Am J Physiol Cell Physiol 287: C468–C474, 2004.
First published April 21, 2004; 10.1152/ajpcell.00182.2003.

A stomatin and a degenerin interact in lipid rafts of the nervous system of *Caenorhabditis elegans*

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Submitted 6 May 2003; accepted in final form 5 April 2004

Sedensky, M. M., J. M. Siefker, J. Y. Koh, D. M. Miller III, and P. G. Morgan. A stomatin and a degenerin interact in lipid rafts of the nervous system of *Caenorhabditis elegans. Am J Physiol Cell Physiol* 287: C468–C474, 2004. First published April 21, 2004; 10.1152/ajpcell.00182.2003.—In *Caenorhabditis elegans*, the gene *unc-1* controls anesthetic sensitivity and normal locomotion. The protein UNC-1 is a close homolog of the mammalian protein stomatin and is expressed primarily in the nervous system. Genetic studies in *C. elegans* have shown that the UNC-1 protein interacts with a sodium channel subunit, UNC-8. In humans, absence of stomatin is associated with abnormal sodium and potassium levels in red blood cells. Stomatin also has been postulated to participate in the formation of lipid rafts, which are membrane microdomains associated with protein complexes, cholesterol, and sphingolipids. In this study, we isolated a low-density, detergent-resistant fraction from cell membranes of *C. elegans.* This fraction contains cholesterol, sphingolipids, and protein consistent with their identification as lipid rafts. We then probed Western blots of protein from the rafts and found that the UNC-1 protein is almost totally restricted to this fraction. The UNC-8 protein is also found in rafts and coimmunoprecipitates UNC-1. A second stomatin-like protein, UNC-24, also affects anesthetic sensitivity, is found in lipid rafts, and regulates UNC-1 distribution. Mutations in the *unc-24* gene alter the distribution of UNC-1 in lipid rafts. Each of these mutations alters anesthetic sensitivity in *C. elegans*. Because lipid rafts contain many of the putative targets of volatile anesthetics, they may represent a novel class of targets for volatile anesthetics.

genetics; anesthetic; membrane; trafficking

IN *CAENORHABDITIS ELEGANS*, the genes *unc-1* and *unc-8* each control locomotion and sensitivity to volatile anesthetics (31). Dominant and recessive alleles of each gene have been isolated; they have different specific effects on locomotion and on anesthetic sensitivity (25, 29, 37). Alleles of *unc-1* share identical phenotypes in air and in anesthetics with alleles of *unc-8*. Each gene also has complex interallelic interactions, indicating that they each function as homo-oligomers, presumably in a protein complex. The protein UNC-1 is a close homolog of the mammalian protein stomatin (30). UNC-8 is a homolog of the α-subunit of epithelial sodium channels (ENaC) (43). In nematodes, both UNC-1 and UNC-8 control locomotion and behavior in anesthetics, whereas in mammals, their homologs appear to be involved in mechanosensation and nociception (10, 11, 15, 17, 43). The nature of the interaction of these gene products is unknown in both *C. elegans* and in mammals.

Multiple lines of evidence suggest that *unc-1* and *unc-8* affect the same physiological function in controlling both locomotion and anesthetic sensitivity. Genetic data indicate that *unc-1* and *unc-8* interact with each other to control movement and behavior in anesthetics (31). Loss-of-function alleles of *unc-1* are able to suppress a neomorphic allele of *unc-8*, and dominant alleles of each interact to produce a novel phenotype. Antibodies to UNC-1 stain major nerve tracts (35); this finding confirmed a similar distribution seen with green fluorescent protein (GFP)-UNC-1 fusion constructs that rescued the null phenotype (31). Tavernarakis et al. (43) originally identified *unc*-8 as a degenerin in specific neurons of *C. elegans*. They localized UNC-8 to a small subset of ventral cord neurons and postulated that UNC-8 is part of a sodium channel that senses stretch/curvature during the sinusoidal movement of the nematode. By responding to this stretch, the sodium channel is thought to control the amplitude of the wave of muscle contractions that propagate along the body of the animal. Chalfie and colleagues (11, 15) showed that the stomatin-like protein (SLP) MEC-2 directly interacts with ENaC subunits in controlling the nematode's response to light touch. They provided corroborating electrophysiological data by expressing the proteins in frog oocytes (11). We have shown that UNC-24, another SLP in *C. elegans*, affects the function of UNC-1. Mutations in *unc-24*, which phenocopy *unc-1(0)* alleles, block the movement of UNC-1 from the perinuclear region to the peripheral cell membrane (35). Together, these data indicate that SLPs interact with each other as well as with ENaCs.

In mammals, stomatin and ENaCs are also found in a wide array of cell types, including those of the central nervous system (5, 10, 22, 34, 36). Work by Fricke et al. (10) localized both stomatin and ENaC subunits to the same cells in the dorsal root ganglion (DRG) of rats. Because direct mechanical stimulation is unlikely to involve the DRG, the stomatin-ENaC interaction may have a function other than directly transducing mechanical input to the nervous system. One possibility is the transduction of painful signals to the central nervous system. Consistent with a broader role in the nervous system, mammalian stomatin has been shown to interact with other proteins, including G protein-coupled receptors and soluble *N*-ethylmaleimide-sensitive factor attachment protein target receptor (SNARE) complex proteins (19, 23, 39). Finally, channels closely related to ENaCs, the acid-sensitive ion channels (ASICs), have been shown to be important in nociception (5, 17).

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Stomatin was originally identified in the membranes of mammalian red blood cells (RBCs); it is absent in RBC membranes from patients with the hemolytic anemia stomatocytosis (6, 40). RBCs from patients with stomatocytosis have abnormal gradients of sodium and potassium across their cell membranes. The original model of stomatin's structure postulated a long intracellular domain that was likened to a "ball and chain" anchored in the plasma membrane by the molecule's transmembrane segment (40). In this manner, stomatin was thought to regulate associated membrane channels. However, new data outlining the role of lipid rafts have modified theories concerning the function of stomatin in the cell membrane.

It is now well established that cellular membranes in mammals do not consist of uniformly distributed lipid and cholesterol components. Instead, microdomains exist within the cell membrane (14, 24). These domains are thought to serve as scaffolds to arrange protein complexes and partially regulate their function. The mammalian protein caveolin is a scaffolding protein in one type of membrane domain known as a cave. Stomatin and caveolin now have been shown to share structural similarities (14, 24, 42). Similarly to caveolin, stomatin has been associated with cell membrane microdomains; these domains are known as lipid rafts (21, 33, 38). Lipid rafts are detergent-resistant, low-density regions of the membrane that are thought to be important in sequestering protein complexes (14, 24). In addition, these membrane fractions are relatively rich in cholesterol and sphingolipids. Stomatin is postulated to play a role similar to that of caveolin, i.e., to regulate the formation and maintenance of membrane domains.

Originally isolated from mammalian endothelial cells in culture (14), lipid rafts have been identified in three invertebrate species: *Drosophila*, *Dictyostelium*, and *Strongylocentrotus* (2, 13, 32). The proposed functions for UNC-1 have led us to study whether lipid rafts exist in *C. elegans* and to determine whether UNC-1 or UNC-8 is present in lipid rafts. We hypothesized that *1*) lipid rafts exist in *C. elegans* and that UNC-1 and UNC-8 are sequestered to rafts; *2*) in the absence of UNC-24 or UNC-1, lipid rafts will not form; and *3*) if rafts form in the absence of UNC-24, the proteins UNC-1 and UNC-8 will not segregate normally to the rafts; because *4*) rafts have been shown to form in the endoplasmic reticulum, UNC-24 will be found in the raft fraction; and, finally, because *5*) ENaCs and stomatins genetically interact and are found in the same cells, UNC-1 and UNC-8 will physically interact.

We have demonstrated that lipid rafts, when isolated under conditions similar to those in other species, do exist in *C. elegans*. UNC-1, UNC-24, and UNC-8 are all found in lipid rafts. Null mutations in *unc-24* and *unc-8* affect the distribution of UNC-1 in rafts, whereas *unc-1(0)* eliminates UNC-8 from lipid rafts. However, none of these mutations eliminate the formation of a discernible raft fraction in *C. elegans*. The importance of lipid rafts as a potential target of the lipid-soluble volatile anesthetics is discussed.

MATERIALS AND METHODS

Nematodes. Nematodes were cultured as previously described (4, 26). The wild-type nematode is N2 in all experiments and was obtained from the Caenorhabditis Genetics Center, as was the null allele, *unc-24(eDf28)*. *unc-1(e580)* was obtained from Carl Johnson (Axys Pharmaceuticals, San Francisco, CA). *unc-1(fc53)* was isolated in our laboratory. *unc-8(e15lb145)* was kindly supplied by Monica Driscoll (Rutgers University). All experiments were performed at 20–22°C.

Antibodies. The cDNA for UNC-1 was isolated as described by Rajaram et al. (30). Monoclonal antibodies against the entire UNC-1 protein were prepared in the laboratory of Man Sun Sy as previously described (35). In the UNC-1 studies presented in this article, we used a single monoclonal anti-UNC-1 antibody; the precise epitope of this antibody is not known. The partial *unc-8* cDNA was supplied by Monica Driscoll. Polyclonal antibodies against the expressed partial UNC-8 protein were isolated from chickens and affinity purified by Pocono Rabbit Farm & Laboratory (Canadensis, PA). Rabbit polyclonal antibodies against UNC-24 were raised against the COOH terminus of the protein (amino acids 400–415) and affinity purified. Western blotting and antibody probing were done according to standard methods (7). Immunohistochemical techniques were followed as previously described (35).

Lipid rafts. Lipid rafts were isolated using a modification of the technique of Moffett et al. (24). Nematodes were grown in 500 ml of liquid culture, spun down in the cold, and separated from *Escherichia coli* and dead nematodes by centrifugation through a sucrose cushion (8, 46). Animals were sonicated on ice with 50 μl of protease inhibitor cocktail (leupeptin, pepstatin A, and 50 μl of PMSF inhibitor, 10 μg/ml each) in homogenization buffer (20 mM Tris, 20 mM HEPES, pH 7.4, 30 mM mannitol, and 10 mM $CaCl_2$) and centrifuged at 1,000 *g* to remove nuclei. The supernatant was removed and then spun at 60,000 *g* at 4°C for 30 min. The membrane pellets were suspended in 2 ml of ice-cold TNE (50 mM Tris-Cl, pH 8.0, 130 mM NaCl, and 5 mM EDTA) containing 1% Triton X-100. The resulting suspension was placed on either a 12-ml (for cholesterol and sphingolipids determination, see Fig. 1, *C* and *D*) or 8-ml (for Western blot analysis) sucrose step gradient and centrifuged at 100,000 *g* for 16–20 h at 4°C. Sucrose step gradients consisted of 2 ml of 5%, 4 ml of 35%, and 2 ml of 40% sucrose (8-ml gradient) or 2 ml of 5%, 6 ml of 35%, and 4 ml of 40% sucrose (12-ml gradient) containing Triton-X 100. The density of gradient fractions was determined by weighing known volumes on an analytical balance. Fractions (1 ml) of the gradient were extracted with methanol-chloroform (20, 24), run on 10–12% SDS-PAGE gels, and stained with Coomassie blue per standard techniques. Western blots were probed with UNC-1, UNC-8, or UNC-24 antibodies.

Phospholipids (lecithin, lysolecithin, and sphingomyelin) were analyzed spectrophotometrically using the phospholipids B reagent (Wako Chemicals, Richmond, VA). Phospholipids are first hydrolyzed by phospholipase D. Choline is released by choline oxidase, which causes the quantitative release of hydrogen peroxide. Hydrogen peroxide production is determined at 505 nm by the peroxidase-catalyzed production of quinone pigment from 4-aminoantipyrine and phenol. Cholesterol content was determined using a similar protocol from Synchron Systems (Beckman Coulter, Fullerton, CA). Free cholesterol is released by cholesterol esterase and oxidized by cholesterol oxidase. This reaction also releases hydrogen peroxide quantitatively, which is analyzed as described above for phospholipids (20).

Coimmunoprecipitation. Total protein was isolated from wild-type worms. Worms were homogenized in NET buffer (150 mM NaCl, 5 mM EDTA, and 50 mM Tris, pH 7.5) plus 1% Triton X-100, PMSF, and 250 mg of protease inhibitor. The resulting homogenate was spun in a clinical centrifuge. The resulting supernatant was exposed to antibodies to UNC-8 bound to Sepharose beads for 1–2 h. The beads were washed with sample buffer and boiled with 60 μl of SDS sample buffer to elute the bound protein. The resulting eluate was run on a 10% PAGE gel, transferred to a Western blot, and probed using established methods (7).

RESULTS

Lipid rafts. Adapting a protocol previously used to isolate lipid rafts in mammals (24), we detected a detergent-insoluble

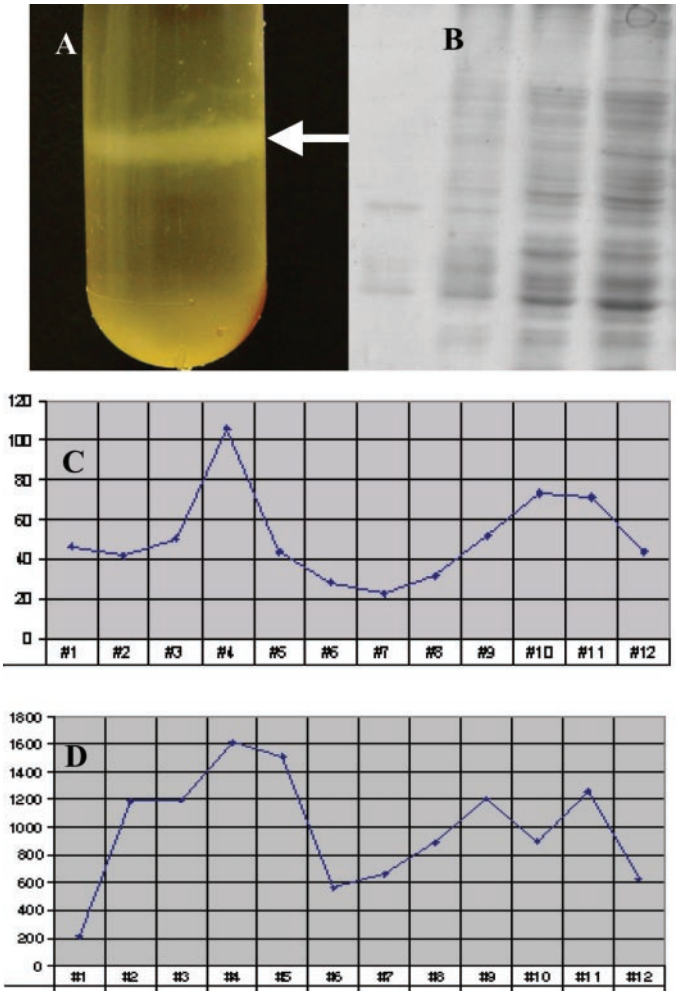

Fig. 1. *A*: representative lipid raft separation on a sucrose step gradient. The position of the rafts is noted as a white band in the middle-density region of the gradient (arrow). The precise position of the band varied between preparations but was always in the middle-density region. The region of highest concentration of cholesterol, sphingolipids, and protein in the middle-density region correlated to the position of the visible band. *B*: Coomassie blue-stained polyacrylamide gel showing the presence of protein associated with lipid rafts from *Caenorhabditis elegans*. An 8-ml sucrose step gradient was used to separate the detergent-insoluble rafts, as described in MATERIALS AND METHODS. In this gel and in the following Western blots (Figs. 1–4), each lane represents a 1-ml fraction isolated from the middle-density portion of the gradients. Only the middle-density fractions (~1.08–1.12 mg/ml) are shown. Protein was also found in the high-density fraction (not shown). *C* and *D*: distribution of cholesterol (*C*) and sphingolipids (*D*) in fractions from the sucrose gradient used to isolate lipid rafts. Samples were isolated by their insolubility in cold Triton X-100. These measurements were done on 1-ml fractions from 12-ml sucrose gradients to maximize separation of the peaks. The position of the cholesterol and sphingolipid peaks coincided with the position of the white band on the sucrose gradient. In both cases, second peaks are also found in the higher density fractions.

fraction in membrane extracts of *C. elegans*. This was usually seen as a conspicuous white ring floating in the middle-density portion (1.08–1.12 g/ml) of the sucrose step gradient (Fig. 1*A*), generally *fractions 3–8* of a 12-ml gradient or *fractions 3–6* of an 8-ml gradient (in Fig. 1, each lane represents a 1-ml fraction). Early studies were performed with 12-ml gradients and later ones with 8-ml gradients (see MATERIALS AND METHODS). Although the absolute fractions in which the raft was most conspicuous were not identical in all preparations, the raft was always within the middle step of the gradient; for each preparation, its position was noted relative to subsequent gradient fractionation. The middle-density fractions contained protein (Fig. 1*B*) as well as cholesterol and sphingolipid (Fig. 1, *C* and *D*). These characteristics are the same as those that define lipid rafts in mammals (14, 24). The density of the middle fractions progressed from 1.08 to 1.12 from the top to the bottom of this step; densities of 1.08–1.12 g/ml are reported for rafts isolated from mammals (14). Second peaks of cholesterol and sphingolipids, as well as protein, were also found in the higher density fractions.

UNC-1. Probing a Western blot of membrane fractions with anti-UNC-1 indicated that UNC-1 localized to those fractions of the membrane that contained lipid rafts (Fig. 2*A*). In rafts, UNC-1 segregates at ~26 kDa, indicating that it is processed from its predicted size of 32 kDa; the larger form is readily detected in whole worm preparations of N2 (35). Essentially no UNC-1 was seen in the membrane pellet fraction in the N2 animal. We subsequently used UNC-1, when present, as a marker for rafts (see below). Rafts do exist in *unc-1(0)* animals, however. A pronounced white band was seen in the middle-density region of the sucrose gradients. Protein, cholesterol, and sphingolipids were present in the same fractions from *unc-1(e580)* or *unc-1(fc53)* animals as in wild-type animals (data not shown). As expected, no UNC-1 could be detected in the rafts of *unc-1(0)* animals (Fig. 2*B*).

UNC-8. The UNC-8 protein was detected in lipid rafts (Fig. 3*A*) in wild-type animals. However, UNC-8, unlike UNC-1, also were localized to the higher density membrane pellet (not shown). In *unc-8(0)* animals, no UNC-8 was detectable in the fractions that contained rafts (data not shown), though rafts were macroscopically visible on sucrose gradients and were localized by the presence of protein, cholesterol, and lipid in the middle-density, detergent-resistant fractions (data not shown). In addition, UNC-8 protein was not detected in lipid raft preparations from *unc-1(0)* animals (Fig. 3*B*). The distribution of UNC-1 in a sucrose gradient was the same in both N2 and *unc-8(0)* animals. However, the predominant form of UNC-1 protein was ~32 kDa in *unc-8(0)* (compared with 26 kDa in N2, Fig. 3*C*); in addition, a band of ~30 kDa also

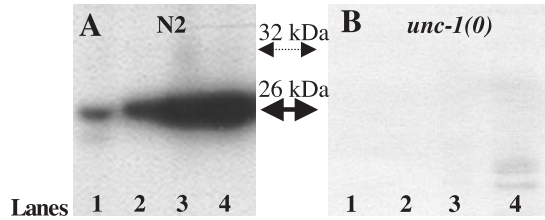

Fig. 2. *A*: Western blot of a sucrose gradient containing membrane fractions from *C. elegans* N2 probed with anti-UNC-1. Only the middle-density fractions (~1.08–1.12 mg/ml) that contain the lipid raft band are shown. Four milliliters were isolated from the middle-density region; each lane corresponds to a 1-ml fraction. The UNC-1 protein is distributed almost exclusively in those fractions containing lipid rafts. UNC-1 is found at 26 kDa in the raft fraction (large arrow), compared with its predicted size of 32 kDa (small arrow). In total protein preparations, UNC-1 appears as two bands, one at 32 kDa and one at 26 kDa (29). An example of staining of total protein by anti-UNC-1 is shown in Fig. 3*C*. *B*: Western blot as in *A*, from the null *unc-1(0)* allele, *fc53*, showing no UNC-1 protein in the middle-density lanes (*lanes 1–4*). In each of these preparations, the raft protein was identified in the middle-density lanes. Coomassie blue staining showed equivalent amounts of protein in the gels from *A* and *B*.

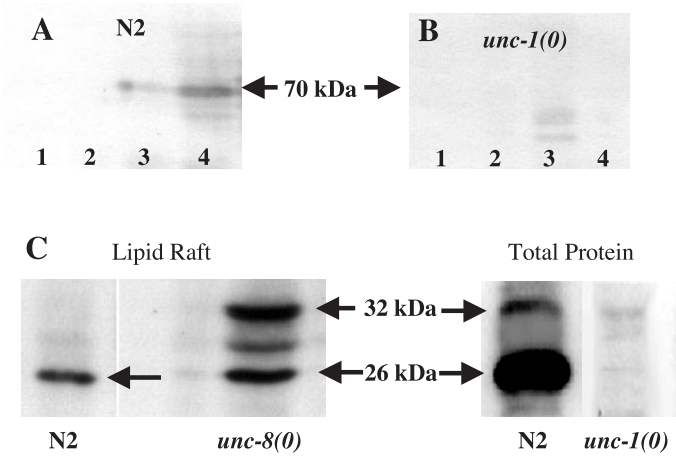

Fig. 3. *A*: Western blot of a sucrose gradient containing membrane fractions from *C. elegans* N2 probed with anti-UNC-8. Only the middle-density fractions (~1.08–1.12 mg/ml) that contain the lipid raft band are shown. The UNC-8 band is found at ~70 kDa (arrow) and appears as a doublet in N2. No band was seen in raft fractions isolated from *unc-8(0)* animals (not shown). *B*: Western blot as in *A*, from the null allele of *unc-1(0)* probed with anti-UNC-8. No UNC-8 protein can be seen in the middle-density lanes (arrow represents 70-kDa position). *C*: Western blot as in *A*, comparing the size of UNC-1 in lipid raft preparations from N2 and *unc-8(0)* (*left*). Only the middle-density lanes from a sucrose gradient are shown. UNC-1 is found as a 26-kDa band (arrow) in N2 but is found primarily as two bands (32 and 26 kDa) in *unc-8*. For comparison, a Western blot of total protein from N2 and the *unc-1(0)* allele, *fc53*, is shown at *right*.

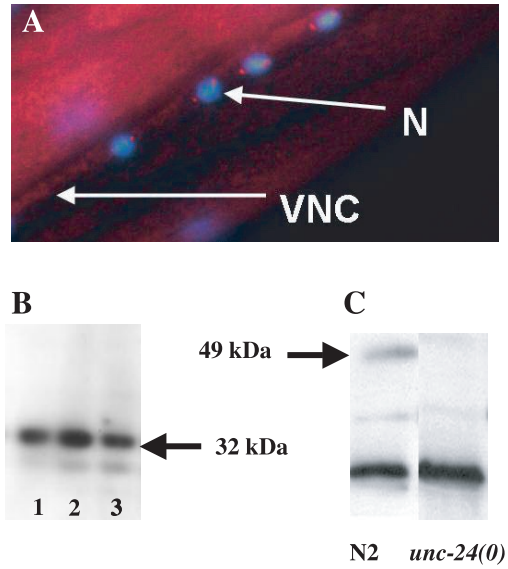

Fig. 4. *A*: staining with anti-UNC-1 of a *unc-24* animal along the ventral nerve cord (VNC). Note the nuclei (N) of the VNC (blue) stained with 4-6-diamidino-2-phenylindole. In N2, UNC-1 is distributed along the axon, but in *unc-24* animals, it is restricted to the perinuclear region, as has been shown previously. *B*: Western blot of a sucrose gradient containing membrane fractions from *unc-24(0)* animals probed with anti-UNC-1. Only the middle-density fractions (~1.08–1.12 mg/ml) that contain the lipid raft band are shown. Note that rafts exist despite the lack of movement of UNC-1 from the nuclei shown in *A*. In addition, the UNC-1 protein is seen in the raft isolate, indicating that UNC-24 is not necessary for sequestration of UNC-1 into rafts even when UNC-1 is restricted to the perinuclear region. However, most of the UNC-1 is present as the higher molecular weight form, 32 kDa (arrow). *C*: Western blot of a sucrose gradient containing membrane fractions from *C. elegans* N2 and *unc-24(0)* allele, *eDf28*, the null mutant, probed with anti-UNC-24. Only the middle-density fractions (~1.08–1.12 mg/ml) that contain the lipid raft band are shown. The UNC-24 band is found at ~49 kDa (arrow). No band at that size was seen in raft fractions isolated from *unc-24(0)* animals (not shown). The UNC-24 antibody consistently identified a band at ~20 kDa (lower band) in both N2 and *unc-24* mutants. We interpret this as cross-reactivity and include it to show that protein levels were comparable between N2 and *unc-24(0)*.

appeared in the *unc-8(0)* background. The distribution and size of UNC-1 in rafts from the dominant mutant, *unc-8(n491)*, were not different from those of N2 (not shown).

UNC-24. In *unc-24(0)* animals, UNC-1 is not distributed to the cell membrane but remains in the perinuclear region, presumably in the endoplasmic reticulum (Fig. 4*A* and Ref. 35). In lipid raft preparations of *unc-24(0)* animals, UNC-1 is primarily localized to the same fractions that normally contain rafts. However, the distribution of UNC-1 was much broader; some UNC-1 was noted in the high-density membrane pellet (not shown). The 32-kDa form of UNC-1 is the primary form seen in rafts of *unc-24(0)* animals (Fig. 4*B*). Earlier results with total protein from *unc-24(0)* indicated that the 32-kDa form is the predominant form of the protein in this mutant (35). The 49-kDa UNC-24 protein does segregate to the raft fraction, but in small quantities compared with UNC-1 (Fig. 4*C*). No change in the distribution of UNC-8 was seen between N2 and *unc-24(0)* animals (data not shown).

Coimmunoprecipitation. When total protein from N2 was incubated with beads bound with anti-UNC-8 antibody and then eluted from the beads, UNC-1 coimmunoprecipitated with UNC-8 (Fig. 5). In addition, about five other unidentified proteins were coimmunoprecipitated with UNC-8 (not shown). These results indicate that UNC-1 and UNC-8 physically interact with each other and with additional, unidentified proteins.

DISCUSSION

Our results indicate that lipid rafts exist in *C. elegans* with physical characteristics similar to those seen in other organisms. These rafts are of a similar density to those found in vertebrates and are enriched in both cholesterol and sphingolipids (14, 24). However, cholesterol and sphingolipids are also found in the high-density fractions of the sucrose gradients. Nusrat et al. (28) also found that sphingolipids were enriched in the high-density fractions of the detergent-insoluble glycolipid. However, they did not find a second peak for cholesterol.

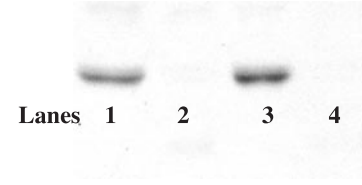

Fig. 5. Coimmunoprecipitation of UNC-1 with UNC-8. Total protein from wild-type worms was exposed to beads with anti-UNC-8 attached. The beads were washed as described in MATERIALS AND METHODS, and the attached protein was eluted. The eluted protein was separated on a polyacrylamide gel and transferred to a Western blot. The resulting Western blot was probed with anti-UNC-1. The eluted fraction contained UNC-8 (not shown) and UNC-1 with a size of 26 kDa. *Lanes 1* and *3* are identical lanes representing anti-UNC-8 attached to beads and then exposed to protein from N2. *Lane 2* represents protein isolated when preimmune serum was used for the coimmunoprecipitation. *Lane 4* represents the eluted protein when the anti-UNC-8 antibody was exposed to protein from the *unc-8* null allele, *e15lb145*. Thus, in the absence of UNC-8 protein, no UNC-1 was immunoprecipitated.

It is not clear whether these fractions identify rafts with different physical properties or whether significant amounts of cholesterol are present in other cellular locations. It is possible that nematodes differ from mammals in their cellular distribution of cholesterol.

It is unknown whether stomatins or similar proteins are necessary for raft formation or maintenance. In the wild-type strain N2, essentially all UNC-1 is associated with rafts. However, UNC-1 expression is not required for all raft formation, since rafts are also present in *unc-1(0)* alleles. Though UNC-1 appears to be essentially a pan-neuronal stomatin and is therefore widely expressed, a total of 10 SLPs are potentially encoded by the *C. elegans* genome. It is therefore likely that other SLPs are also associated with rafts in *C. elegans* and may serve to support raft formation.

It is important to note that the UNC-1 protein is found in the raft isolates even when it is sequestered to the perinuclear region. UNC-24, which is primarily restricted to the perinuclear region (Koh JY, Meir J, and Miller DM, unpublished data), is also found in rafts. These findings indicate that we have identified both mature rafts in the plasma membrane and probable immature rafts in the endoplasmic reticulum. At present, we cannot separate these populations.

Our previous immunohistochemical studies of UNC-1 in *unc-24* mutants indicate that UNC-1 may move from the perinuclear region of neurons to axonal membranes (35). Data presented here show that UNC-1 associates with lipid rafts in both wild-type and *unc-24* animals, although more of the 32-kDa form is seen in *unc-24* animals. In total protein preparations from *unc-24(0)* animals, the 32-kDa form is virtually the only identifiable species of the UNC-1 protein (35). In addition, in isolates from *unc-24* animals, some UNC-1 is found in the higher density fractions of the step gradient (not shown). We interpret these data to indicate that lipid rafts are formed in the endoplasmic reticulum and contain an incompletely processed UNC-1, which is seen as the predominant 32-kDa form of UNC-1 in raft fractions. UNC-24 is necessary for moving UNC-1 as part of a raft to the cell membrane, and UNC-24 has both a stomatin domain and a lipid transfer domain (1). We postulate that UNC-24 binds both lipids and stomatins and facilitates the processing of UNC-1 into its usual 26-kDa form in lipid rafts in neuronal membranes. Lack of functional UNC-24 also results in UNC-1 accumulation in a higher density membrane fraction than a raft, which may represent immature rafts located in the endoplasmic reticulum.

The protein UNC-8 is also associated with the lipid raft fraction on sucrose gradients. Interestingly, previous studies have noted that ENaCs are not associated with rafts in mammalian kidney cells (12). However, since UNC-1 strongly associates with rafts and coimmunoprecipitates UNC-8, it is not surprising that UNC-8 also associates with rafts in *C. elegans*. Its presence in rafts is dependent on the presence of UNC-1, based on its absence from rafts in the *unc-1(0)* animal. We have previously reported genetic data to link the functions of UNC-1 and UNC-8 (31). Coupled with the coimmunoprecipitation results and their colocalization to lipid rafts in nematodes, as well as the association of their homologs in other systems, these proteins appear to physically interact in vivo.

In *unc-8(0)*, the size of the predominant UNC-1 in lipid rafts is increased; it may be that binding to UNC-8 facilitates final processing of the UNC-1 protein in a raft. As would be expected, both the 32- and 26-kDa forms of the UNC-1 protein are seen on Western blots of total worm protein in wild-type animals (35). However, it appears that the predominant form of UNC-1 found in the mature raft, as well as in total protein, is the 26-kDa form.

As noted in the Introduction, stomatin was originally identified as a protein missing from the membranes of RBCs in patients with a hereditary hemolytic anemia. These changes were presumed to be the result of mutations in the stomatin gene that resulted in loss of the protein. However, no mutations were found in stomatin in patients with stomatocytosis (6). In addition, a mouse knockout of this gene does not have hemolytic anemia (6, 47). This has led to reconsideration of what might be the precise role of stomatin.

Chalfie and colleagues (11, 15) elucidated the physiological relationship of the SLP MEC-2 with two ENaCs, MEC-4 and MEC-10. Mutations in these proteins disrupt the nematode's response to light touch; thus they are postulated to be necessary for the transduction of a mechanosensory signal in *C. elegans*. Chalfie et al. (15) further showed that the presence of MEC-2 is necessary for maximal sodium current conduction by MEC-4 homomeric channels in *Xenopus* oocytes. It remains to be seen whether all ENaCs in *C. elegans* associate with rafts or with SLPs. However, our results, coupled with those showing that MEC-2 interacts with MEC-4, indicate that this association is likely of a general nature. It remains unclear whether stomatin plays a primary role in raft homeostasis or functions primarily as an interacting partner with other raft proteins.

We previously showed (25, 31) that mutations in *unc-1*, *unc-8*, and *unc-24* each alter sensitivity to volatile anesthetics. In addition, ENaCs are now known to be crucial to neuronal function in the response to pain (3, 9). One class of the ENaC family is the ASIC. ASICs are proton-gated channels found in both the peripheral and central nervous systems in mammals. These channels have been shown to be responsible for the transduction of pain and mechanosensation. These data, coupled with the colocalization of ENaCs and stomatins in the DRG of mammals (10), demonstrate the ubiquitous nature of the association of these two families of proteins across very different phyla and may have important implications for understanding neuronal functions crucial to processing of sensory information.

For the past two decades, considerable debate has centered on the molecular sites of action of volatile anesthetics and how these agents cause loss of consciousness and alleviate pain (18). These unique compounds are very lipophilic; their potency is a direct function of their oil/gas partition coefficient (18, 27). However, they also can affect protein function in a lipid-free environment (27). As a result, both proteins and lipids have been postulated to be primary target of these unique agents. The list of proteins associated with lipid rafts includes ligand-gated channels, G protein-coupled receptors, and members of the SNARE complex (24, 41, 44). Each of these has been postulated to be a target of volatile anesthetics (16, 18, 45). Because rafts form a complex containing both lipids and proteins, it is possible that volatile anesthetics can perturb specific protein complexes embedded in this unique lipid milieu. These data may eventually serve as a unifying model for understanding how volatile anesthetics work at the molecular level.

In summary, we found that rafts do exist in nematodes and that UNC-1, UNC-8, and UNC-24 are present in lipid rafts in *C. elegans*. Loss of UNC-1 alters the distribution of UNC-8 into rafts, but does not alter the formation of rafts. Mutations in *unc-24* and *unc-8* alter the processing of UNC-1 found in lipid rafts. Mutations in *unc-24* do not alter the distribution of UNC-8 into lipid rafts. Because these proteins are known to play a role in anesthetic response, we propose that rafts are possible targets for volatile anesthetics.

ACKNOWLEDGMENTS

We thank Monica Driscoll for sharing nematode strains and cDNAs. We also thank Judy Preston, Qiao-yun Jiang, and Melinda Hubbard for excellent technical assistance and Helen Salz and Pat Hunt for thoughtful input.

GRANTS

This work was supported in part by National Institutes of Health (NIH) Grant GM-45402 (to P. G. Morgan and M. M. Sedensky). J. Y. Koh was supported by NIH Grants NS-26115 and DK-58212 (to D. M. Miller III) and by NIH Institutional Research Training Grants T32 NS-07491 and T32 MH-65215.

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