Endophilin 1 (Endo1) participates in synaptic vesicle biogenesis through interactions of its Src homology 3 domain with the polyphosphoinositide phosphatase Synaptojanin and the GTPase Dynamin. Endo1 has also been reported to affect endocytosis by converting membrane curvature via its lysophosphatidic acid acyltransferase activity. Here we report that a closely related isoform of Endo1, Endo3, inhibits clathrin-mediated endocytosis. Mutational analyses showed that the variable region of Endo3 is important in regulating transferrin endocytosis. In the brain, Endo3 is co-localized with dopamine D2 receptor in olfactory nerve terminals and inhibits its clathrin-mediated endocytosis in COS-7 cells. Furthermore, overexpression of Endo3 in an olfactory epithelium-derived cell line suppressed dopamine D2 receptor-mediated endocytosis and therefore accelerated its dopamine-induced differentiation. These results indicate that Endo3 may act as a negative regulator of clathrin-mediated endocytosis in brain neurons.

The mammalian Endophilin proteins are encoded by the endophilin 1–3 (A1–3) and B1–2 genes. Endophilins 1–3, originally named SH3p4/p8/p13 (1, 2), SH3GL1–3 (3), and SH3d2a-c (4), were identified as binding partners of Synaptojanin (5, 6), whereas Endophilins B1 and B2, also called SH3GLB1/Bif-1 (7, 8) and SH3GLB2 (7) respectively, were identified as Endophilin-related molecules (9) or apoptosis-inducing Bax-binding proteins (8). The most extensively studied of these Endophilin isoforms is Endo1, which functions during synaptic vesicle recycling (10–14). Endo1 binds, via its C-terminal SH3 domain, to proline-rich domains of Amphiypophosine (15), Dynamin (2), and Synaptojanin (2, 5), all of which are involved in the endocytosis of synaptic vesicles (6). Recent genetic studies using Drosophila and Caenorhabditis elegans have shown that the Endophilin-mediated recruitment and stabilization of Synaptojanin on synaptic vesicles is crucial for clathrin-mediated endocytosis (16, 17).

In addition to the activity of its SH3 domain, the N-terminal domain of Endo1 has been shown to have lysophosphatidic acid acyltransferase (LPA-AT) activity; this enzyme converts lysophosphatidic acid (LPA) and acyl-CoA to phosphatidic acid (PA) (11). Since the cone shape of PA is more compatible with a high curvature than the inverted cone shape of LPA, Endo1 may promote negative membrane curvature by altering the composition of the donor membrane at the invaginating bud, thereby promoting the conversion of a shallow pit (11). Moreover, the binding of Endo1 to liposomes is sufficient to deform them into narrow tubules (18). These observations provide direct evidence to support the hypothesis that Endo1 generates membrane curvature during synaptic vesicle endocytosis (19).

In contrast to the neural expression of Endo1, the closely related protein Endophilin 2 (Endo2) is ubiquitously distributed throughout many tissue types (2). Endo2 has been reported to interact with Endo1 via its coiled-coil domain, thus linking a number of neuronal targets to the endocytic machinery (20). In this study, we assayed the involvement of a third mammalian Endophilin isoform, Endophilin 3 (Endo3), in receptor-mediated endocytosis. We show that, while structurally similar to Endo1 and -2, Endo3 is functionally distinct in its ability to inhibit clathrin-mediated transferrin endocytosis. We also show that Endo3 is expressed in the brain, including the olfactory nerve (ON) terminals, where presynaptic D2Rs are co-localized. We therefore investigated the ability of Endo3 to regulate D2R-mediated endocytosis in COS-7, HEK293T, and olfactory epithelium-derived cell lines.

**EXPERIMENTAL PROCEDURES**

Dopamine was purchased from Sigma; (−)-Nmethoxy-NHsalipride (specific activity, 73.7 Ci/mmol) from PerkinElmer Life Sciences; and restriction enzymes, DNA ligase, and KOD Dash DNA polymerase from Toyobo Co. (Osaka, Japan). FuGENE 6, alkaline phosphatase, and monoclonal antibody against human mhc (9E10) were obtained from Roche Diagnostics (Basel, Switzerland) and pBlueScript II SK was purchased from Stratagene (La Jolla, CA). EGFP-N1 and DsRed2-N1 were purchased from Clontech. Monoclonal antibody against human dopamine D2 receptor was obtained from Santa Cruz Biotechnology (Santa Cruz, CA); monoclonal antibody against Dynamin-1 (H153-1) was purchased from Upstate Biotechnology (Lake Placid, NY); anti-Synaptojanin antibody was purchased from Medical and Biological
The cells were therefore incubated with 2.8 nM \( ^{3}H \)ulpireline in 1 ml of HEPES-buffered saline (pH 7.4) at 4 °C for 150 min, washed twice with ice-cold PBS (−), and treated with 1% Triton X-100 solution. To each sample, 3.5 ml scintillation mixture (Aquasol-2, PerkinElmer Life Sciences), and the amount of \( ^{3}H \)ulpireline remaining was quantified with a scintillation counter (Tri-Carb, PerkinElmer Life Sciences). Nonspecific binding of \( ^{3}H \)ulpireline was determined by incubation in the presence of 10 nM haloperidol, a D2R ligand, and was found to be a maximum of 5% of the total binding. Each sample was assayed in triplicate.

Production of Recombinant Proteins and Antibodies—cDNA encoding full-length Endo3 (aa 1–347) was subcloned into pGEX-4T-3 (Amersham Biosciences, Uppsala, Sweden), and the construct was verified by sequencing both strands. The host strain BL21 (DE3) was transformed with the resulting expression vector, and the recombinant protein was purified on glutathione-Sepharose (Amersham Biosciences) using standard methods. New Zealand White rabbits were immunized with GST-Endo3 fusion protein, and the antisera were affinity-purified by chromatography on GST-Endo3 immobilized on Affi-Gel (Bio-Rad). The antibodies were further purified by passing them through glutathione-Sepharose columns coupled with GST-Endo1, and GST-Endo2.

Western Blot Analysis—Western blot analyses of rat brain and transfected cells lysates with anti-Endo3 or anti-pan-Endophilin antibody were performed as described previously (29).

Immunoprecipitation—An olfactory bulb was extracted in 1.2 ml of Tris-buffered saline (10 mM Tris, pH 7.6, 250 mM NaCl, 5 mM EDTA, 1 mM EDTA, 1 μM phenylmethylsulfonyl fluoride). The extract was pre-cleared by incubation for 1 h at 4 °C with 20 μl of protein A-Sepharose and divided into two parts. 2 μl of affinity-purified anti-Endo3 antibody was added to one part, while 2 μl of preimmune serum was added to the other part, and the lysates were incubated for 1 h at 4 °C. The immune complexes were precipitated with 10 μl of protein A-Sepharose (Amersham Biosciences) and eluted into 20 μl of SDS-PAGE sample buffer. Following electrophoresis on 7.5% SDS-PAGE, the proteins were transferred and incubated with monoclonal antibody to Dynamin-1 or Synaptotagmin-1.

Immunohistochemistry—Adult female Wistar rats (8 weeks old, 200 g) were perfused with 2% paraformaldehyde fixative for 20 min. The brain was postfixed in the same fixative for 2 h, cryoprotected in 30% sucrose in PBS (−) solution and frozen in Tissue-Tek OCT Compound (Miles, Elkhart, IN) at −80 °C. All experiments were conducted in accordance with the Guideline for the Care and Use of Animals (Tokyo Metropolitan Institute for Neuroscience, 2000). Frozen olfactory bulbs were cut into 20-μm-thick sections with a cryostat. The sections were mounted onto silanized slides, air-dried, and stored at −80 °C until used. The brain sections were incubated with 2.5% bovine serum albumin and 10% Block Ace (Snow Brand Milk, Sapporo, Japan) for 1 h and then with rabbit anti-Endo3 (1:100 dilution) and mouse anti-D2R (1:500 dilution) antibodies at 4 °C for 4 days. Endo3 was visualized with the Alexa568-labeled secondary antibody. D2R was visualized with the Alexa488-labeled secondary antibody. For signal amplification, the sections were incubated with biotinylated secondary antibody, followed by biotinyl-tyramide and horseradish peroxidase, and visualized using Alexa 488-conjugated streptavidin.

In Situ Hybridization—Olfactory epithelium samples were collected from female rats, fixed in 4% paraformaldehyde, and dehydrated in 8% EDTA for 1 day. Sections 12 μm in thickness were attached to silanized slides and incubated at 56 °C for 16 h with RNA probes of endo1 and endo3 labeled with digoxigenin in a hybridization solution containing 50% formamide, 10 mM Tris-HCl (pH 8.0), 500 μg/ml tRNA, 1× Denhardt’s solution, 300 μg/ml NaCl, 10% dextran sulfate, and 1 mM EDTA (pH 8.0, 300). Hybridized RNAs were detected with alkaline phosphatase-conjugated anti-DIG Fab fragment antibody (Roche Diagnostics), and signals were visualized using 4-nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate (Roche Diagnostics) as chromogenic substrates. Inhibition of Transferrin Endocytosis by Endo3—To clarify the role of Endo3 in clathrin-mediated endocytosis, we assayed the effect of Endo3 on transferrin uptake (31) by transient expression of endo3 in COS-7 cells, with an empty vector or endo1 as controls. In contrast to control cells, which showed marked transferrin uptake, we unexpectedly observed an inhibition of transferrin uptake in endo3-expressing cells (Fig. 16).
Inhibition of Receptor-mediated Endocytosis by Endo3

To confirm this inhibition and to determine the Endo3 domain responsible, we interchanged the variable and SH3 regions of Endo1 and Endo3. Replacement of Endo1 domains with those of Endo3 (Endo1–3) resulted in the Endo3-phenotype for transferrin uptake; conversely, replacement of Endo3 domains with those of Endo1 (Endo3–1) resulted in the Endo1 phenotype (Fig. 1, a–c). When we interchanged the variable regions of Endo1 and Endo3, we found that Endo1(V3) showed robust inhibition, whereas Endo3(V1) stimulated transferrin uptake (Fig. 1, a–c). These results strongly indicate that the variable domain of Endophilin is responsible for the regulation of endocytosis.

To test the role of the Endophilin variable region in the regulation of endocytosis, we introduced additional structural perturbations by site-directed mutagenesis (Fig. 1a). Secondary structure analyses of the Endophilins revealed that both Endo1 and 2 have short N-terminal α-helices in their variable regions, whereas the corresponding region of Endo3 has a β-turn. We therefore inserted the α-helical structure of Endo1 (MSLEF, corresponding to amino acids 261–265) into the N terminus of the variable region of Endo3 (Endo3(+II)), and we found that this fragment stimulated transferrin uptake (Fig. 1, b and c). Converesely, introduction of a point mutation into the middle of the α-helix of the variable region of Endo1 (Endo1L263P) resulted in marked inhibition of transferrin uptake (Fig. 1, c).

Expression of Endo3 in D2R-containing Olfactory Glomeruli—To address the functional role of Endo3 in neurons, we searched brain neurons, which express endo3 but not endo1, by in situ hybridization (30) for optimal expression of endo3. We found that olfactory mucosae were suitable for determining the function of Endo3. In situ hybridization revealed that endo3 mRNA was strongly expressed in the middle two-thirds of the olfactory glomeruli.
olfactory epithelium (Fig. 2, a and c), corresponding to the location of the primary ON perikarya (30). In contrast, the expression of endo1 mRNA in olfactory mucosae was much weaker than that of endo3 (Fig. 2, b and d). In addition, little signal was detected in sections hybridized with the endo3-sense control (data not shown). To determine the distribution of Endo3 protein in olfactory neurons, we generated an anti-Endo3 antibody and performed Western blotting, and we found that Endo3 protein was expressed in all brain regions, including the olfactory bulb (Fig. 2e).

Among the G protein-coupled receptors expressed in the olfactory bulb, D2R is highly and selectively expressed on the ON terminals (32). To determine whether Endo3 is co-expressed with presynaptic D2R in this region, we incubated olfactory bulbs with anti-Endo3 and anti-D2R antibodies, and we found that D2Rs were co-localized with Endo3 at the same glomerular synapses (Fig. 2, h–j). Immunoelectron microscopy showed that Endo3 was highly enriched in the presynaptic ON terminals, which are characterized by the presence of numerous synaptic vesicles (data not shown). These data strongly suggest that Endo3 is co-localized with presynaptic D2Rs on the terminals and may be involved in D2R-mediated endocytosis.

Identification of Dynamin-1 and Synaptotagmin-1 as Endo3-binding Proteins in the Brain—Since Endo3 has been reported to be testis-specific and to associate with testis-specific Dynamin-3 (2), we used the variable and SH3 domains of endo3 cDNA as a bait in a yeast two-hybrid screening system to identify the brain proteins that interact with it (21). This screening led to the isolation of dynamin-1 and -3. To confirm that Endo3 is associated with Dynamin-1 in vivo, we immuno-precipitated Endo3 from an extract of olfactory bulbs and found that Dynamin-1 was co-precipitated (Fig. 2f). In addition, Synaptotagmin-1 was co-precipitated with Endo3 (Fig. 2g), indicating that Endo3 forms a stable complex with Dynamin-1 and Synaptotagmin-1 in olfactory bulbs.

Effects of Endo3 Proteins on Presynaptic D2R-mediated Endocytosis—To confirm the involvement of Endo3 in presynaptic D2R-mediated endocytosis, we reconstituted the assay system of D2R-mediated internalization after dopamine stimulation (26–28). D2Rs are reported to be internalized in an agonist-dependent manner when GRK2 is co-expressed (26). We assayed the effect of Endo3 on D2R internalization, which was detected as a decrease in [3H]sulpiride binding activity in cells treated with dopamine (Fig. 3a). Control cells, which were transfected with the expression vector alone, showed robust D2R internalization (41.9 ± 0.5%, n = 10) after 120 min of dopamine treatment. In contrast, there was little decrease in [3H]sulpiride binding (6.5 ± 0.7%, n = 10) when endo3-expressing cells were treated with dopamine (Fig. 3a). Overexpression of endo1 led to an increase in the basal level of D2R endocytosis even before dopamine treatment (Fig. 3g), and dopamine-induced internalization of D2R in endo1-expressing cells was slightly reduced compared with that in cells expressing vector (Fig. 3b).

When we examined D2R internalization in transiently infected cells by confocal microscopy, we found that Endo3 suppressed dopamine-induced internalization of the c-myc-tagged D2Rs. In cells expressing D2R and GRK2, D2R was located predominantly at the cell surface in the absence of dopamine (Fig. 3c). When these cells were treated with dopamine, the receptors were distributed in the intracellular endosomes (Fig. 3d). In endo3-expressing cells, however, there was almost a complete absence of D2R redistribution into the endosomes (Fig. 3f). Since Endo2 is the primary Endophilin isomorph in COS-7 cells (Fig. 3i), our results suggest that exogenous Endo3 may inhibit D2R endocytosis by disrupting the interaction of Dynamin with endogenous Endo2.

Promotion of Dopamine-induced Differentiation of an Olfactory Epithelial Cell Line Expressing Endo3—To confirm that Endo3 inhibits D2R endocytosis in olfactory neurons, we used a neuronal cell line generated by transfection of rat olfactory epithelium with the immortalizing recombinant oncopogene E1A of adenovirus-2 (33). This 13.5.1.24 cell line has the phenotype of olfactory neuronal progenitors, and addition of dopamine has been shown to induce their differentiation into bipolar olfactory neurons. When we expressed the EGFP-Endo3 fusion protein in this cell line and treated it with the dopamine agonist apomorphine (10 μM), we observed that a large proportion of epitheloid cells underwent morphological differentiation into bipolar cells (Fig. 4, e and h). In contrast, apomorphine treatment of cells expressing EGFP or EGFP-Endo1 fusion protein
resulted in the differentiation of a significantly smaller proportion of cells (Fig. 4, f and g). In addition, when we co-transfected myc-tagged D2R and the pEF-endo3 into these 13.S.1.24 cells line and immunostained them with anti-myc antibody, we observed inhibition of D2R-mediated endocytosis; in contrast, endocytosis was stimulated in cells co-transfected with myc-endo1 (c), or D2R and GRK2 plus endo3 (d). All cells, other than those in a, were treated with 10 μM dopamine for 120 min. Fixed cells were permeabilized and incubated with anti-myc antibody. e-h, 13.S.1.24 cells were transfected with vector alone (f), endo1 (g), or endo3 (c, h). Cells other than those in e were treated with 10 μM apomorphine for 3 days. i, time course of bipolar cell number in cultures treated with 10 μM apomorphine. One-hundred cells were counted from each experiment. Each point represents the mean ± S.E. of bipolar cells from seven independent experiments. * p < 0.01; ** p < 0.001; and *** p < 0.0001, as assessed by Student’s t test.

**FIG. 4.** Accelerated differentiation of an endo3-expressing olfactory epithelium-derived cell line by apomorphine. a–d, inhibition of D2R endocytosis in 13.S.1.24 cells by endo3. 13.S.1.24 cells were transfected with D2R and GRK2 plus vector (a, b), D2R and GRK2 plus endo1 (c), or D2R and GRK2 plus endo3 (d). All cells, other than those in a, were treated with 10 μM dopamine for 120 min. Fixed cells were permeabilized and incubated with anti-myc antibody. e–h, 13.S.1.24 cells were transfected with vector alone (f), endo1 (g), or endo3 (c, h). Cells other than those in e were treated with 10 μM apomorphine for 3 days. i, time course of bipolar cell number in cultures treated with 10 μM apomorphine. One-hundred cells were counted from each experiment. Each point represents the mean ± S.E. of bipolar cells from seven independent experiments. * p < 0.01; ** p < 0.001; and *** p < 0.0001, as assessed by Student’s t test.

**DISCUSSION**

We have shown here that Endo3 is an inhibitor of receptor-mediated endocytosis. While overexpression of endo3 in COS-7 cells was associated with the arrest of transferrin endocytosis, this inhibition of transferrin uptake was abolished by replacing the variable region of Endo3 with that of Endo1. In olfactory neurons, only Endo3, not Endo1, was expressed. Endo3 inhibited the endocytosis of dopamine D2 receptors localized on olfactory nerve terminals, and this inhibition facilitated the dopamine-induced differentiation of a cell line derived from olfactory epithelium.

Endo1 is required for the transition from early to late stages of clathrin-mediated endocytosis, as shown by the microinjection of anti-Endophilin antibodies at the giant synapse of the lamprey, which resulted in the accumulation of clathrin-coated pits at the presynaptic membrane (10). Furthermore, mutant Drosophila larvae lacking endophilin failed to take up FM1–43 dye in synaptic boutons, indicating an inability to retrieve synaptic membrane (13, 14). Based on these findings and the similarity of Endo3 to Endo1 and Endo2, we initially expected that Endo3 would activate the endocytosis of synaptic vesicles. Unexpectedly, however, our results showed that expression of Endo3 almost completely inhibited receptor-mediated endocytosis.

The variable domain of Endo3 is composed of a β-turn, whereas that of Endo1 has an additional α-helix at its aminoterminal end. As the crystal structures of the Endophilins are not yet known, the sites responsible for inhibition of endocytosis were identified by mutagenesis. For example, when we inserted five residues (MSELF aa261–265) from Endo1, which have a high potential to form an α-helix, into the corresponding portion of the variable region of Endo3, we observed reduced inhibition of transferrin endocytosis compared with wild type Endo3. Conversely, when we replaced a leucine in the α-helix of Endo1 with proline, we observed an inhibition of transferrin uptake equal to that seen with Endo3. The proline residue introduced a kink into the α-helix, which bends away from the side of the helix continuing the proline (34). Taken together, these results suggest that an amino-terminal α-helical structure in the variable region of the Endophilins regulates transferrin endocytosis.

Similar findings have been described for Endo2, in that the presence of glutamate at amino acid 264 was shown to be required for its binding to voltage-gated calcium channels (35). A point mutation at this amino acid was found to eliminate this calcium-dependent interaction, as well as impairing synaptic vesicle endocytosis, indicating that this region is necessary for facilitation of endocytosis. Although there is as yet no direct evidence that this glutamate binds Ca2+, the replacement of glutamate with serine at the corresponding position of Endo3 suggests that it may work as a dominant negative regulator for other Endophilins.

NH2-terminal LPA-AT activity was also shown to be involved in clathrin-mediated endocytosis. When we compared LPA-AT activity of the three Endophilins, we found that, although Endo3 had some LPA-AT activity, it was significantly lower than that of Endo2 (data not shown). Thus, overexpression of Endo3 protein in COS-7 cells may act as a dominant negative regulator by replacing endogenous Endo2, which induces positive-negative lipid membrane curvature along with conversion of the inverted cone-shaped LPA into cone-shaped PA. This N-terminal low LPA-AT model does not necessarily exclude inhibition via a variable region model. Either or both models may explain the ability of Endo3 to inhibit receptor-mediated endocytosis.

The in vivo functional role of Endo3 in the ON terminals is of great interest. Axons from olfactory epithelium form the ON, which innervates the olfactory bulb (36). ON terminals make synapses with dendrites of both mitral and tufted and periglomerular cells, and periglomerular neurons can form reciprocal dendro-dendritic synapses with mitral and tufted cells (37). Dopamine and D2R agonists are known to block synaptic transmission from the olfactory nerve to mitral and tufted cells (38). In other words, the activation of D2Rs presynaptically inhibits olfactory nerve terminals and decreases the ability to detect odors. We therefore hypothesize that dopamine-induced D2R internalization is suppressed by co-expression of Endo3 in ON terminals. For example, if an animal is tonically exposed to a
strong odor, the glomeruli activated by this odor might release more dopamine, resulting in greater presynaptic inhibition. In the absence of Endo3 from the glomeruli, however, D2R would be internalized rapidly after odor stimulation. Under these conditions, feedback inhibition would not work satisfactorily, leading to sensing of the offensive smells. Thus, presynaptic inhibition of ON terminals via D2R is a potential mechanism for increasing the range of adaptation to strong odors.

We have demonstrated here that Endo3 inhibits transferrin uptake and that its variable region is critical for the regulation of clathrin-mediated endocytosis, suggesting a new role of Endo3 in modulating physiological sensations.

Acknowledgments—We thank Dr. R. J. Lefkovitz for GRK2 cDNA, Dr. D. K. Grandy for D2R cDNA, Dr. S. Nagata for pEF-BOS plasmid, Dr. T. Uchihara for confocal laser microscopy.

REFERENCES

1. Sparks, A. B., Hoffman, N. G., McConnell, S. J., Fowlkes, D. M., and Kay, B. K. (1996) Nat. Biotechnol. 14, 741–744
2. Ringstad, N., Nemoto, Y., and De Camilli, P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8569–8574
3. Giachino, C., Lantelme, E., Lanzetti, L., Saccone, S., Bella Valle, G., and Migone, S. (1997) Genomics 41, 427–434
4. Torrey, T., Kim, W., Morse, H. C., and Konak, C. A. (1998) Mamm. Genome 9, 74–75
5. de Heuvel, E., Bell, A. W., Ramjaun, A. R., Wong, K., Sossin, W. S., and McPherson, P. S. (1997) J. Biol. Chem. 272, 8710–8716
6. Slepnev, V. I., and De Camilli, P. (2000) Nat. Rev. Neurosci. 1, 161–172
7. Piazzolla, H., Ciajolo, N., and Betz, E. A. (2001) J. Neurosci. 21, 757–767
8. Cattel, B. S., Yamaguchi, H., Komatsu, K., Miyashita, T., Yamada, M., Watanabe, K., and Wang, H. G. (2001) J. Biol. Chem. 276, 20559–20565
9. Modregger, J., Schmidt, A. A., Ritter, B., Huttner, W. B., and Plomann, M. (2003) J. Biol. Chem. 278, 4160–4167
10. Ringstad, N., Gad, H., Rasmussen, D. O., Di Polo, G., Nemoto, Y., and Brodin, L. (2000) Neuron 27, 301–312
11. Guichet, A., Wucherpfennig, T., Dufu, V., Ettor, S., Wilsch-Brauninger, M., Hellweg, A., Gonzalez-Gaitan, M., Huttner, H. W., and Schmidt, A. (2002) EMBO J. 21, 1661–1672
12. Verstreken, P., Kjaerulf, O., Lloyd, T. E., Atkinson, R., Zhou, Y., Mehtap, E., and Bellen, H. J. (2002) Cell 109, 111–112
13. McEwen, K. D., Ramjaun, A. R., Kay, B. K., and McPherson, P. S. (1997) FEBS Lett. 414, 308–312
14. Schuske, K. R., Richmond, J. E., Matthies, D. S., Davis, W. S., Runz, S., Rube, D. A., van der Bie, A., and Jorgensen, E. M. (2003) Neuron 40, 749–762
15. Schuske, P., Koh, T. W., Schulze, K. L., Zhai, R. G., Hiesinger, P. R., Zhou, Y., Mehta, S. Q., Cao, Y., Roos, J., and Bellen, H. J. (2003) Neuron 40, 733–748
16. Farsad, P., Ringstad, N., Takei, K., Floyd, S. R., Rose, K., and De Camilli, P. (2001) J. Cell Biol. 155, 193–200
17. Huttner, W. B., and Schmidt, A. A. (2002) Trends Cell Biol. 12, 155–158
18. Ringstad, N., Nemoto, Y., and De Camilli, P. (2001) J. Biol. Chem. 276, 40424–40430
19. Irie, Y., Yamagata, K., Gan, Y., Miyamoto, K., Do, E., Kuo, C. H., Taira, E., and Miki, N. (2000) J. Biol. Chem. 275, 2687–2695
20. Yamagata, K., Matsumura, K., Inoue, W., Shiraki, T., Suzuki, K., Yasuda, S., Sugihara, H., Cao, C., Watanabe, Y., and Kobayashi, S. (2001) J. Neurosci. 21, 2669–2677
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., pp. 2108–2117, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
22. Mizushima, S., and Nagata, S. (1990) Nucleic Acids Res. 18, 5322
23. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989) Gene (Amst.) 77, 51–59
24. Ito, K., Haga, T., Lameh, J., and Sadee, W. (1999) Eur. J. Biochem. 260, 112–119
25. Hotta, K., Takahashi, Y., and Matsumoto, M. (2000) Mol. Pharmacol. 59, 496–506
26. Iwata, K., Ito, K., Fukuzaki, A., Inoue, K., and Haga, T. (1999) Eur. J. Biochem. 263, 596–602
27. Sugihara, H., and Yamauchi, T. (1992) Brain Res. 593, 97–104
28. Sauer, T., and Schmitz, C. J. (1995) Histochemistry 100, 431–440
29. Swanson, L. W., and Warden, W. S. (2001) J. Comp. Neurol. 435, 231–235
30. Woolfson, D. N., and Williams, D. H. (1990) FEBS Lett. 277, 185–188
31. Chen, Y., Deng, L., McEwen, N., Lai, M., Chang, S., Chen, G., and Zhang, J. F. (2003) Cell 115, 37–48
32. Shipley, M. T., and Ennis, M. (1996) J. Neurosci. 16, 123–176
33. Pinching, A. J., and Powell, T. P. (1971) J. Comp. Neurol. 141, 9–347
34. Ennis, M., Zhou, F. M., Ciumbur, K. J., Arziumian-Anderjaska, H., Hayar, A., Borrelli, E., ZINNER, L. A., Margolis, P., and Shipley, M. T. (2001) J. Neurophysiol. 86, 2986–2997
Inhibitory Role of Endophilin 3 in Receptor-mediated Endocytosis
Hiroko Sugiura, Ken Iwata, Masato Matsuoka, Hiroshi Hayashi, Takako Takemiya, Shin Yasuda, Masumi Ichikawa, Takashi Yamauchi, Patrick Mehlen, Tatsuya Haga and Kanato Yamagata

J. Biol. Chem. 2004, 279:23343-23348.
doi: 10.1074/jbc.M312607200 originally published online April 5, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M312607200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 37 references, 11 of which can be accessed free at http://www.jbc.org/content/279/22/23343.full.html#ref-list-1