in all brain regions (35, 36). The expression of synaptotagmins III and VI, however, exhibited a different pattern in different brain regions, with much higher levels of expression in hippocampal and neocortical regions in contrast to lower levels in other brain regions.
pattern: both synaptotagmins were present in all brain regions but displayed large regional variations. Synaptotagmin III was almost undetectable in the cerebral cortex and olfactory bulb but was highly expressed in the hippocampus and cerebellum. In addition, it was present at moderate levels in spinal cord and hindbrain (Fig. 3). Synaptotagmin VI was synthesized at rather low levels in all brain areas except for the olfactory bulb, which contained exceptionally high levels of this isoform.

Previous data suggested that synaptotagmin III is localized to synapses (10). This result was supported in the current study by two independent observations. First, we examined the developmental expression profile of synaptotagmins. The level of synaptic vesicle proteins goes up dramatically postnatally in parallel with synaptogenesis (37). When we analyzed brain proteins from rat forebrain as a function of age, we found that synaptotagmin I levels increased postnatally, as expected (10) (Fig. 4). By contrast, the general trafficking protein rab5 exhibited only a moderate developmental change. Synaptotagmins III and VI, similar to synaptotagmin I, experienced a striking postnatal increase in levels that paralleled the time course of synaptogenesis (Fig. 4). In adults, the levels of synaptotagmin VI, but not synaptotagmins I and III, decreased slightly. Two closely migrating bands were observed for synaptotagmin VI that may be due to alternative splicing; the molecular basis for this heterogeneity is unclear.

The second observation confirming a synaptic localization of synaptotagmin III was obtained by immunocytochemistry. Unfortunately, the quality of our synaptotagmin VI antibodies precluded a morphological localization; even the signal for synaptotagmin III in immunocytochemical stains was insufficient to allow immunoelectronmicroscopy. At the light level, however, synaptotagmin III was present in a pattern strongly suggestive of a synaptic localization (Fig. 5; data not shown). This was most clear in the retina, which contains two sharply delineated synaptic layers, the thin outer plexiform layer composed of photoreceptor synapses and the broader inner plexiform layer comprising multiple sublayers of synapses (38). Synaptotagmin III was highly concentrated in the two synaptic layers in the retina, similar to synaptotagmin I (Fig. 5).
only difference between synaptotagmins I and III was that in the inner plexiform layer, synaptotagmin I was present throughout the synaptic zone, whereas synaptotagmin III was enriched in a few sharply delineated sublayers (Fig. 5). These data confirm that synaptotagmin III is highly enriched in synapses.

Because synaptotagmins I and II are known stoichiometric components of synaptic vesicles (1) and are highly homologous to synaptotagmins III and VI (3, 12), a logical presumption is that synaptotagmins III and VI are also vesicle proteins. This is supported by the finding that all synaptotagmins are co-enriched in brain, where they appear to be concentrated in the synapses, and that at least some synaptotagmin III protein can be detected in the vesicles (10, 12). However, the relative distributions of synaptotagmins between various brain fractions was never quantitatively examined. Therefore, we studied this question systematically by subcellular fractionation (Fig. 6). As expected, synaptotagmin I was enriched in two fractions: 1) free synaptic vesicles (Fig. 6, lane 6), and 2) synaptic plasma membranes that contain active zones with firmly docked vesicles (lane 7). Synaptotagmin III was also detectable in free synaptic vesicles but, surprisingly, was not enriched in the vesicles (Fig. 6, lane 6). Instead, it was highly concentrated in synaptic plasma membranes (lane 7). The pattern of synaptotagmin III closely resembled that of neurexins, which are known components of the presynaptic plasma membrane (39). Synaptotagmin VI was evenly present in all fractions and was also not particularly enriched in synaptic vesicles. The distributions of synaptogyrin I as an additional synaptic vesicle marker (32) and of complexins as a marker for the cytosol (40) were analyzed as controls in the same blots and found to be localized in the appropriate fractions (Fig. 6).

The subcellular fractionation data raised the possibility that synaptotagmin III, although present in synapses, may not actually be a synaptic vesicle protein. To test this hypothesis, we performed systematic studies on the enrichment of synaptotagmins with synaptic vesicles. We used two complementary techniques in these studies: 1) purification of synaptic vesicles by CPG chromatography (Fig. 7), which is widely accepted as the method that yields the most highly purified vesicles (28, 29), and 2) immunoprecipitation of synaptic vesicles by antibodies to synaptotagmin I or synaptobrevin II (Fig. 8), which allows separation of the synaptic vesicles from similarly sized organelles (30, 31).

First, we examined the levels of various synaptotagmins by quantitative immunoblotting in four fractions obtained during the isolation of highly purified vesicles. In this purification procedure, starting homogenate (Fig. 7, lane 1) is used to purify

2 The abbreviation used is: CPG, controlled pore glass.
Synaptosomes (lane 2) that yield free synaptic vesicles (lane 3; see also Fig. 6) that are further purified by CPG chromatography (lane 4). Coomassie Blue-stained SDS-gels revealed that the protein composition of synaptic vesicles changed dramatically upon purification of the vesicles by CPG chromatography. We then measured the relative enrichment of various synaptotagmins by immunoblotting with 125I-labeled secondary antibodies and phosphorimager detection; all blots were reacted with antibodies to either synaptotagmin I, III, or VI and to synaptogyrin I followed by 125I-labeled secondary antibodies. The ratio of the synaptotagmin to synaptogyrin signal was quantified on a phosphorimager and set at 1.0 for the starting homogenate. Data shown are from three independent experiments (TH, total brain homogenate; P2, synaptosomes; LP2, LP2 vesicles obtained from synaptosomes; CPG, synaptic vesicles purified by CPG chromatography).

To confirm this unexpected result by an independent method, we performed experiments in which organelles were immunoisolated from rat brain homogenates with beads coated with antibodies to either synaptotagmin I or synaptobrevin II, which are known synaptic vesicle proteins. As a control, incubations were performed with beads that were coated only with glycine (30, 31) (Fig. 8A). The resulting fractions were again analyzed by quantitative immunoblotting, and the signals obtained for glycine beads were subtracted from the synaptotagmin I or synaptobrevin II bead signals. As in the biochemical preparations, we normalized all results for synaptogyrin analyzed on the same gels. As before, immunoisolation dramatically increased the levels of synaptotagmin I and synaptogyrin I in the samples, and their ratio remained constant. In contrast, a quantitative loss of synaptotagmins III and VI from the immunoisolated vesicles was observed; their ratio to synaptogyrin decreased significantly in the immunoisolated samples. (Fig. 8B). The two kinds of beads coated with the different antibodies gave identical results, thereby confirming each other.

Conclusion—Synaptotagmins are a large family of homologous proteins that are enriched in brain and thought to function in Ca2+-regulated membrane traffic. Although synaptotagmins I and II have known functions in synaptic vesicle exocytosis, little is known about the localizations, properties,
and functions of the other synaptotagmins. In the current study, we examined the localizations of synaptotagmins III and VI. Unexpectedly, we have found that synaptotagmin III, although synaptic, is not on synaptic vesicles, and that synaptotagmin VI also fails to co-purify with synaptic vesicles. Our data suggest that the functions of nerve terminals must involve other, as yet unidentified Ca^{2+}-regulated membrane trafficking pathways that involve these synaptotagmins. The high concentration of synaptotagmin III in synaptic plasma membranes raises the possibility that this isoform may, in fact, not be on an intracellular vesicle but may be localized to the plasma membrane. One possibility is that synaptotagmin III is a plasma membrane protein that could regulate the expansion of presynaptic plasma membranes as a function of activity; another possibility is that an as yet unidentified organelle co-purifies with synaptic plasma membranes and represents the true localization of this isoform. Future experiments will have to test this and other possible hypotheses about what the functions of these proteins might be in synapses that do not directly involve synaptic vesicles.

Acknowledgments—We thank A. Roth, I. Leznicki, and E. Borowicz for technical support and Drs. G. Lonart and S. Sugita for help with brain dissections.

REFERENCES
1. Sudhof, T. C., and Rizo, J. (1996) Neuron 17, 379–388
2. Perin, M. S., Brose, N., Jahn, R., and Sudhof, T. C. (1991) J. Biol. Chem. 266, 623–629
3. Li, C., Ullrich, B., Zhang, J. Z., Anderson, R. G. W., Brose, N., and Sudhof, T. C. (1995) Nature 375, 594–599
4. Chapman, E. R., Blasi, J., An, S., Brose, N., Johnston, P. A., Sudhof, T. C., and Jahn, R. (1996) Biochem. Biophys. Res. Commun. 235, 526–532
5. Veit, M., Sollner, T. H., and Rothman, J. E. (1996) FEBS Lett. 385, 119–123
6. Rizo, J., and Sudhof, T. C. (1998) J. Biol. Chem. 273, 15879–15882
7. von Poser, C., Ichtcchenko, K., Shao, X., Rizo, J., and Sudhof, T. C. (1997) J. Biol. Chem. 272, 14314–14319
8. Perin, M. S., Fried, V. A., Mignery, G. A., Jahn, R., and Sudhof, T. C. (1990) Nature 345, 260–261
9. Geppert, M., Archer, B. T., III, and Sudhof, T. C. (1991) J. Biol. Chem. 266, 13548–13552
10. Ullrich, B., Li, C., Zhang, J. Z., McMahon, H., Anderson, R. G. W., Geppert, M., and Sudhof, T. C. (1994) Neuron 13, 1281–1291
11. Geppert, M., Geda, Y., Hammer, R. E., Li, C., Rosahl, T. W., Stevens, C. F., and Sudhof, T. C. (1994) Cell 79, 717–727
12. Mizuta, M., Inagaki, N., Nemoto, Y., Matsukura, S., Takahashi, M., and Seino, S. (1994) J. Biol. Chem. 269, 11675–11678
13. Hillbush, B. S., and Morgan, J. I. (1994) Proc. Natl. Acad. U. S. A. 91, 8195–8199
14. Craxton, M., and Goedert, M. (1995) FEBS Lett. 361, 196–200
15. Hudon, A. W., and Birnbaum, M. J. (1995) Proc. Natl. Acad. U. S. A. 92, 5895–5899
16. Babity, J. M., Armstrong, J. N., Plummer, J. C., Currie, R. W., and Robertson, H. A. (1997) Proc. Natl. Acad. U. S. A. 94, 2638–2641
17. Thompson, C. C. (1996) J. Neurosci. 16, 7832–7840
18. Li, C., Davletov, B. A., and Sudhof, T. C. (1995) J. Biol. Chem. 270, 24989–24992
19. Fukuda, M., Kojima, T., and Mikoshiba, K. (1997) Biochem. J. 323, 421–425
20. Geda, Y., and Stevens, C. F. (1994) Proc. Natl. Acad. U. S. A. 91, 12942–12946
21. Chapman, E. R., Desai, R. C., Davis, A. F., and Tornehl, C. K. (1998) J. Biol. Chem. 273, 32966–32972
22. Mizuta, M., Kurose, T., Miki, T., Shoji-Kasai, Y., Takahashi, M., Seino, S., and Matsukura, S. (1997) Diabetes 46, 2002–2006
23. Guan, K. L., and Dixon, J. E. (1991) Anal. Biochem. 192, 262–267
24. Rosahl, T. W., Spillane, D., Missler, M., Herz, J., Selig, D. K., Wolff, J. R., Hammer, R. E., Malenka, R. C., and Sudhof, T. C. (1995) Nature 375,
Synaptic Synaptotagmins Not on Synaptic Vesicles

488–493
25. Wood, G. S., and Warnke, R. (1981) J. Histochem. Cytochem. 29, 1196–1204
26. Lichtlé, B., Veh, R. W., Meyer, H. E., and Kilimann, M. W. (1992) EMBO J. 11, 2521–2530
27. Jones, D. H., and Matus, I. (1974) Biochim. Biophys. Acta 356, 276–287
28. Nagy, A., Baker, R. R., Morris, S. J., and Whittaker, V. P. (1976) Brain Res. 109, 285–309
29. Huttner, W. B., Schiebler, W., Greengard, P., and De Camilli, P. (1983) J. Cell Biol. 96, 1374–1388
30. Burger, P. M., Hell, J., Mehl, E., Krasel, C., Lottspeich, F., and Jahn, R. (1989) Neuron 7, 287–293
31. Fischer von Mollard, G., Stahl, B., Walsh-Solimena, C., Takei, K., Daniels, L., Khokhlatchev, A., De Camilli, P., Sudhof, T. C., and Jahn, R. (1994) Eur. J. Cell Biol. 65, 319–326
32. Stenius K., Janz, R., Sudhof, T. C., and Jahn, R. (1994) J. Cell Biol. 131, 1801–1809
33. Laemmli, U. K. (1970) Nature 227, 680–685
34. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. U. S. A. 76, 4350–4354
35. Hata, Y., Butz, S., and Sudhof, T. C. (1996) J. Neurosci. 16, 2488–2494
36. Hata, Y.-P., Yang, F.-C., Khurzitzia, V., Naisbitt, S., Cohen, A. R., Weinberg, R. J., and Sheng, M. (1998) J. Cell Biol. 142, 139–151
37. Daly, C., and Ziff, E. B. (1997) J. Neurosci. 17, 2365–2375
38. Dowling, J. (1987) The Retina, Harvard University Press, Cambridge, MA
39. Ushkaryov, Y. A., Petrenko, A. G., Geppert, M., and Sudhof, T. C. (1992) Science 257, 50–56
40. McMahon, H. T., Missler, M., Li, C., and Sudhof, T. C. (1995) Cell 83, 111–119