Mammalian Homologues of *Caenorhabditis elegans* unc-13 Gene Define Novel Family of C₂-domain Proteins*

(Received for publication, June 13, 1995, and in revised form, August 14, 1995)

Niels Brose†§, Kay Hofmann¶, Yutaka Hata‡, and Thomas C. Südhof**

From the †Department of Molecular Genetics and Howard Hughes Medical Institute, University of Texas Southwestern Medical School, Dallas, Texas 75235, the §Max-Planck-Institut für Experimentelle Medizin, 37075 Göttingen, Federal Republic of Germany, and the ¶Swiss Institute for Experimental Cancer Research, 1066 Epalinges, Switzerland

The unc-13 gene in *Caenorhabditis elegans* is essential for normal presynaptic function and encodes a large protein with C₁- and C₂-domains. In protein kinase C and synaptotagmin, C₁- and/or C₂-domains are regulatory domains for Ca²⁺, phospholipids, and diacylglycerol, suggesting a role for unc-13 in regulating neurotransmitter release. To determine if a similar protein is a component of the presynaptic machinery for neurotransmitter release in vertebrates, we studied unc-13 homologues in rat. Molecular cloning revealed that three homologues of unc-13 called Munc13-1, -13-2, and -13-3 are expressed in rat brain. Munc13s are large, brain-specific proteins with divergent N termini but conserved C termini containing C₁- and C₂-domains. Specific antibodies demonstrated that Munc13-1 is a peripheral membrane protein that is enriched in synaptic vesicles and localized to plasma membranes but absent from synaptic vesicles. Our data suggest that the function of unc-13 in *C. elegans* is conserved in mammals and that Munc13s act as plasma membrane proteins in nerve terminals. The presence of C₁- and C₂-domains in these proteins and the phenotype of the C. elegans mutants raise the possibility that Munc13s may have an essential signaling role during neurotransmitter release.

In presynaptic nerve terminals, synaptic vesicles store neurotransmitters and release them by exocytosis. Major progress has been made in recent years in the identification of proteins important for synaptic vesicle functions and in the understanding of the molecular interactions that underlie exocytosis. At this point, the molecular composition of synaptic vesicles is well described, and the functions for key proteins in membrane fusion have been explored (for review, see Refs. 1 and 2). However, the identification of the proteins that mediate synaptic vesicle functions is far from complete. Even in the case of the most intensely studied proteins, our understanding is limited. For example, synaptotagmin I is an intrinsic membrane protein of synaptic vesicles that is essential for Ca²⁺-triggered fast neurotransmitter release (3). Structurally, synaptotagmin I contains two C₂-domains, binds phospholipids as a function of Ca²⁺, and interacts with several synaptic proteins in vitro (4–11). However, the exact reactions performed by synaptotagmin I in the nerve terminal are unknown, as are the number of protein-protein interactions it performs physiologically. It seems likely that many more proteins will ultimately be found to be required for neurotransmitter release than the few proteins that are currently being studied.

Pioneering experiments by Sydney Brenner identified a collection of mutants in *Caenorhabditis elegans* called unc-13 mutants that are completely or partially paralyzed (12). A sub-group of these unc mutants is characterized by the presence of high levels of acetylcholine, resistance to acetylcholine esterase inhibitors such as aldicarb, and normal activities of acetylcholine esterase and choline acetyltransferase (13, 14). These characteristics suggest that in this subgroup of unc genes, presynaptic neurotransmitter release is impaired, a hypothesis that is supported by the molecular identification of several unc genes of this subgroup. unc-18 encodes a protein whose mammalian homologue, Munc18, stoichiometrically binds to syntaxin, a component of the synaptic vesicle fusion complex that is essential for release (15–17). The protein encoded by another gene, unc-17, encodes the vesicular transporter for acetylcholine (18). Finally, mutants in the gene encoding the *C. elegans* homologue of synaptotagmin have the same phenotype as the unc mutants that accumulate acetylcholine and are aldicarb-resistant (19).

Although putative functions of several of the unc genes have been identified, many remain to be studied. unc-13 is a particularly interesting member of this group because of its severe phenotype (14). Characterization of the unc-13 gene (20) revealed that it encodes a large protein (1734 amino acids) with no homology to other proteins except for two regions: a C₁-domain homologous to the phorbol ester- and diacylglycerol binding region of protein kinase C that as a recombinant protein also binds phorbol esters (21, 22) and a C₂-domain that in synaptotagmin constitutes a Ca²⁺-binding and a protein-protein interaction domain (4–11). These characteristics suggested a potential role for unc-13 in neurotransmitter release that involves a diacylglycerol-dependent and Ca²⁺-dependent step. No such step has yet been identified in synaptic vesicle exocytosis, but phorbol esters have profound effects on neurotransmitter release that cannot be wholly attributed to protein kinase C (23). In order to determine if unc-13 and its presumptive function is conserved in vertebrates and to gain insight into its nature, we have now cloned multiple mammalian homologues for unc-13 that we have named Munc13–1, -13–2, and -13–3. Our data show that these proteins form a highly conserved family of plasma membrane proteins with a probable function in neurotransmitter release.

* This study was supported in part by a grant from the Deutsche Forschungsgemeinschaft. 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.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U24070, U24071, and U24072.

† Present address: Research Development Corp., Kobe 651-22, Japan.

** To whom correspondence should be addressed.

1 Y. Goda and C. F. Stevens, personal communication.
Cloning of Munc13–1, –13–2, and –13–3—A rat brain library in λZAP-PII was screened at low stringency (24) with an insert probe derived from the C. elegans unc-13 cDNA clone pZC1T (20). 15 positive clones were isolated from 5 × 10⁶ plaques, subcloned into M13 vectors, and sequenced, revealing that four clones encode parts of Munc13–1, three clones encode parts of Munc13–2, and two clones encode parts of Munc13–3, with the remaining six clones representing artifacts. Overlapping cDNA clones covering the entire coding region of Munc13–1 and -13–2 and part of the coding region of Munc13–3 were isolated in subsequent screens. The amino acid sequences of Munc13–1, –13–2, and –13–3 were derived from their nucleotide sequences assembled from multiple cDNAs and are aligned with each other and with that of the unc-13 gene product in Fig. 1, revealing a high degree of conservation between the different mammalian isoforms and the C. elegans protein. This conservation suggests that the Munc13s are true mammalian unc-13 homologues.

Munc13s are large proteins; Munc13–1 has 1735 residues, and Munc13–1 and -13–2 has 1985 residues, resulting in sizes of 196 and 222 kDa respectively. Munc13s are highly homologous to each other in their C-terminal two-thirds, but dissimilar in their N-terminal third (Fig. 1). 50% of all residues of the C-terminal two-thirds of unc-13 and Munc13–1, –13–2, and -13–3 are invariant (621 of 1243 residues). Blocks of almost 100% identical residues are separated by small islands of nonconserved sequences. At three positions, a 13–19 amino acid gap is present in the C. elegans sequence compared with the Munc13–1 sequence. At two of these positions, overlapping Munc13–1 cDNA clones either contained or lacked the corresponding sequence, suggesting that these regions are subject to alternative splicing.

Analysis of the C1- and C2-domains of Munc13s—Sequence analyses revealed that Munc13s, similar to unc-13, contain C1- and C2-domains (Fig. 2). No additional significant homologies were detected in the current databanks. The C2-domains of Munc13s and unc-13 are most homologous to those of protein kinase Cα, β, and γ. They also appear to be functionally similar since the C2-domain of unc-13 binds phorbol esters with an affinity similar to that of protein kinase C (21, 22). Although only a single C2-domain in the middle of unc-13 was originally detected (20), a more detailed analysis demonstrated that the C terminus of all Munc13s and of unc-13 contain a second C2-domain (marked by a solid line in Fig. 1). In addition, the N terminus of Munc13-1 but not of the other members of this family contains a third, distantly related C2-domain (Fig. 2).

We systematically analyzed the sequence homology between different C2-domains of Munc13s and various proteins in order to examine their relationship. Databank searches showed that C2-domains are found in a large number of proteins, with a total of more than 50 C2-domains. Many of these proteins are involved in signal transduction or membrane trafficking pathways, but others have only been defined at the sequence level. All Munc13s contain a single C2-domain immediately N-terminal to the middle C2-domain; only protein kinase C isoforms contain a similar arrangement of C2- and C2-domains (Fig. 2A). Several proteins in addition to Munc13s, including synaptotagmins and raphembrins, contain multiple C2-domains. The sequences of the C2-domains of Munc13s are aligned with each other and with the sequences of the most conserved C2-domains in the databanks in Fig. 2B, revealing that some residues are conserved in all C2-domains, others only in subgroups of C2-domains, and others vary between almost all C2-domains.

The crystal structure of the first C2-domain of synaptotagmin I showed that it is composed of 8 β-sheets that are arranged in a compact greek key motif (37). Synaptotagmin I probably contains multiple Ca²⁺ binding sites, at least one of which was identified in the crystal as formed by two loops connecting β-sheets. When the sequence of the first C2-domain of synaptotagmin I is compared with those of the other C2-domains, the sequences corresponding to the β-strands in synaptotagmin I are the most conserved sequences of the C2-domains. The sequences corresponding to loops connecting the β-strands are less conserved except for the Ca²⁺ binding loops (see Ref. 37). However, even here some of the residues are replaced in the C2-domains from Munc13s, particularly the
FIG. 1. Primary structures of Munc13–1, -13–2, and -13–3. The amino acid sequences of the rat proteins were deduced from the nucleotide sequence assembled from multiple overlapping cDNA clones (GenBank accession numbers U24070, U24071, and U24072). Sequences are shown in single letter amino acid code and are aligned for maximal homology with the C. elegans unc-13 gene product. Residues that are identical in at least 50% of the sequences are shown on a black background, and residues that are similar are shaded (similarity groups: F, Y, W; I, L, V, M; H, R, K; D, E; G, A; T, S; N, Q). C1-domains are marked by a dotted line, and C2-domains are marked by continuous lines. Sequences are identified and numbered on the left. For Munc13–3, only a partial cDNA sequence was obtained. Two sequence stretches encoding residues 1434–1456 and residues 1541–1559, respectively, were either present or absent in Munc13–1 cDNAs, suggesting that these sequences are alternatively spliced. Note that the C. elegans sequence contains gaps at the corresponding positions.
FIG. 2. Domain structure of Munc13s: Comparison with other C2-domain proteins. A, diagram of the domain structures of Munc13–1 and other representative proteins containing C2-domains. The domain structures of the other Munc13s and of unc-13 are similar to that of Munc13–1 except that the N-terminal C2-domain is absent. Below Munc13–1, representative members of other protein families containing C2-domains are depicted: synaptotagmins, protein kinase C isozymes (PKC), phospholipase C (PLC), and phospholipase A2 (PLA2), rabphilin-3A and its C. elegans homologue f37a4.7, and a number of databank entries of sequences of unknown function. C1- and C2-domains are labeled C1 and C2, respectively. Kinase domains, kinase; pleckstrin homology regions, PH; and transmembrane regions, TM. X denotes a conserved region in phospholipase C of unknown significance, and catal denotes their catalytic domain; SH2 and SH3 stands for src-homology regions 2 and 3; rabph is for the rabphilin homology domain; and r and URE-B1 for the rsp5- and the URE-B1-domains (40). B, sequence alignment of C2-domains from representative members of the protein families depicted in A. Sequences are aligned for maximal homology. Dots indicate gaps, and numbers in brackets are residues that are absent from other C2-domains and have been deleted from the alignment. Conserved residues are shown on a black background. Sequences are identified on the left; C2-domains from proteins with multiple domains are labeled A, B, C, and D. C, dendrogram of the C2-domains aligned in B. The length of the lines connecting the different C2-domains is a measure for their sequence distance. The tree was calculated by the neighbor joining method (29) and tested by bootstrapping, which recalculates the tree for random subsets of the data (100 identical alignments in which random sequences were substituted by Xs). Bifurcation points that were confirmed by bootstrapping analysis in 80–100 replicates are marked by filled triangles, in 50–80 replicates by filled circles, in 20–50 replicates by open circles, and in less than 20 replicates by no label. Notethat the different C2-domains can be divided in subclasses based on the sequence alignment and the dendrogram. One subclass contains the different C2-domains from synaptotagmins, Ca2+-dependent protein kinase C isozymes, and rabphilins; a second subclass contains the phospholipases, and a third contains the Munc13s, with the reliability of the tree being the highest at bifurcation points between closely related proteins.
C-terminal and N-terminal C2-domains. The biggest sequence variations are observed in the loops between the fourth, fifth, and sixth \(\beta\)-strands that contain insertions of up to 24 amino acids (Fig. 2B).

Quantitation of the similarities between different C2-domains demonstrated that they fall into distinct classes (Fig. 2C). The dendrogram revealed that the middle C2-domain (referred to as C2-B in all Munc13s because Munc13–1 contains an additional N-terminal C2-domain called C2-A) and the last C2-domain of Munc13s (C2-C) form separate groups that are most similar to the corresponding domains from other isoforms of the same gene family. It seems likely that the different C2-domains originated from a single evolutionary ancestor but evolved and duplicated independently in precursor proteins to gene families such as the synaptotagmins and Munc13s. Their independent evolution suggests that C2-domains perform specialized functions in different proteins.

The C2-domains of Munc13s Are Not Ca\(^{2+}\) /Phospholipid Binding Domains—Some but not all C2-domains from synaptotagmins bind phospholipids as a function of Ca\(^{2+}\) (8, 11, 38).

To examine if C2-domains from Munc13s are Ca\(^{2+}\)-dependent phospholipid binding domains, we expressed the middle C2-domains of Munc13–1, -13–2, and -13–3 as glutathione S-transferase-fusion proteins in bacteria and measured their Ca\(^{2+}\)-dependent phospholipid binding (Fig. 3). The middle C2-domains were chosen because they are most similar to the C2-domains of protein kinase C and synaptotagmin. In contrast to the first C2-domain of synaptotagmin I, no Ca\(^{2+}\)-dependent phospholipid binding was observed for the Munc13 C2-domains. Interestingly, background binding of phospholipids in the absence of Ca\(^{2+}\) was reproducibly reduced by both Ca\(^{2+}\) and Mg\(^{2+}\) in all three Munc13 C2-domains tested. This finding suggests that divalent cations affect the conformation of the C2-domains from Munc13s. The C-terminal C2-domain from Munc13–1 also failed to bind phospholipids as a function of Ca\(^{2+}\) (data not shown). The absence of Ca\(^{2+}\)-dependent phospholipid binding to the middle C2-domains of Munc13s supports the conclusion from the dendrogram (Fig. 2C) that the group of C2-domains of protein kinase C, rabphilin, and synaptotagmin forms a separate group from that of the Munc13s.

Tissue-specific Expression of Munc13s—RNA blots loaded with poly(A)\(^{+}\)-enriched RNA from different rat tissues were hybridized at high stringency with probes specific for either Munc13–1, -13–2, or -13–3. mRNAs for all three Munc13 isoforms were only detected in brain (Fig. 4). The sizes of the mRNAs for the different isoforms vary from 7.5 (Munc13–1) to 9 (Munc13–3) and 10 kilobases (Munc13–2).

Characterization of Munc13 Antibodies—In order to study Munc13s, we raised antibodies against bacterially expressed fusion proteins from Munc13–1 and Munc13–2. Immunoblotting of proteins from COS cells transfected with full-length expression vectors encoding Munc13–1 and Munc13–2 showed that the antibodies recognize a protein of approximately 200 kDa in the transfected COS cells (Fig. 5). The Munc13–1 antibody does not react with Munc13–2, and the Munc13–2 antibody does not react with Munc13–1, suggesting that the antibodies are specific.

We next examined the distribution of Munc13 proteins. Munc13–1 was only detected in the nervous system, confirming the RNA blotting data (Fig. 6). The size of the protein in brain corresponds to that of the protein expressed by transfection in COS cells, suggesting that the expression vector encodes full-length Munc13–1 (Fig. 5). By contrast, antibodies to Munc13–2 failed to identify an immunoreactive protein in all tissues tested, possibly because of low protein levels and/or low anti-
Munc13–1 is a peripheral membrane protein—Although the primary structure of Munc13–1 includes several hydrophobic regions, it does not contain a classical transmembrane region, suggesting that it is not an intrinsic membrane protein (Fig. 1). Centrifugations of total brain homogenates in the presence or absence of Ca\(^{2+}\) demonstrated that Munc13–1 fractionates with particulate material under both conditions (Fig. 7A). Treatment of the membranes with several detergents resulted in the complete solubilization of Munc13–1 even at low salt concentrations, suggesting that Munc13–1 is a peripheral membrane protein (data not shown).

In order to examine the nature of the association of Munc13–1 with membranes, synaptic plasma membranes that are enriched for Munc13–1 (see below) were treated with increasing amounts of NaCl in the presence and absence of Ca\(^{2+}\) (Fig. 7B). Even at high salt concentrations, Munc13–1 remained membrane-bound. Some peripheral membrane pro-
Subcellular distribution of Munc13-1. Equal amounts of subcellular fractions from rat brain (20 μg/tube) were analyzed by immunoblotting with an affinity-purified Munc13-1 antibody. Antibodies to synaptophysin and the NM2A receptor subunit NM2A R1 were used as controls for synaptic vesicle proteins and postsynaptic membrane proteins, respectively. Subcellular fractions are designated as follows: HOM, homogenate; P1, nuclear pellet; P2, crude synaptosomal pellet; P3, light membrane pellet; S1, cytosolic fraction; LP1, lysed synaptosomal membranes; LP2, crude synaptic vesicle fraction; LS2, cytosolic synaptosomal fraction; SPM, synaptic plasma membranes.

Fig. 8. Subcellular distribution of Munc13-1. Equal amounts of subcellular fractions from rat brain (20 μg/tube) were analyzed by immunoblotting with an affinity-purified Munc13-1 antibody. Antibodies to synaptophysin and the NM2A receptor subunit NM2A R1 were used as controls for synaptic vesicle proteins and postsynaptic membrane proteins, respectively. Subcellular fractions are designated as follows: HOM, homogenate; P1, nuclear pellet; P2, crude synaptosomal pellet; P3, light membrane pellet; S1, cytosolic fraction; LP1, lysed synaptosomal membranes; LP2, crude synaptic vesicle fraction; LS2, cytosolic synaptosomal fraction; SPM, synaptic plasma membranes.

Proteins that are tightly attached to membranes can be removed by chaotropic agents or alkaline carbonate. We therefore washed the synaptic plasma membranes with carbonate buffer at pH 11. Now the majority of Munc13-1 was removed from the membranes, whereas intrinsic membrane proteins remained attached (Fig. 7C and data not shown). This result demonstrates that Munc13-1 is tightly bound to membranes. Treatment of membranes from transfected COS cells gave an identical result, suggesting that membrane binding of Munc13-1 is an intrinsic property of the protein and not dependent on nerve-terminal specific proteins (data not shown).

Munc13-1 Is a Component of Synaptic Plasma Membranes—Since the antibody against Munc13-1 did not give a signal in immunocytochemistry, we performed subcellular fractionations as a first step to localizing Munc13-1. Synaptosomes were isolated from brain homogenates (Fig. 8, P2) and fractionated into synaptic vesicles (LP2), synaptosomal cytosol (LS2), and synaptic plasma membranes (SPM). Most of the Munc13-1 in brain co-purified with synaptic markers in synaptosomes. Subfractionation of synaptosomes demonstrated that Munc13-1 was highly enriched in the synaptic plasma membranes. The synaptic vesicle fraction, although containing substantial quantities of synaptophysin, lacked Munc13-1 (Fig. 8). These findings suggest that Munc13-1 is a plasma membrane protein.

Discussion

The C. elegans unc-13 gene belongs to a subgroup of unc genes that encode proteins with putative functions in neurotransmitter release (13, 14). Worms with mutations in this subgroup are characterized by elevated levels of acetylcholine, resistance to the acetylcholinesterase inhibitor aldicarb, normal acetylcholine agonist responses, and wild-type levels of choline acetyltransferase and acetylcholinesterase. Several genes in this subgroup of unc mutants have been identified, including unc-17 as the vesicular acetylcholine transporter (18), unc-18 as a component of the synaptic vesicle docking-fusion complex (15–17), and synaptotagmin (19). However, the functions of many unc genes in this group of presynaptic mutants are unknown. Of these, unc-13 is remarkable because of its particularly severe phenotype (14). Cloning revealed that the unc-13 gene encodes a protein with C1- and C2-domains similar to protein kinase C, suggesting that it is involved in Ca2+- and diacylglycerol-signaling (20). However, little is known about the properties of this protein, its localization, its functions in the nerve terminal, and its general presence in organisms other than C. elegans. In order to address these questions, we have now examined the presence and properties of unc-13 homologues in mammalian brain.

cDNA clones encoding three rat brain proteins that are homologues to unc-13 were isolated and characterized. The high degree of sequence homology between the rat proteins and unc-13 suggests that the newly identified proteins are true mammalian homologues of the nematode protein, and therefore we named them Munc13–1, -13–2, and -13–3 (Fig. 1). RNA blotting experiments demonstrated that all Munc13s are expressed only in brain. Similar to other mammalian homologues of C. elegans unc genes with neuronal functions, unc-13 is highly conserved in evolution but expressed in multiple isoforms instead of the single form observed in C. elegans. Munc13s are large proteins with an interesting domain structure (Fig. 2B): an N-terminal region that is not conserved between the different family members and is followed by a domain doublet composed of adjacent C1- and C2-domains, a large middle segment that is highly conserved between different Munc13s but exhibits no homologies to other proteins in the current databases, and a C-terminal C2-domain that is also present in all family members. Biochemical experiments demonstrated that Munc13–1 is a peripheral membrane protein that is tightly bound to the plasma membrane and enriched in synaptic plasma membranes. Considering the homology between the different family members, it is likely that all Munc13s are plasma membrane proteins and have similar functions. Since unc-13 probably has an essential presynaptic function based on its phenotype, Munc13s probably also function in neurotransmitter release, a role that would fit very well with its localization to the presynaptic plasma membrane.

The presence of a C1-domain in Munc13s and the demonstrated binding of phorbol esters to the C1-domain of unc-13 (21, 22) suggest that these proteins are regulated by phorbol esters and diacylglycerol. Phorbol esters have multiple effects on synaptic transmission that have largely been attributed to their effects on protein kinase C. Interestingly, part of the effect of phorbol esters on synaptic transmission cannot be inhibited by protein kinase C inhibitors (23), suggesting that there may be additional phorbol ester receptors at a synapse. It is possible that some of these effects are mediated by Munc13s. In addition to C1-domains, the C2-domains of Munc13s are also likely to participate in intracellular signaling reactions. The sequence comparisons showed that they form a subgroup of C2-domains distinct from those of synaptotagmin and protein kinase C isoforms (Fig. 2). Binding measurements revealed that the middle C2-domains of Munc13s do not bind phospholipids as a function of Ca2+ (Fig. 3). However, this does not imply that the Munc13 C2-domains are not Ca2+-binding modules since recent experiments on C2-domains of synaptotagmins have discovered Ca2+-dependent activities that are independent of phospholipid binding (11).

The identification of mammalian homologues of unc-13 now allows a biochemical investigation into their functions in neurotransmitter release. Based on the similarity between the phenotypes of the unc-13 and unc-18 mutants in C. elegans, it has been proposed that unc-13 may be involved in synaptic

2 S. Sugita, Y. Hata, and T. C. Südhof, unpublished observation.
vesicle docking like unc-18 (39). Future experiments will have
to determine if Munc13s are components of the active zone that
function in docking and what its interacting partners are in
this function.

Acknowledgments—We thank Dr. Murayama for the C. elegans
unc-13 cDNA clone, Dr. R. Jahn for antibodies, Drs. J. Boulter and
J. Sullivan for the rat brain cDNA library, Dr. J. Felsenstein for the
PHYLIP program, Dr. M. Geppert for initial RNA blotting experiments,
and I. Leznicki, A. Roth, and E. Borowicz for excellent technical
assistance.

REFERENCES
1. Bennett, M. K., and Scheller, R. H. (1994) Annu. Rev. Biochem. 63, 63–100
2. Südhof, T. C. (1995) Nature 375, 645–653
3. Geppert, M., Goda, Y., Hammer, R. E., Li, C., Rosahl, T. W., Stevens, C. F., and
Südhof, T. C. (1994) Cell 79, 717–727
4. Perin, M. S., Fried, V. A., Mignery, G. A., Jahn, R., and Südhof, T. C. (1990)
Nature 345, 260–261
5. Brose, N., Petrenko, A. G., Sul, T. C., and Jahn, R. (1992) Science 256, 1021–1025
6. Bennett, M. K., Calakos, N., and Scheller, R. H. (1992) Science 257, 255–259
7. Yoshida, A., Oho, C., Omori, A., Kuwahara, R., Ito, T., and Takahashi, M.
(1992) J. Biol. Chem. 267, 24925–24928
8. Davletov, B. A., and Südhof, T. C. (1993) J. Biol. Chem. 268, 26386–26390
9. Hata, Y., Davletov, B., Petrenko, A. G., Jahn, R., and Südhof, T. C. (1993)
Neuron 10, 307–315
10. Zhang, J. Z., Davletov, B. A., Südhof, T. C., and Anderson, R. G. W. (1994)
Cell 78, 751–760
11. Li, C., Ullrich, B., Zhang, J. Z., Anderson, R. G. W., Brose, N., and Südhof, T.
C. (1995) Nature 375, 594–599
12. Brenner, S. (1974) Genetics 77, 71–94
13. Hosono, R., Sasaki, T., and Kuno, S. (1987) J. Neurochem. 49, 1820–1823
14. Hosono, R., and Kamiya, Y. (1981) Naunyn-Schmiedeberg’s Arch. Pharmacol.
319, 243–244
15. Hata, Y., Slaughter, C. A., and Südhof, T. C. (1993) Nature 366, 347–351
16. Pevsner, J., Hsu, S.-C., and Scheller, R. H. (1994) Proc. Natl. Acad. Sci.
U. S. A. 91, 1445–1449
17. Garcia, E. P., Gatti, E., Butler, M., Burton, J., and De Camilli, P. (1994) Proc.
Natl. Acad. Sci. U. S. A. 91, 2003–2007
18. Alfonso, A., Grundahl, K., Durst, J. S., Han, H.-P., and Rand, J. B. (1993)
Science 261, 617–619
19. Nonet, M. L., Grundahl, K., Meyers, B. J., and Rand, J. B. (1993) Cell 73,
1291–1305
20. Maruyama, I. N., and Brenner, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88,
5729–5733
21. Ahmed, S., Maruyama, I. N., Kozma, R., Lee, J., Brenner, S., and Lim, L.
(1992) Biochem. J. 287, 995–999
22. Kaizaneziet, M. G., Lewin, N. E., Bruns, J. D., and Blumberg, P. M. (1995)
J. Biol. Chem. 270, 10777–10783
23. O’Dell, T. J., Kendall, E. R., and Grant, S. G. N. (1991) Nature 353, 558–560
24. Sudhof, T. C. (1990) J. Biol. Chem. 265, 7849–7852
25. Devereux, J., Haeberli, P., and Smithies, O. (1984) Nucl. Acids Res. 12,
387–395
26. Luthy, R., Xenarios, I., and Bucher, P. (1994) Protein Sci. 3, 139–142
27. Henikoff, S., and Henikoff, J. G. (1992) Proc. Natl. Acad. Sci. U. S. A. 89,
10915–10919
28. Benson, D., Boguski, M., Lipman, D. J., and Ostell, J. (1994) Nucl. Acids Res.
22, 3441–3444
29. Saitou, N., and Nei, M. (1987) Mol. Biol. Evol. 4, 406–425
30. Guan, K. L., and Dixon, J. E. (1991) Anal. Biochem. 192, 262–267
31. Gorman, C. (1985) in DNA Cloning (D. M. Glover, ed) Vol. II, pp. 143–190, IRL
Press, Oxford, United Kingdom
32. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring
Harbor Laboratory, Cold Spring Harbor, NY
33. Laemmli, U. K. (1970) Nature 227, 680–685
34. Towbin, H., Staehelin, T., and Gordon, J. (1979) Proc. Natl. Acad. Sci. U. S. A.
76, 4350–4354
35. Brose, N., Gasco, G. P., Vetter, D. E., Sullivan, J. M., and Heinemann, S. F.
(1993) J. Biol. Chem. 268, 22663–22678
36. Johnston, P. A., Jahn, R., and Südhof, T. C. (1989) J. Biol. Chem. 264,
1268–1273
37. Sutton, B., Davletov, B. A., Berghuis, A. M., Sudhof, T. C., and Sprang, S. R.
(1995) Cell 80, 929–938
38. Ullrich, B., Li, C., Zhang, J. Z., McMahon, H., Anderson, R. G. W., Geppert, M.,
and Südhof, T. C. (1994) Neuron 13, 1281–1291
39. Jorgensen, E. M., and Nonet, M. L. (1995) Seminar. Dev. Biol. 6, 207–220
40. Hoffmann, K., and Bucher, P. (1995) FEBS Lett. 358, 153–157
Mammalian Homologues of *Caenorhabditis elegans unc-13* Gene Define Novel Family of C$_2$-domain Proteins

Nils Brose, Kay Hofmann, Yutaka Hata and Thomas C. Südhof

*J. Biol. Chem.* 1995, 270:25273-25280.
doi: 10.1074/jbc.270.42.25273

Access the most updated version of this article at [http://www.jbc.org/content/270/42/25273](http://www.jbc.org/content/270/42/25273)

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

[Click here](http://www.jbc.org/content/270/42/25273.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 39 references, 16 of which can be accessed free at [http://www.jbc.org/content/270/42/25273.full.html#ref-list-1](http://www.jbc.org/content/270/42/25273.full.html#ref-list-1)