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Munc18-2/syntaxin3 complexes are spatially separated from syntaxin3-containing SNARE complexes

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Abstract Exocytosis of mast cell granules requires a vesicular- and plasma membrane-associated fusion machinery. We examined the distribution of SNARE membrane fusion and Munc18 accessory proteins in lipid rafts of RBL mast cells. SNAREs were found either excluded (syntaxin2), equally distributed between raft and non-raft fractions (syntaxin4, VAMP-8, VAMP-2), or selectively enriched in rafts (syntaxin3, SNAP-23). Syntaxin4-binding Munc18-3 was absent, whereas small amounts of the syntaxin3-interacting partner Munc18-2 consistently distributed into rafts. Cognate SNARE complexes of syntaxin3 with SNAP-23 and VAMP-8 were enriched in rafts, whereas Munc18-2/syntaxin3 complexes were excluded. This demonstrates a spatial separation between these two types of complexes and suggests that Munc18-2 acts in a step different from SNARE complex formation and fusion.

Key words: SNARE; Munc18; Raft; Membrane fusion

1. Introduction

Lipid rafts are specialized membrane microdomains with a specific composition of lipids and proteins that can be isolated by their ability to float to low density after sucrose gradient fractionation following lysis [1]. The selective enrichment of components allows these microdomains to serve as platforms for signal transduction [2,3] and intracellular trafficking [4]. Abundant information exists on the involvement of lipid rafts in signaling initiated by aggregation of the mast cell high affinity IgE receptor (FcεRI) [5,6]. Aggregation recruits FcεRI into raft domains that contain a selective panel of molecules such as Lyn, Fyn, LAT, Cbp/PAG etc. in order to achieve effective signaling and cellular responses [7–9]. One of the physiological consequences is degranulation with release of allergic inflammatory mediators, a process that involves extensive membrane fusion [10,11]. Evidence in support of the hypothesis that fusion can similarly rely on the enrichment of proteins in rafts is rapidly accumulating [4]. Depletion of lipid-enriched cholesterol in rafts was shown to seriously perturb secretory trafficking in a variety of cell types [12–14]. SNAREs (soluble *N*-ethylmaleimide-sensitive fusion factor attachment protein receptors), a large family of membrane fusion proteins, were found to be present in lipid rafts. They can be divided into vesicular or v-SNAREs and target or t-SNAREs localized on opposing cellular compartments [15]. Fusion of these compartments is facilitated by their ability to form stable macromolecular complexes [16]. The SNAREs Ti-VAMP and syntaxin3 were shown to reside in lipid rafts at the *trans*-Golgi level in MDCK cells, where they functioned in apical sorting [17]. The neuronal SNAREs syntaxin1 and SNAP-25 were also enriched in rafts, as revealed by their association with detergent-resistant domains in PC12 cells [18]. Although Lang et al. found that the same SNAREs are solubilized by detergent, detailed fluorescence imaging of these SNAREs in plasma membrane sheets demonstrated, however, that they concentrate in cholesterol-dependent clusters that define docking and fusion sites for exocytosis [19]. Several accessory proteins interact with SNAREs [15,20]. Among these is the sec1/Munc18 (SM) family of proteins. Like SNAREs they are essential components of membrane fusion as revealed by gene inactivation studies in several organisms [21]. Munc18 family members, in particular, are involved in exocytosis of secretory vesicles with the plasma membrane. In addition to the neuronal isoform Munc18-1, two ubiquitously expressed mammalian isoforms, Munc18-2 and Munc18-3, with different syntaxin binding properties, have been described. Munc18-2 binds to syntaxin1, 2 and 3, while Munc18-3 binds to syntaxin2 and 4 [22,23]. Only Munc18-1 has been studied for lipid raft localization and was found to be excluded [18]. Their precise role in exocytosis is still unknown and likely complicated, given that they can also be considered inhibitors of exocytosis because they bind to SNAREs of the syntaxin family and prevent them from participating in SNARE complex formation [23,24].

In this study we compared the distribution of mast cell-expressed SNARE and Munc18 proteins [25–27] within and outside lipid rafts. Substantial heterogeneity in the distribution of the individual proteins was observed. Examination of proteins co-immunoprecipitating with syntaxin3 revealed that within rafts syntaxin3 does not form a complex with Munc18-2, whereas outside rafts these proteins are complexed.

2. Materials and methods

2.1. Antibodies and reagents

Antibodies directed against the following SNARE proteins were produced in our laboratory and have been described: syntaxin2, 3 and 4, VAMP-8 [26]. The VAMP-2- and SNAP-23-specific antibodies were purchased from Synaptic Systems (Göttingen, Germany).

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Antibodies to Munc18-2 and Munc18-3 have been described [27]. Mouse IgE specific for dinitrophenyl (DNP) [28] was used as ascites fluid or was affinity-purified. For some experiments the IgE was labelled with ^{125}I as described [29]. Fab′2 fragments of peroxidase-conjugated anti-rabbit and anti-mouse were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). DNP-HSA (human serum albumin) was purchased from Sigma (St. Louis, MO, USA).

2.2. Cell culture and cell stimulation

RBL-2H3 cells were maintained in Dulbecco's modified Eagle's medium–glutamax (Invitrogen, France), supplemented with 10% fetal calf serum, 100 IU/ml penicillin G and 100 μg/ml streptomycin (Invitrogen), at 37°C in a humidified 5% CO_2 incubator. Forty-eight hours before each experiment cells were plated at $3–4\times10^6$ cells in 15 cm culture dishes (Becton Dickinson) with complete medium and sensitized overnight with 500 ng/ml anti-DNP IgE (or in the case of ascites a 1:10 000 dilution). In some instances cells were sensitized with [^{125}I]IgE anti-DNP. Before stimulation, cells were washed and resuspended in prewarmed activation buffer (PIPES 25 mM pH 7.2 containing 150 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 0.5 mM $CaCl_2$). Cells were stimulated by aggregating receptor-bound IgE with 100 ng/ml DNP-HSA or were left unstimulated. The reaction was stopped by adding an excess of ice-cold Tris–HCl 10 mM, pH 7.5, containing 150 mM NaCl (TNV).

2.3. Preparation of lipid rafts and immunoprecipitations

Cells were solubilized in lysis buffer (TNV containing 0.1% (1.6 mM) Triton X-100, sodium orthovanadate 1 mM (Sigma), aprotinin 1000 U/ml (Sigma), pepstatin 10 μg/ml, leupeptin 20 μg/ml, aminoethyl benzenesulfonyl fluoride 2 μM (all Alexis, San Diego, CA, USA)) by directly adding 1 ml of lysis buffer to adherent cells before harvesting them by scraping as previously described [30]. The suspension was then transferred to a Dounce homogenizer and subjected to 15 strokes. The homogenate was centrifuged at $1000\times g$ for 10 min at 4°C and the supernatant was mixed in an Ultra-clear TM centrifuge tube (Beckman) with an equal volume of 85% sucrose. This mixture was successively overlaid with 6 ml of 30% sucrose and 3.5 ml of 5% sucrose, neither of which contained Triton X-100. The tubes were then centrifuged at $200\,000\times g$ at 4°C, in a Beckman SW40Ti rotor for 16 h. Sequential 1 ml fractions were harvested from the top of the gradient. An opaque band at the interface between the 5% and 30% layers was routinely harvested in fraction 4 and contained the lipid rafts. ^{125}I-containing fractions were counted in a gamma counter, and aliquots were analyzed by the bicinchoninic acid method for total protein [31], and by immunoblotting for specific proteins resolved by electrophoresis on sodium dodecyl sulfate (SDS)–polyacrylamide gels.

For immunoprecipitation, Triton X-100 was added to individual fractions to give a final concentration of 0.2%. Proteins were immunoprecipitated from pooled fractions using 5 μg of indicated antibody and protein A-Sepharose overnight. Immunoprecipitated proteins were resolved by SDS–polyacrylamide gel electrophoresis (PAGE), transferred to a nitrocellulose membrane and probed with the indicated antibodies.

2.4. Immunoblotting

Proteins resolved by SDS–PAGE were transferred to nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany) and membranes were blocked by incubation in Tris-buffered saline containing 0.1% Tween 20 and 5% non-fat dry milk. Blots were then incubated for 1 h at room temperature with primary antibodies. After several washes, peroxidase-labelled anti-rabbit or anti-mouse IgGs were used as secondary antibodies and incubated for 1 h at room temperature. The blots were washed and developed using the enhanced chemoluminescence assay (Amersham Pharmacia Biotech).

2.5. Data analysis

All experimental data shown are representative of each series of experiments and are from at least three independent experiments.

3. Results

Studies on the accumulation of SNARE proteins in lipid raft fractions using sucrose gradient fractionation revealed conflicting results [18,19]. Therefore, before investigating whether SNARE proteins in mast cells are localized in lipid rafts, we calibrated raft isolation according to a well established procedure in RBL mast cells [5] by looking at the distribution of FcεRI in these domains. Cultured RBL cells were loaded with radioiodinated IgE anti-DNP as a measure for FcεRI, as its short-term binding (hours) is essentially irreversible due to its slow dissociation rate [29]. Cells were then either left unstimulated or were stimulated by aggregating cell surface-bound IgE with DNP-HSA for 6 min before solubilization in a low concentration of Triton X-100 and fractionation through a discontinuous sucrose gradient. The distribution of FcεRI in the 12 individual recovered fractions was then evaluated by measuring the [^{125}I]IgE counts. The results in Fig. 1A show that surface FcεRI in resting cells was predominantly found in fractions 10–12 corresponding to the high density non-lipid raft fractions. Only a minor fraction of the receptor was associated with fractions 4–6, which correspond to the low density lipid raft fraction. No receptor was found in the very low density fractions 1–3. However, a small amount was localized in intermediate fractions 7–9. Following antigen-induced aggregation, the presence of FcεRI in fractions 4–6 was increased by two-fold or more as well as its presence in the intermediate fractions 7–9. A corresponding decrease in fractions 10–12 was observed indicating substantial recruitment into lipid rafts. This is in agreement with previous studies [5,6]. We also investigated the distribution of the adapter protein, LAT, and the cytoskeleton-associated protein, paxillin, in the pooled fractions using immunoblot analysis. These proteins are well characterized markers of lipid rafts and high density fractions, respectively

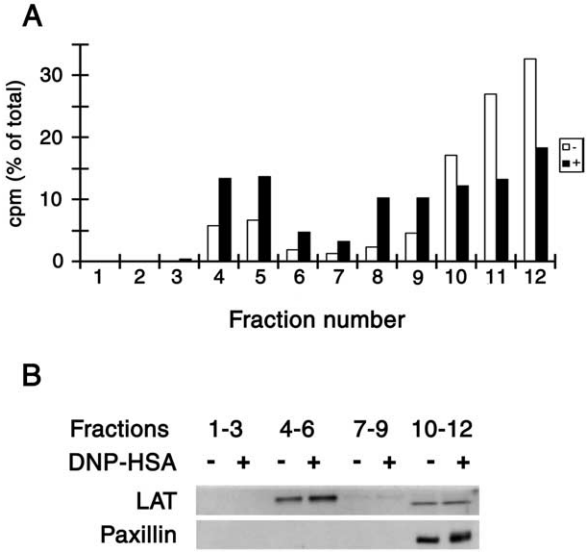

Fig. 1. Analysis of lipid raft distribution of the FcεRI (A) and two known marker proteins (B). [^{125}I]IgE-loaded or IgE-loaded RBL cells were either left unstimulated (−) or were stimulated (+) with antigen (DNP-HSA) for 6 min before lysis under low detergent conditions. Lysates were fractionated on a discontinuous sucrose gradient to separate high and low density fractions. A: Twelve individual fractions were collected and analyzed for [^{125}I]IgE counts. B: Twelve individual fractions were collected and pooled by three before immunoblot analysis using LAT- and paxillin-specific antibodies. Fractions 4–6 correspond to the low density lipid raft fractions and fractions 10–12 correspond to the high density non-lipid raft fractions.

[32]. As expected, paxillin did not distribute to the low density fractions 4–6, but was found exclusively in the high density non-lipid raft fractions 9–12 (Fig. 1B). In contrast, the dually acylated LAT [9] was primarily found in the low density lipid raft fraction and a slight increase of LAT in lipid rafts was observed in stimulated cells, as previously described [32].

Having established the validity of our fractionation procedure, we explored the raft distribution of SNARE proteins expressed in RBL mast cells [25–27]. Fig. 2 shows an immunoblot analysis of proteins in pooled fractions from a sucrose gradient separation, using specific antibodies directed to v- and t-SNARE proteins. Interestingly, marked differences were found. The t-SNARE syntaxin2 distributed almost exclusively into the high density fraction, with only minimal amounts present in the low density membrane domains. Syntaxin4 was found in higher proportion in the low density fractions but some also was found in intermediate fractions. However, a slight majority was found in the high density fractions. In contrast, both syntaxin3 and SNAP-23 showed a selective enrichment in low density lipid raft fractions. For v-SNAREs an almost equal distribution was seen for VAMP-8 in all fractions. In contrast, VAMP-2 showed some enrichment in the intermediate density fractions. No significant change in these patterns was noticed for any of the detected SNARE proteins after FcεRI stimulation. The SM proteins Munc18-2 and Munc18-3, which interact with syntaxin2/syntaxin3 or syntaxin4, respectively, also showed a distinctive pattern. While the majority of Munc18-2 distributed to the high density fraction we consistently saw a considerable amount of this protein in the low density fraction (Fig. 2). In contrast, Munc18-3 was not found in the low density fraction and distributed exclusively to the high density fractions. As for SNAREs, Munc18 proteins showed no significant changes in distribution following cell stimulation.

The differential compartmentation of the various syntaxins and Munc18 proteins on the membrane allowed us to tackle the question of whether differences might be observed in the interaction of Munc18 with syntaxins. This might reflect a

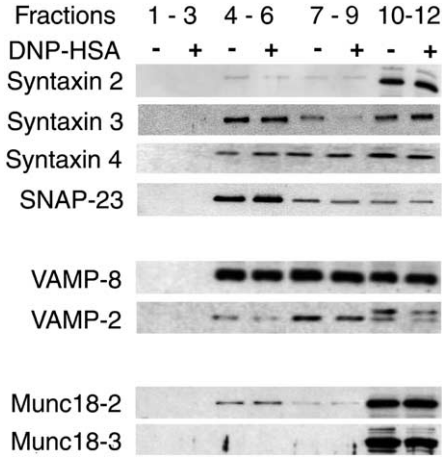

Fig. 2. Analysis of lipid raft distribution of SNARE and Munc18 proteins expressed in RBL mast cells. IgE-loaded RBL cells were either left unstimulated (−) or were stimulated (+) with antigen (DNP-HSA) for 6 min before lysis under low detergent conditions. Lysates were fractionated on a discontinuous sucrose gradient to separate high and low density fractions. Twelve individual fractions were collected and pooled by three before immunoblot analysis using the indicated SNARE- and Munc18-specific antibodies.

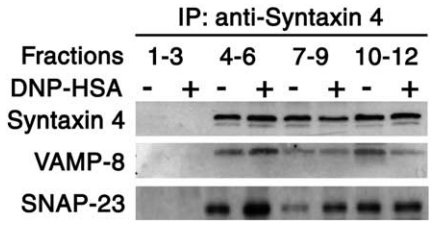

Fig. 3. Analysis of the lipid raft distribution of complexes of syntaxin4 with SNARE proteins. IgE-loaded RBL cells were either left unstimulated (−) or were stimulated (+) with antigen (DNP-HSA) for 6 min before lysis under low detergent conditions. Lysates were fractionated on a discontinuous sucrose gradient to separate high and low density fractions. Twelve individual fractions were collected and pooled by three. After addition of Triton X-100 to bring detergent concentrations to 0.2%, pooled fractions were subjected to immunoprecipitation with syntaxin4-specific antibody. Complexes with SNAP-23 and VAMP-8 were examined by immunoblot analysis using specific antibodies.

functional role as the fusion ability of syntaxins is inhibited by its interaction with Munc18 proteins [33]. Thus, co-immunoprecipitation experiments were performed on fractions of low detergent concentration lysates of resting and stimulated RBL cells separated through a discontinuous sucrose gradient. Syntaxin3 and syntaxin4 were immunoprecipitated from pooled fractions and analyzed for associated proteins using specific antibodies. Fig. 3 shows that this method allowed detection of macromolecular complexes of syntaxin4 with SNAP-23 and VAMP-8. For the most part, the distribution of syntaxin4 with co-immunoprecipitated VAMP-8 and SNAP-23 largely resembled that seen in total extracts (Fig. 2). No enrichment of the syntaxin4-containing complexes within or outside lipid rafts was observed regardless of whether cells were resting or stimulated. Thus, evaluation of any changes in its interaction with Munc18-3 within or outside lipid rafts did not merit investigation since Munc18-3 was found exclusively in the high density fraction. In contrast, because Munc 18-2 is found in low and high density fractions we investigated its association with syntaxin3, which is also found in both fractions. As depicted in Fig. 4, the recovery of

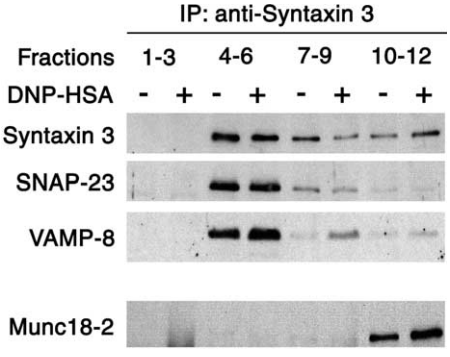

Fig. 4. Analysis of the lipid raft distribution of complexes of syntaxin 3 with SNARE and Munc18 proteins. IgE-loaded RBL cells were either left unstimulated (−) or were stimulated (+) with antigen (DNP-HSA) for 6 min before lysis under low detergent conditions. Lysates were fractionated on a discontinuous sucrose gradient to separate high and low density fractions. Twelve individual fractions were collected and pooled by three. After addition of Triton X-100 to bring detergent concentrations to 0.2%, pooled fractions were subjected to immunoprecipitation with syntaxin3-specific antibody. Complexes with SNAP-23, VAMP-8 and Munc18 were examined by immunoblot analysis using specific antibodies.

syntaxin3 in the immunoprecipitates showed a similar distribution as in total extracts with a selective enrichment of this protein in low density lipid raft fractions. Given that the distribution of SNAP-23 in total extracts was primarily in low density fractions, it was not surprising that syntaxin3–SNAP 23 interactions were enriched in the low density fraction. In contrast, given that VAMP-8 distributed equally between fractions 4–12 in total extracts (Fig. 2), complexes of this v-SNARE protein with syntaxin3 were selectively enriched in lipid rafts. Strikingly a different pattern from that of total lysates was seen in the case of Munc18-2, as all of the co-immunoprecipitated protein was found in the high density fractions despite the fact that they contained lower amounts of syntaxin3. Thus, the Munc18-2 present in lipid raft domains, as seen in total extracts, was not associated with syntaxin3. These results demonstrate a spatial separation of syntaxin3 SNARE complex formation from the site of Munc18-2 action in lipid rafts.

4. Discussion

There is substantial evidence that SNARE and SM proteins are essential components in membrane fusion [21,34,35]. SNARE family proteins act by forming energetically favored macromolecular complexes that diminish the energetic barrier that blocks the merger of two lipid phases during fusion [16,20,21]. The role of Munc18 proteins in membrane fusion is not well understood because of the dichotomy of being required for exocytosis and their ability to bind to syntaxins and inhibit SNARE complex formation [23,24]. Nonetheless, proposed models to explain this dichotomy invoke an interaction with a potential effector, like a Rab or Munc13 protein, to allow the transition from its function in sequestering syntaxins to its function in fusion [36–38].

To gain further insight into the interaction of SNARE and Munc18 proteins in membrane fusion we examined their distribution in lipid raft domains. We confirmed the enrichment of certain SNAREs, such as syntaxin3 and SNAP-23, in lipid rafts [17], while others, such as syntaxin4, VAMP-2 and VAMP-8, were more or less equally distributed between raft and non-raft fractions. One of the examined SNAREs, syntaxin2, did not significantly localize to rafts but was found in high density fractions. This heterogeneity of distribution in membranes suggests functional differences between individual SNAREs expressed in mast cells that could relate to the reported localization and function within different cellular compartments [25,26]. It could also be a result of the isolation procedure, however, the consistent difference in localization observed between experiments, at a minimum, implies that these proteins have properties that cause them to partition differently. Considerable heterogeneity of membrane compartments has indeed been revealed by lysing the cells under different detergent condition [18] or by electron microscopic studies of membrane sheets coupled to immunogold labeling [39].

Concerning SM proteins, we found Munc18-3 to be completely excluded from lipid raft domains. This finding is similar to data obtained with the neuronal isoform Munc18-1 [18]. In contrast, although the majority of Munc18-2 was found outside lipid raft domains a small but significant portion reproducibly appeared in the lipid raft fractions. This could indicate a specific function for this small fraction of Munc18-2 that may relate to its reported secretory granule localization [27]. Despite its presence in rafts, complexes of Munc18-2 with syntaxin3 were not found in low density fractions demonstrating that they are spatially separated from the sites of syntaxin3–SNARE complex formation that are highly enriched in rafts. The loss of interaction of syntaxin3 with Munc18-2 in lipid rafts is in agreement with previous evidence for these sites as being preferential platforms for membrane fusion [19] since this loss presumably renders syntaxin3 competent for fusion. This is also supported by our observation of the preferential interactions occurring between v-SNAREs (like VAMP-8) and t-SNAREs (like syntaxin3) in these domains. The lipid raft-independent binding of Munc18-2 with syntaxin3 agrees with models suggesting that this interaction precedes membrane fusion and that it may have a role beyond maintaining the syntaxins in a fusion-incompetent state. In fact, the additional role of a chaperone has been shown for a yeast Munc18 homologue, which functions to regulate the expression levels of its corresponding syntaxin partner [40]. This chaperone-like action was also seen in gene-inactivated mice deficient for the neuronal Munc18-1 isoform as the corresponding syntaxin levels were decreased by approximately 70% [34]. Another possible role lies probably at the steps involved in the docking of secretory granules. Indeed, ultrastructural analysis of the distribution of large dense core vesicles (LDCV) of chromaffin cells obtained from Munc18-1-deficient mice has revealed an about 10-fold reduction in morphologically docked LDCVs [41].

Because we consistently found a small amount of Munc18-2 in lipid raft domains, which was not complexed to syntaxin3, other functional interactions and molecular partners are possible in these domains. This may now allow the means to identify potential new partners because the lipid raft localization holds promise of being a positive effector for Munc18-2 action. Such identification would reconcile the data that revealed a late action of Munc18-1 in fusion pore expansion [42] with those that suggested an action in the steps preceding fusion [7,34,41]. This would also promote the concept that Munc18-2 has multiple roles in regulating fusion. Revealing these roles is likely to significantly advance our knowledge of vesicle fusion and exocytosis.

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References

[1] Simons, K. and Ikonen, E. (1997) Nature 387, 569–572.
[2] Simons, K. and Toomre, D. (2000) Nat. Rev. Mol. Cell Biol. 1, 31–39.
[3] Langlet, C., Bernard, A.M., Drevot, P. and He, H.T. (2000) Curr. Opin. Immunol. 12, 250–255.
[4] Ikonen, E. (2001) Curr. Opin. Cell Biol. 13, 470–477.
[5] Field, K.A., Holowka, D. and Baird, B. (1995) Proc. Natl. Acad. Sci. USA 92, 9201–9205.
[6] Field, K.A., Holowka, D. and Baird, B. (1997) J. Biol. Chem. 272, 4276–4280.
[7] Brdicka, T. et al. (2000) J. Exp. Med. 191, 1591–1604.
[8] Parravicini, V. et al. (2002) Nat. Immunol. 3, 741–748.
[9] Rivera, J. (2002) Curr. Opin. Immunol. 14, 688–693.

[10] Fernandez, J., Neher, E. and Gomperts, B. (1984) Nature 312, 453–455.
[11] Rohlich, P., Anderson, P. and Uvnas, B. (1971) Acta Biol. 22, 197–213.
[12] Dhanvantari, S. and Loh, Y.P. (2000) J. Biol. Chem. 275, 29887–29893.
[13] Wang, Y., Thiele, C. and Huttner, W.B. (2000) Traffic 1, 952–962.
[14] Schmidt, K., Schrader, M., Kern, H.F. and Kleene, R. (2001) J. Biol. Chem. 276, 14315–14323.
[15] Chen, Y.A. and Scheller, R.H. (2001) Nat. Rev. Mol. Cell Biol. 2, 98–106.
[16] Sutton, R.B., Fasshauer, D., Jahn, R. and Brunger, A.T. (1998) Nature 395, 347–353.
[17] Lafont, F., Verkade, P., Galli, T., Wimmer, C., Louvard, D. and Simons, K. (1999) Proc. Natl. Acad. Sci. USA 96, 3734–3738.
[18] Chamberlain, L.H., Burgoyne, R.D. and Gould, G.W. (2001) Proc. Natl. Acad. Sci. USA 98, 5619–5624.
[19] Lang, T., Bruns, D., Wenzel, D., Riedel, D., Holroyd, P., Thiele, C. and Jahn, R. (2001) EMBO J. 20, 2202–2213.
[20] Jahn, R. and Sudhof, T.C. (1999) Annu. Rev. Biochem. 68, 863–911.
[21] Rizo, J. and Sudhof, T.C. (2002) Nat. Rev. Neurosci. 3, 641–653.
[22] Tamori, Y., Kawanishi, M., Niki, T., Shinoda, H., Araki, S., Okazawa, H. and Kasuga, M. (1998) J. Biol. Chem. 273, 19740–19746.
[23] Tellam, J., Macaulay, S., McIntosh, S., Hewish, D., Ward, C. and James, D. (1997) J. Biol. Chem. 272, 6179–6186.
[24] Pevsner, J., Hsu, S., Braun, J., Calakos, N., Ting, A., Bennett, M. and Scheller, R. (1994) Neuron 13, 353–361.
[25] Guo, Z., Turner, C. and Castle, D. (1998) Cell 94, 537–548.
[26] Paumet, F., Le Mao, J., Martin, S., Galli, T., David, B., Blank, U. and Roa, M. (2000) J. Immunol. 164, 5850–5857.
[27] Martin-Verdeaux, S., Pombo, I., Iannascoli, B., Roa, M., Varin-Blank, N., Rivera, J. and Blank, U. (2003) J. Cell Sci. 116, 325–334.
[28] Liu, F.T., Bohn, J.W., Ferry, E.L., Yamamoto, H., Molinaro, C.A., Sherman, L.A., Klinman, N.R. and Katz, D.H. (1980) J. Immunol. 124, 2728–2737.
[29] Kulcycki, A. and Metzger, H. (1974) J. Exp. Med. 140, 1677–1695.
[30] Pombo, I., Martin-Verdeaux, S., Iannascoli, B., Le Mao, J., Deriano, L., Rivera, J. and Blank, U. (2001) J. Biol. Chem. 12, 12.
[31] Smith, P.K. et al. (1985) Anal. Biochem. 150, 76–85.
[32] Arudchandran, R., Brown, M.J., Peirce, M.J., Song, J.S., Zhang, J., Siraganian, R.P., Blank, U. and Rivera, J. (2000) J. Exp. Med. 191, 47–60.
[33] Jahn, R. (2000) Neuron 27, 201–204.
[34] Verhage, M. et al. (2000) Science 287, 864–869.
[35] Schoch, S., Deak, F., Konigstorfer, A., Mozhayeva, M., Sara, Y., Sudhof, T.C. and Kavalali, E.T. (2001) Science 294, 1117–1122.
[36] Lupashin, V. and Waters, M. (1997) Science 276, 1255–1258.
[37] Misura, K.M.S., Scheller, R.H. and Weis, W.I. (2000) Nature 404, 355–362.
[38] Richmond, J.E., Weimer, R.M. and Jorgensen, E.M. (2001) Nature 412, 338–341.
[39] Wilson, B.S., Pfeiffer, J.R., Surviladze, Z., Gaudet, E.A. and Oliver, J.M. (2001) J. Cell Biol. 154, 645–658.
[40] Bryant, N.J. and James, D.E. (2001) EMBO J. 20, 3380–3388.
[41] Voets, T. et al. (2001) Neuron 31, 581–591.
[42] Fisher, R.J., Pevsner, J. and Burgoyne, R.D. (2001) Science 291, 875–878.