UNC-31 (CAPS) Is Required for Dense-Core Vesicle But Not Synaptic Vesicle Exocytosis in Caenorhabditis elegans

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Previous studies indicated that CAPS (calcium-dependent activator protein for secretion) functions as an essential component for the Ca\(^{2+}\)-dependent exocytosis of dense-core vesicles in neuroendocrine cells. However, recent mouse knock-out studies suggested an alternative role in the vesicular uptake or storage of catecholamines. To genetically assess the functional role of CAPS, we characterized the sole Caenorhabditis elegans CAPS ortholog UNC-31 (uncoordinated family member) and determined its role in dense-core vesicle-mediated peptide secretion and in synaptic vesicle recycling. Novel assays for dense-core vesicle exocytosis were developed by expressing a prepro-atrial natriuretic factor–green fluorescent protein fusion protein in C. elegans. unc-31 mutants exhibited reduced peptide release in vivo and lacked evoked peptide release in cultured neurons. In contrast, cultured neurons from unc-31 mutants exhibited normal stimulated synaptic vesicle recycling measured by FM4-64 [N-(3-triethylammoniumpropyl)-4-(6-(4-diethylamino)phenyl)hexatrienyl]pyridinium dibromide] dye uptake. Conversely, UNC-13, which exhibits sequence homology to CAPS/UNC-31, was found to be essential for synaptic vesicle but not dense-core vesicle exocytosis. These findings indicate that CAPS/UNC-31 function is not restricted to catecholaminergic vesicles but is generally required for and specific to dense-core vesicle exocytosis. Our results suggest that CAPS/UNC-31 and UNC-13 serve parallel and dedicated roles in dense-core vesicle and synaptic vesicle exocytosis, respectively, in the C. elegans nervous system.

Key words: CAPS; UNC-13; dense-core vesicle; exocytosis; synaptic vesicle; C. elegans

Introduction

Neurotransmitters are secreted from neurons by two types of vesicles. Small clear synaptic vesicles contain classical transmitters such as glutamate, GABA, and acetylcholine that activate postsynaptic ionotropic receptors and mediate fast synaptic transmission. Dense-core vesicles contain neuropeptides and monoamines that bind and activate G-protein-coupled receptors and modulate presynaptic or postsynaptic function. There are many similarities between synaptic vesicle and dense-core vesicle exocytosis. Both classes of vesicles require Ca\(^{2+}\) to trigger exocytosis, and several proteins [e.g., SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptor), Munc-18, and synaptotagmin] are common required constituents for the fusion of either vesicle type (Rettig and Neher, 2002; Jahn et al., 2003; Sorensen, 2004). Despite similarities in the fusion machinery for both vesicle types, there are differences in the kinetics of exocytosis and in the physiological regulation of release (Rettig and Neher, 2002; Martin, 2003). These differences suggest that there are proteins and mechanisms that are distinct for synaptic vesicle and dense-core vesicle exocytosis.

CAPS1 (calcium-dependent activator protein for secretion) was isolated from brain cytosol as a factor that restored Ca\(^{2+}\)-triggered dense-core vesicle exocytosis in permeable neuroendocrine cells (Martin and Walent, 1989; Walent et al., 1992; Ann et al., 1997; Grishanin et al., 2004). CAPS1 is present on dense-core crine cells (Martin and Walent, 1989; Walent et al., 1992; Ann et al., 1997; Grishanin et al., 2004). CAPS1 is present on dense-core but not synaptic vesicles in brain homogenates (Berwin et al., 1997; Grishanin et al., 2004). Despite similarities in the fusion machinery for both vesicle types, there are differences in the kinetics of exocytosis and in the physiological regulation of release (Rettig and Neher, 2002; Martin, 2003). These differences suggest that there are proteins and mechanisms that are distinct for synaptic vesicle and dense-core vesicle exocytosis.

A study of a CAPS1\(^{+/−}\)/CAPS2\(^{+/+}\) mouse concluded that CAPS1 is instead essential for the uptake or storage of catecholamines in dense-core vesicles (Speidel et al., 2005). However, a role for CAPS1 in vesicle exocytosis could not be directly assessed because of redundancy with CAPS2. In contrast, a study of a CAPS1\(^{+/−}\)/CAPS2\(^{−/−}\) mouse found strong deficits in dense-core vesicle-mediated neurotrophin secretion in the cerebellum.
worm (Livingstone, 1991; Ann et al., 1997; Charlie et al., 2006), we analyzed CAPS/UNC-31 function in C. elegans. We developed a novel in situ assay for peptide secretion and novel cultured neuron assays for peptide secretion and synaptic vesicle recycling. With these, we directly assessed whether CAPS is required for regulated peptide secretion and whether CAPS functions selectively for dense-core vesicle exocytosis or also for synaptic vesicle exocytosis. We found that CAPS is expressed throughout the nervous system, that unc-31 mutants developed normally, and that CAPS/UNC-31 was required for the regulated release of dense-core vesicle peptide contents but not for evoked synaptic vesicle recycling. Conversely, UNC-13, which exhibits sequence homology to CAPS, was required for synaptic vesicle cycling but not for dense-core vesicle exocytosis.

Materials and Methods

Strains. The following mutant strains were used: EG3404 unc-31(e928), EG3405 unc-31(u280), and BC168 unc-13(e69). The green fluorescent protein (GFP)-expressing strains used in this study were as follows: EG3344 oxIs180 [Pex-3::ANF::GFP], EG3346 unc-31(e928) oxIs207 [Pex-3::ANF::GFP], EG3680 oxIs206 [Pex-3::ANF::GFP], EG3681 unc-31(e928) oxIs206 [Pex-3::ANF::GFP], EG3682 unc-31(u280) oxIs206 [Pex-3::ANF::GFP], EG3345 unc-13(e69) oxIs206 [Pex-3::ANF::GFP], EG3683 unc-13(e51) oxIs206 [Pex-3::ANF::GFP], EG3410 oxEx608 [Punc-31::GFP], EG3407 unc-31(e928) oxEx605 [Punc-31::GFP], EG1285 oxIs12 [Punc-47::GFP], EG1846 unc-31(u280); oxIs12 [Punc-47::GFP], MTR827 lin-15(n765) nIs52 [Punc-25:SNB-1::GFP; lin-15(+)I], EG1845 unc-31(u280) nIs52 [Punc-25:SNB-1::GFP; lin-15(+)I], and EG2623 unc-31(e928); lin-15(n765) osEx93 [Pacr-5::SNB::GFP; lin-15(+)I]. Additional strains used include the following: EG2844 unc-31(e928) oxIs206 [Punc-31::GFP], EG1846 unc-31(u280); oxIs12 [Punc-47::GFP], MTR827 lin-15(n765) nIs52 [Punc-25:SNB-1::GFP; lin-15(+)I], and EG2623 unc-31(e928); lin-15(n765) osEx93 [Pacr-5::SNB::GFP; lin-15(+)I]. Additional strains used include the following: EG2844 unc-31(e928) oxIs206 [Punc-31::GFP], EG1846 unc-31(u280); oxIs12 [Punc-47::GFP], MTR827 lin-15(n765) nIs52 [Punc-25:SNB-1::GFP; lin-15(+)I], and EG2623 unc-31(e928); lin-15(n765) osEx93 [Pacr-5::SNB::GFP; lin-15(+)I].

Molecular biology. The Punc-31 GFP transcriptional and UNC-31::GFP translational constructs (Fig. 1E) were generated from a common vector called pSS1.5. To construct pSS1.5, a 15.5 kb Ael–Xhol fragment containing the entire unc-31 intron/exon structure and ~2 kb of upstream sequence was cut from cosmid ZK897 and ligated into the EcoRV–Xhol sites of pLHitus28 (New England Biolabs, Beverly, MA). The rescuing Punc-31::UNC-31::GFP translational construct (pSS1.6) was generated by cutting an Xhol fragment containing the open reading frame for GFP without a stop codon, and flanked by splice acceptor and donor sequences, from the vector pPD14.24 (provided by A. Fire, Stanford University, Palo Alto, CA). The Xhol fragment was ligated into a unique Nhel site in pSS1.5, which lies in the first intron of unc-31. The Punc-31::GFP translational construct (pSS1.7) was constructed by PCR amplification of the GFP open reading frame preceded by a nuclear localization sequence and ending with a stop codon, followed by the unc-54 3’ untranslated region from the vector pPD95–67. Oligonucleotides for the PCR added a 3’
Table 1. unc-31 alleles

| Allele | Molecular nature | Protein |
|--------|-----------------|---------|
| unc-31(e299) | I (530) → X | Yes |
| unc-31(e169) | Disrupts 5’ splice donor of intron 5 | Yes |
| unc-31(e71) | Q (680) → STOP | No |
| unc-31(e69) | Q (680) → STOP | No |
| unc-31(e68) | R (1,260) → STOP | No |
| unc-31(e64) | Unknown breakpoints | ND |
| unc-31(e65) | Q (330) → STOP | No |
| unc-31(e67) | W (503) → STOP | No |
| unc-31(e74) | G (538) → E | Yes |
| unc-31(e104) | Disrupts 3’ splice acceptor of intron 10 | No |
| unc-31(e22) | Disrupts 3’ splice acceptor of intron 10 | No |
| unc-31(e77) | Disrupts 5’ splice donor of intron 5 | Yes |
| unc-31(e34) | Disrupts 5’ splice donor of intron 5 | Yes |
| unc-31(e69) | Disrupts 5’ splice donor of intron 9 | No |
| unc-31(e49) | Disrupts 5’ splice donor of intron 9 | No |
| unc-31(e7) | W (754) → STOP | No |
| unc-31(e68) | Q (821) → STOP | No |

ND, Not determined.

splice acceptor at the 5’ end of the product and XbaI cleavage sites at both the 5’ and 3’ ends. The PCR product was digested with XbaI and ligated into the unique Nhel site of pSS1.5. Primers used in the PCR were as follows: forward, GTCTCTAGATTTTCAGATGACTGCTCCAAA-GAAGAAGCG; and reverse, GTCTCTAGAATCTACAAAAAGCTTAT-GGTTGGTATATTGGGAATGTATTC. The Paex-3:ANF::GFP construct (generously provided by E. Levitan, University of Utah, Salt Lake City, UT), lin-15(+)(+) (20 ng/µl), and 1 kb DNA ladder (64 ng/µl) into the wild-type N2 strain. Unc-31::GFP is expressed in the pharynx and is used as a coinjection marker. Three independent stable extrachromosomal lines were recovered: oxEx607, oxEx608, and oxEx609. Note that GFP is inserted in-frame after exon 1, but that the construct contains the entire intron/exon structure of unc-31 downstream of the GFP stop codon.

The following DNA fragments were coinjected into unc-31(e928) animals to generate the unc-31(e928) translational line: Pmyo-2::HIS::GFP (1 ng/µl), Pmyo-2::HIS::GFP (1 ng/µl) from Susan Mango (University of Utah, Salt Lake City, UT), lin-15(+)(+) (20 ng/µl), and 1 kb DNA ladder (64 ng/µl) into the wild-type N2 strain. Pmyo-2::HIS::GFP is expressed in the pharynx and is used as a coinjection marker. Three independent stable extrachromosomal lines were recovered: oxEx604, oxEx605, and oxEx606. ANF::GFP lines were generated by injecting lin-15(+)(+) (pblH98) and Paex-3::ANF::GFP-4 into lin-15(+)(±) (pblH98) animals. The extrachromosomal array containing Paex-3::ANF::GFP-4 in strain KYS212 was integrated into the genome by gamma irradiation and outcrossed four times to generate the allele tgl65 [Paex-3::ANF::GFP::Punc-31::GFP].

unc-31 rescue. Rescue experiments were performed using animals carrying an extrachromosomal array coding for UNC-31::GFP [unc-31(e928); oxEx604]. Note that the rescue experiment is not a single gene rescue: there is a U5–10 snRNA in intron 11 and U5–11 snRNA in intron 18 of the unc-31 locus, which are included in the rescue construct. However, it is not likely that these genes are disrupted in the e928 allele because the deletion is 3’ to the U5–10 snRNA and the U5–11 snRNA locus is duplicated and still present in the genome according to our PCR analysis of the e928 allele. To ensure that we are rescuing defects in unc-31 in the e928 allele, we were able to show that unc-31(u280), which does not disrupt either snRNA, has similar phenotypes. In particular, the defection defects were quantitatively similar (Fig. 2A, B). Additionally, the movement defects of the two alleles were also similar.

Acute expression of unc-31 from a heat shock promoter is able to rescue the anterior body muscle contraction (aboc) defect in unc-31(e928). Animals were heat shocked at 33°C for 1 h and allowed to recover at room temperature for 6 h. Six cycles were observed for each animal scored to determine the percentage of cycles that had an aboc event. Heat shock treatment partially rescued the uncoordinated defect of unc-31(e928) animals in the absence of oxEx59, making it difficult to assay locomotion rescue by the transgene.

Movement and defection cycle analysis. Video tracking was used to
The number of axons crossing the midline was determined by manually counting the number of axons crossing to and making contact with the DNC in confocal Z-series images obtained for strains EG1285 lin-15(n763ts); ods12 and EG1846 unc-31(u280); ods12. Cell body counts of GABA motor neurons were obtained by manually counting under wide-field fluorescence. ANF–GFP endocytosis into coelomocytes. Confocal settings used for image capture were held constant from animal to animal and across genotypes. To control for changes in laser power, we monitored the intensity of InspecK calibration microspheres (Invitrogen) before and after each imaging session. In addition, animals with different genotypes were interlaced within each imaging session. To maintain complete objectivity in sampling of coelomocytes, the cells were identified, and the Z-series was established while imaging the cells using bright-field illumination and differential interference contrast (DIC) optics. To control for light scattering issues that could affect the GFP signal collected, only posterior coelomocytes that were not behind other tissues (gut and gonads) and were easily identifiable via DIC were imaged. Collected images were analyzed using the three-dimensional quantitation software Volocity (Improvision, Lexington, MA). Briefly, the total sum of pixel intensities arising from ANF–GFP signal in each coelomocyte was quantitated in three dimensions. This number more closely represents the total amount of ANF–GFP in a particular coelomocyte than the average pixel intensity from a two-dimensional maximum pixel intensity projection. Averages of the total sum of pixel intensities per coelomocyte were used as a basis of comparing wild-type, unc-31, and unc-13 mutants.

Preparation of C. elegans dense-core vesicles and synaptic vesicles. Fluorescent dense-core vesicles and synaptic vesicles were isolated from homogenates of the tgl5[Peak-3:ANF-GFP] or js219[synaptogyrin:GFP] strains, respectively. Homogenates were prepared from worms on 20 10-cm nematode growth medium (NGM) plates seeded with E. coli strain NA22 (CCG) and grown to slight starvation. Sucrose-washed worms were resuspended in either cold 0.02 M HEPES, 0.25 M sucrose, 0.002 M EGTA, and protease cocktail (for dense-core vesicles) or a similar buffer with 0.05 M sucrose (for synaptic vesicles) and passed (∼100 ×) through a ball homogenizer with a 12 μm clearance until cuticles were no longer visible. All subsequent steps were conducted at 4°C. For dense-core vesicle preparation, the homogenate was clarified by centrifugation at 5000 × g and the supernatant was centrifuged at 100,000 × g for 20 min in a T80 rotor. For synaptic vesicle preparation, the homogenate was centrifuged at 25,000 × g for 20 min in a JA20 rotor, and the supernatant was centrifuged at 165,000 × g in a Ti80 rotor for 2 h. Dense-core vesicle and synaptic vesicle pellets were resuspended in corresponding homogenization buffers and fractionated on 0.3–1.5 M sucrose gradients in a
For assays of synaptic vesicle recycling, the jls219["syntaptogyrin::GFP"] strain was crossed with unc-31(e928) or unc-13(e69) strains to generate cells of the desired genotypes. Cultured embryonic cells were prepared (Christensen et al., 2002), and grown for 5 d in L-15–10 medium at 20°C in cover slips dishes. Medium was removed, and cells were incubated in depolarization buffer (in mM: 25 HEPES, 48 KCl, 2 CaCl₂, and 2 MgCl₂, pH 7.4) containing 5 μm FM4-64 [6-(4-diethylamino)phenyl)hexatrienyl]pyridinium dibromide] (Inkaya, Japan) TE2000 microscope equipped with epifluorescence and a 1.4 numerical aperture objective immediately after buffer exchange and at subsequent 30 and 60 min intervals. Fluorescence intensity in neuronal cell bodies was quantified using MetaMorph software (Universal Imaging, Downingtown, PA).

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**Results**

The unc-31 locus encodes the C. elegans homolog of CAPS

The unc-31 locus was originally encoded in a screen based on its locomotion defects and mapped to chromosome IV (Brenner, 1974). This region contains an open reading frame of 4062 nucleotides encoding a 1353 aa protein with a molecular weight of 154 kDa (Fig. 1A). The C. elegans UNC-31 protein is 51% identical (70% similar) to the human CAPS and 58% identical (74% similar) to D. melanogaster dCAPS. The domain structure of the UNC-31 protein (Fig. 1B) includes a pleckstrin homology (PH) domain. The PH domain in rat CAPS has been shown to bind phosphatidylinositol-4, 5-biphosphate to facilitate membrane trafficking, or nonsense mutations, and strains harboring these also lacked CAPS/UNC-31 protein (Fig. 1A, Table 1). Of the seven nonsense mutants sequenced, three (e375, e69, and u280) contained the same amber-suppressible mutation (Avery et al., 1993). mRNAs from mutants bearing the u377 and sa534 alleles contained the same 60 bp deletion (Table 1) at the 3’ end of exon 5 and thus resulted in a 20 aa deletion. Nevertheless, these strains contained near wild-type levels of CAPS/UNC-31 protein (Fig. 1C). Because these alleles resemble the nulls in phenotype, these data suggest that amino acids 273–293, which are conserved in vertebrate CAPS (50% identical; 85% similar), correspond to a novel N-terminal region of the UNC-31 protein that is required for function (Fig. 1B). Of interest, this region of unc-31 corresponds to a portion of a reported p150Glued-binding domain in human CADPS2 (CAPS2) that is deleted in some autistic patients (Sadakata et al., 2007b).

Two mutants (ox299 and e714), which produce full-length CAPS/UNC-31 protein (Fig. 1C), contain missense mutations (Ile530Lys and Gly538Glu, respectively) in highly conserved residues in the C2 domain (Fig. 1D). Although ox299 only exhibits subtle defects in the worm, e714 exhibits a moderately strong defect in locomotion and nose tap response. For the latter, we cannot eliminate the possibility that increased degradation of the UNC-31 protein (Fig. 1C) contributes to the phenotype. However, functional studies of the cognate rat CAPS mutant proteins indicate that these mutations cause a severe loss-of-function as well as dominant-negative properties in secretion assays (Grishanin et al., 2004) (M.P. and T.F.J.M., unpublished observations).

The data suggest that the C2 domain of UNC-31/CAPS plays an important functional role. In summary, these studies define an N-terminal region of UNC-31/CAPS that may be part of a functional domain and suggest the functional importance of the C2 domain. In addition, mutant alleles confirm that the unc-31 locus encodes the invertebrate homolog of CAPS.

**unc-31 mutants exhibit defects in locomotion and defection**

unc-31 mutants were isolated on the basis of defects in locomotion (Brenner, 1974) but were later shown to exhibit broad nervous system dysfunction extending to pharyngeal pumping, egg-laying, and recovery from the dauer state (Avery et al., 1993). Similar defects have been observed in mutants affecting monoamines and neuropeptides (Nelson et al., 1998; Waggoner et al., 2000; Niacaris and Avery, 2003; Rogers et al., 2003; Chase et al., 2004). Because the role of monoamines and neuropeptides in locomotion is modulatory rather than essential, we considered whether unc-31 mutants exhibit defects in the modulation of movement rather than in the basic locomotor process. It was observed previously that well fed unc-31 mutants are paralyzed but generate normal sinusoidal waves and move at near wild-type speeds when removed from food (Hodgkin et al., 1988; Avery et al., 1993). To investigate the movement defect in unc-31 mutants, we performed video tracking assays in the presence or absence of food. unc-31(e928) animals are nearly paralyzed on food, although older animals are slightly more active. We found that unc-31(e928) animals that have just been removed from food were able to move at faster speeds (Fig. 2C). unc-31(e928) animals food deprived for 5 h and assayed off food spent more time at higher speeds, approaching speeds achieved by the wild type on food. The ability of unc-31 animals to move well off food indicates that UNC-31 is not essential for basal movement. The lack of movement on food is likely attributable to loss of a modulatory input, which would be consistent with dysfunction of neuropeptide or monoamine release in unc-31 mutants.

The C. elegans defecation cycle is a stereotypic motor program that occurs approximately every 50 s (Thomas, 1990). This cycle begins with a posterior body contraction that forces gut contents to the anterior of the worm and is followed by an anterior body muscle contraction (aBoc) that pushes gut contents toward the
which CAPS1 expression was restricted to the nervous and endocrine systems (Walent et al., 1992; Ann et al., 1997; Renden et al., 2001; Wassenberg and Martin, 2002). All reporter strains also displayed expression in the vulval muscles VM1 and VM2 (Fig. 3B) and occasional expression in what is likely VulE and VulF. Consistent expression was also noted in the UV1 cells (Fig. 3B) and the spermatheca (Fig. 3A). The UV1 cells are secretory and express other neuropeptides, monoamines, and other synaptic components (Nonet et al., 1993; Li et al., 1999; Zahn et al., 2001; Alkema et al., 2005). In summary, CAPS/UNC-31 is expressed throughout the nervous system and in other secretory cells.

CAPS/UNC-31 localization in neurons
Unlike synaptic vesicle release, dense-core vesicle release is not restricted to sites of synaptic contact. Components of dense-core vesicles in C. elegans, including FMRFamide-related peptides, neuropeptide-processing enzymes (Kass et al., 2001; Jacob and Kaplan, 2003), and IDA-1 (related to islet cell diabetes autoantigen-1) (Zahn et al., 2004), are localized throughout soma, axons, and synapses. We analyzed the localization of UNC-31 using a GFP fusion construct capable of rescuing the mutant phenotypes. UNC-31::GFP localized to neuronal cell bodies and axonal projections similarly to other dense-core vesicle components. Intense fluorescence was observed in regions containing synaptic contacts such as the nerve ring and the ventral and dorsal nerve cords (Fig. 3C). Because these areas are also dense with axonal projections, it was not possible to distinguish whether the UNC-31::GFP was present at synaptic contacts. To assess synaptic localization, we analyzed the presynaptic terminals of the SAB neurons of the head. Punctate expression as well as colocalization with the synaptic vesicle marker synaptobrevin (α-SN1-1) indicate that UNC-31 is localized to synaptic terminals, reported previously by Charlie et al. (2006).

CAPS/UNC-31 is not required for neuronal development
The widespread nervous system dysfunction displayed by unc-31 null worms could indicate that UNC-31 plays a role in nervous system development. We examined neuronal cell differentiation, axon outgrowth, and synaptogenesis in unc-31 null strains. To assess differentiation and axon outgrowth, a GFP marker that is expressed in the cell body and axons of GABA motor neurons was crossed into the unc-31(u280) background. The GABA motor nervous system consists of 26 cells with a subset of cells sending commissures across the midline to the DNC. Analysis revealed no defect in GABA motor neuron cell body number (Fig. 4C) or in the number of commissures (Fig. 4A) sent to the dorsal nerve cord in unc-31(u280) animals. No gross defects in axon projections were detected in this strain, indicating that pathfinding was normal. Furthermore, no defects in the density of synapses were detected by scoring synaptobrevin–GFP puncta in the GABA neuromuscular junctions of unc-31(u280) animals (Fig. 4B).

Figure 3. UNC-31 is expressed in neurons and is enriched at synapses. A, Confocal image of an adult hermaphrodite expressing GFP under the unc-31 promoter (ex608[Punc-31::GFP]). Expression of unc-31 is pan-neuronal with additional expression in the spermatheca and vulval muscles. Anterior is left. B, Confocal image of the ventral surface of an adult hermaphrodite expressing GFP under the unc-31 promoter. Expression is observed in the vulval muscles VM1 and VM2. Expression is also seen in the UV1 cells. C, Confocal images of unc-31(e928) ex608(unc-31::GFP). UNC-31::GFP protein localizes to the synaptic-rich region of the nerve ring as well as the dorsal and ventral nerve cords. GFP expression inside the dashed circle around the posterior bulb of the pharynx can be disregarded because it arises from the coinjection marker (Pmyo-2::GFP). Additional diffuse expression of the coinjection marker can also be seen in the isthmus of the pharynx. D, Subcellular localization of UNC-31::GFP in the SAB neurons of the head. Punctate expression as well as colocalization with the synaptic vesicle marker synaptobrevin (α-SN1-1) indicate that UNC-31 is localized to synaptic terminals, reported previously by Charlie et al. (2006).
neuron outgrowth indicates there are no defects in pathfinding in cord of cholinergic neurons in pathfinding (data not shown). Synapse density in the dorsal nerve/H9262ta/10mal in gene containing a gain-of-function mutation in G results demonstrating that a heat shock promoter-driven trans-
adult animals (Fig. 4
nervous system development. Expression of UNC-31 using a heat
hesive defects in mutant animals after the completion of Sadakata et al., 2007a).

Figure 4. Absence of developmental defects in unc-31. A, Quantification of GABA motor neuron outgrowth indicates there are no defects in pathfinding in unc-31(u280). Confocal Z-series were captured of each animal at 40×, and the numbers of axons that crossed the midline and terminated on the dorsal nerve cord were counted. B, Synaptogenesis was evaluated by using GFP-tagged synaptobrevin (SNB-1:GFP). Quantification of SNB-1:GFP puncta density indicates that unc-31 mutants have normal numbers of GABA motor neuron synapses in the dorsal nerve cord. Puncta density was determined for a single animal by taking images of the dorsal nerve cord with a 63× lens at the posterior and anterior reflexes of the gonad. Puncta density in these two images was averaged to get the puncta per 10 μm. C, By counting the number of GFP-positive cells in a strain expressing GFP in GABA neurons (EG1846), it was determined that all GABA neurons are properly specified in unc-31 mutants. D, Acute expression of unc-31 from a heat shock (HS) promoter is able to rescue the anterior body contraction defect in unc-31(e928). Six cycles were observed for each animal scored to determine the percentage of cycles that had a contraction. All animals in these developmental assays were analyzed as young adults.

Development of the cholinergic nervous system was also normal in unc-31 mutants. Analysis of axon morphology and midline crossing in unc-31(u280) animals expressing GFP in the acetylcholine motor neurons indicated there were no defects in axon pathfinding (data not shown). Synapse density in the dorsal nerve cord of cholinergic neurons in unc-31(u280) animals (3.34 puncta/10 μm) was similar to that in wild-type animals (3.38 puncta/10 μm). Together, studies of the GABA and acetylcholine neurons indicated no defects in differentiation, axon outgrowth, or synaptogenesis in unc-31 null mutants. This is consistent with the absence of gross developmental defects in CAPS mutants in mice and D. melanogaster (Renden et al., 2001; Speidel et al., 2005; Sadakata et al., 2007a).

To further rule out possible defects in neuronal development, we determined whether acute expression of UNC-31 would rescue phenotypic defects in mutant animals after the completion of nervous system development. Expression of UNC-31 using a heat shock promoter completely rescued defecation cycle defects in adult animals (Fig. 4D). These experiments are consistent with results demonstrating that a heat shock promoter-driven transgene containing a gain-of-function mutation in Gας (Korswagen et al., 1997) strongly rescues the locomotion defects of unc-31 null mutants (Charlie et al., 2006). Thus, CAPS/UNC-31 is not required for development but instead is required for proper regulation of neurally controlled behaviors in the mature animal.

ANF–GFP is targeted to dense-core vesicles
Dense-core vesicles are prevalent in C. elegans neurons in which at least 92 distinct neuropeptides are expressed (Li et al., 1999; Nathoo et al., 2001; Pierce et al., 2001), but there is no suitable functional assay for dense-core vesicle exocytosis in C. elegans. To directly assess the role of CAPS/UNC-31 in dense-core vesicle exocytosis, we established a novel assay using cultured embryonic neurons (Christensen et al., 2002) from C. elegans strains that express an ectopic neuropeptide, ANF–GFP.

The prodomain of a preproANF–GFP fusion protein directs the ectopically expressed neuropeptide to dense-core vesicles in PC12 cells (Burke et al., 1997) and in D. melanogaster (Shakiryanova et al., 2005, 2006) so that it can be used as a reliable fluorescent reporter of dense-core vesicle transport and exocytosis. We generated a C. elegans strain with an integrated extrachromosomal array that expresses preproANF–GFP under the control of the pan-neuronal ace-3 promoter. As anticipated, ANF–GFP was expressed throughout the nervous system in the integrated strain with fluorescence localized to the nerve ring as well as in the dorsal and ventral nerve cords (Fig. 5A). The ANF–GFP was concentrated in synaptic regions particularly in the nerve ring in which it colocalized with the synaptic proteins synaptotagmin and Rab3 (data not shown). The ANF–GFP was also distributed in neuronal cell bodies as would be anticipated for Golgi transport and dense-core vesicle packaging. ANF–GFP exhibited normal localization in unc-31 mutants (Fig. 5A).

ANF–GFP was properly targeted to dense-core vesicles in C. elegans based on two criteria. First, dense-core vesicle transport along axons can be observed using fluorescently tagged vesicle proteins (Zahn et al., 2004) and requires the kinesin UNC-104 for transport to the synapse (Jacob and Kaplan, 2003). Live imaging of ANF–GFP fluorescence in the nervous system revealed that GFP puncta moved along the length of commissural axons. Moreover, ANF–GFP was no longer present in synaptic regions but was mislocalized to cell bodies in unc-104 mutants (Fig. 5A). Second, ANF–GFP-containing vesicles exhibited a size distribution characteristic of dense-core vesicles. Postnuclear supernatants from homogenates of the ANF–GFP- and synaptogyrin (SNG)–GFP-expressing strains were analyzed by velocity sedimentation on sucrose gradients (Fig. 5B, top). Western blotting of gradient fractions with a monoclonal GFP antibody demonstrated that synaptogyrin–GFP (SNG-1:GFP, βsIs219), which is a synaptic vesicle component, sediments in fraction 14 (Fig. 5B, top). Synaptotagmin, which is localized to both synaptic vesicles and dense-core vesicles, exhibited peaks in fractions 14 with other synaptic vesicle components and with larger vesicles in fraction 10 (data not shown). Western blotting of gradient fractions from ANF–GFP–expressing strain (glsIs5[Paex-3:ANF::GFP]) revealed an expected ~50 kDa ANF–GFP fusion protein with a peak distribution in fractions 9–12. Because 70 nm dense-core vesicles from PC12 cells sediment to fraction 6 in similar gradients (Martin and Kowalchyk, 1997), fractions 9–12 would be the expected location of the 37–52 nm dense-core vesicles in C. elegans (White et al., 1986). This was confirmed by finding that the mature form of the dense-core vesicle transmembrane IDA-1 protein was distributed in fractions 9–12 (Fig. 5B, bottom) (Zahn et al., 2004). Faster sedimenting material (fractions 2–7) in the gradient likely represented ANF–GFP in Golgi elements (Fig. 5B, top), which co-sedimented with the uncleaved IDA-1 precursor (Fig. 5B, bot-
Figure 5. UNC-31 but not UNC-13 is essential for evoked dense-core vesicle exocytosis in cultured neurons. A. Images of the tglS[Pax-3:ANF::GFP] strain in either wild-type (WT), unc-31(e928) or unc-104(e1265) backgrounds. B. Quantitation of ANF–GFP fluorescence in cultured neurons. C. The neuronal dense-core vesicle exocytosis assay was performed as described. The vesicle exocytosis assay was performed as described. The ANF–GFP fluorescence in the nerve ring was quantitated. D. Western blotting was used to detect mature IDA-1 in cell bodies and neurites from unc-31(u280) (m), unc-31(e928) (n), and wild-type (WT) neurons. E. Mean ± SEM values of percentage release correspond to percentage fluorescence loss from cell bodies.

To determine whether ANF–GFP is released from neurons in vivo, we quantitated the endocytic accumulation of ANF–GFP in coelomocytes. Coelomocytes are scavenger cells that continuously take up fluid from the pseudocoelomic space and would be expected to accumulate ANF–GFP that was released into the coelomic space. Indeed, ANF–GFP fluorescence was readily detected in coelomocytes in the wild-type strain. The overall expression level of ANF–GFP in the nervous system was comparable between the wild-type unc-31 and unc-13 mutant strains. However, lower levels of ANF–GFP fluorescence were observed in coelomocytes of both unc-31 mutant strains assayed (Fig. 6 A, B). Most coelomocytes from wild-type animals accumulated high levels of ANF–GFP with fluorescence near saturation, whereas those from unc-31(e928) or unc-31(u280) animals accumulated low levels of ANF–GFP and only rarely exhibited high fluorescence. We excluded the possibility that lack of ANF–GFP uptake by unc-31(e928) coelomocytes was attributable to an endocytosis defect because coelomocytes in unc-31(e928) mutants were able to endocytose Texas Red-conjugated bovine serum albumin that was injected into the pseudocoelomic space.

To determine whether ANF–GFP release defects might arise generally from reduced levels of neuronal activity, we assayed coelomocyte ANF–GFP uptake in unc-13 mutants that are deficient in synaptic vesicle fusion (Richmond et al., 1999). We found that unc-13(s69) mutants displayed significant decreases in coelomocyte ANF-GFP levels as reported recently (Sieburth et al., 2007) but that unc-13(s51) mutants displayed wild-type coelomocyte ANF-GFP levels (Fig. 6A, B). These allele-specific effects are likely attributable to molecular differences in the two unc-13 alleles (Kohn et al., 2000), which disrupt synaptic activity to differing extents (Richmond et al., 1999). Although the coelomocyte ANF-GFP uptake results were consistent with defects in dense-core vesicle-mediated release in unc-31 mutants, they also indicated that decreased synaptic activity could also decrease ANF-GFP secretion. To circumvent the complication of synaptic activity effects on peptide secretion, we developed a cell culture assay for dense-core vesicle exocytosis.

CAPS/UNC-31 but not UNC-13 is essential for regulated dense-core vesicle exocytosis

To more directly assess the role of UNC-31 in activity-dependent dense-core vesicle exocytosis, we used cultured C. elegans neurons. Using the protocol established by Christensen et al. (2002) for culturing embryonic neurons, we developed a direct assay for the regulated release of ANF–GFP from dense-core vesicles. The primary cultured neurons exhibited ANF–GFP in mobile vesicular structures in neurite extensions as well as in cell bodies (Fig. 5C, D). Incubations of the cells under resting conditions (without depolarization or divalent cations) over a 60 min period did not affect ANF–GFP fluorescence intensity. Depolarization in Ba2+-containing buffers effectively stimulates extensive neuropeptide secretion over an extended time period (Ng et al., 2002). We conducted incubations with depolarizing Ba2+-containing buffers (in mMs: 86 KCl, 5 CaCl2, and 5 BaCl2), which led to the extensive (~70%) reduction in ANF–GFP fluorescence from cell bodies of the neurons (Fig. 5D, E) and the appearance of ANF–GFP fluorescence in the extracellular buffer (data not shown). To determine the function of CAPS/UNC-31 and other proteins in dense-core vesicle exocytosis, we crossed the ANF::GFP integrated strain with mutant strains to generate cultured neurons expressing ANF–GFP in different genetic backgrounds.

UNC-13 has a well characterized role in the priming of synaptic vesicles for exocytosis (Richmond et al., 1999, 2001). However, the role of UNC-13 in neuronal dense-core vesicle exocytosis has not been established. To assess this function for UNC-13, we measured evoked ANF–GFP release from cultured neurons derived from C. elegans harboring a strong loss-of-function unc-13(s69) mutation. There was no detectable defect in stimulated ANF–GFP release (Fig. 5D, E), which indicates that UNC-13 does not play an essential role in neuronal dense-core vesicle exocytosis.
Similar assays conducted with cultured neurons derived from *C. elegans* harboring a null allele of unc-31(e928) revealed that evoked ANF–GFP release was nearly abolished and was indistinguishable from release observed in unstimulated wild-type neurons (Fig. 5D,E). unc-31(e928) neurons exhibited a normal subcellular distribution of vesicular ANF–GFP, indicating that dense-core vesicle synthesis was normal in unc-31(e928) neurons. The results indicate that evoked dense-core vesicle exocytosis requires CAPS/UNC-31.

**UNC-13 but not CAPS/UNC-31 is required for regulated synaptic vesicle recycling**

The cultured neuron preparation also provided the opportunity to directly assess the role of CAPS/UNC-31 in regulated synaptic vesicle exocytosis by conducting assays for synaptic vesicle recycling. Synaptic vesicles in synaptic terminals undergo stimulated exocytosis and compensatory endocytosis, which can be detected as the stimulated uptake of a membrane-binding styryl dye such as FM4-64 (Betz and Bewick, 1992). Cultured neurons expressing GFP-tagged synaptogyrin (jsIs219) were used to identify synaptic terminals (Nonet, 1999). Synaptic uptake of FM4-64 was readily detected in cultures incubated in depolarizing buffers with calcium (56 mM KCl and 5 mM CaCl2) (Fig. 7A, top row). In contrast, FM4-64 uptake was not observed for incubations in buffers lacking depolarizing K+ or Ca2+ (Fig. 7A, rows 3 and 5). Thus, FM4-64 dye loading by cultured *C. elegans* neurons was Ca2+- and depolarization dependent as expected for stimulated synaptic vesicle recycling.

Synaptic transmission is essentially abolished in the motor neurons of unc-13(s69) mutants (Richmond et al., 1999), indicating a virtually complete failure of synaptic vesicle exocytosis. Consistent with this, neurons from unc-13(s69) worms exhibited no stimulated uptake of FM4-64 (Fig. 7A, row 4, B). This result provides an important control for the specificity of FM4-64 uptake for synaptic vesicle recycling in the culture system and independently confirms the essential role of UNC-13 in synaptic vesicle exocytosis (Aravamudan et al., 1999; Augustin et al., 1999; Richmond et al., 1999). In marked contrast, the stimulated uptake of FM4-64 into neurons from the unc-31(e928) strain was indistinguishable from that of wild-type neurons (Fig. 7A, row 2, B). Because the synapses in primary culture display heterogeneity with variation in FM4-64 fluorescence loading, we binned the data to derive a profile of loaded synapse intensities (Fig. 7C). Unstimulated wild-type and stimulated unc-13 neurons displayed the same intensity distribution, whereas stimulated unc-31 neurons displayed an intensity distribution that was indistinguishable from that of stimulated wild-type neurons. The combined data from cultured neurons indicate that CAPS/UNC-31 is essential for evoked dense-core vesicle but not synaptic vesicle recycling. Conversely, UNC-13 is essential for evoked synaptic vesicle but not dense-core vesicle exocytosis.

**Discussion**

Our results lead to four major conclusions. First, the unc-31 gene in *C. elegans* encodes a CAPS protein that is very similar to vertebrate orthologs and is expressed in most if not all neurons and in a few secretory cells outside the nervous system in *C. elegans*. Second, the loss-of-function unc-31 phenotype, which includes widespread nervous system dysfunction, does not arise from de-
UNC-31 (CAPS) Is Required for Dense-Core Vesicle Exocytosis

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Figure 7. UNC-13 but not UNC-31 is essential for evoked synaptic vesicle exocytosis in cultured neurons. A, Cultured neurons were prepared from jsk219 [synaptogyrin::GFP] strains of different genotypes. FM4-64 dye uptake was stimulated in K+-depolarization buffer for wild-type or unc-31(e928) or unc-13(s69) neurons as indicated. Left column corresponds to FM4-64 fluorescence, and right column corresponds to synaptogyrin–GFP in terminals. Bottom two rows correspond to control studies (high K+ buffer without Ca2+ or low K+ buffer) in wild-type or unc-31 neurons, indicating that FM4-64 uptake fails to occur under control conditions. Scale bar, 2 μm. B, Quantitation of FM4-64 uptake in wild-type (WT), unc-31(e928), and unc-13(s69) neurons in depolarization buffer and in wild-type neurons in resting buffer (control). For each condition, n = 50. Mean ± SEM values are shown. C, Binned data of FM4-64 uptake. Intensity distributions are shown for unstimulated wild-type neurons (×) and stimulated unc-13(s69) (▲), wild-type (●), or unc-31(e928) (■) neurons.

developmental defects. Third, UNC-31 function is required for dense-core vesicle exocytosis but not synaptic vesicle recycling in the nervous system of C. elegans. Fourth, UNC-13, which shares sequence homology with CAPS/UNC-31 and is essential for evoked synaptic vesicle exocytosis, is not required for neuronal dense-core vesicle exocytosis. Together, our results suggest that CAPS/UNC-31 and UNC-13 are functional homologs that operate selectively in dense-core vesicle and synaptic vesicle exocytosis, respectively, in the nervous system of C. elegans.

Vertebrate CAPS1 is exclusively expressed in neurons and peptide hormone-secreting endocrine cells (Valent et al., 1992; Ann et al., 1997; Wassenberg and Martin, 2002), whereas vertebrate CAPS2 is expressed throughout the nervous system but also widely in other secretory tissues (Cisternas et al., 2003; Speidel et al., 2003; Sadakata et al., 2007b). Our analysis of a transcriptional GFP reporter indicated that UNC-31 is expressed in most neurons of the hermaphrodite. Recent immunocytochemical studies (Charlie et al., 2006) indicated a more restricted distribution of UNC-31 protein in cholinergic and some noncholinergic synapses, but this may reflect different sensitivities of the techniques used. We also observed expression of UNC-31 in the UV1 uterine secretory cells, which is consistent with previous findings that these cells express IDA-1, a homolog of the dense-core vesicle membrane protein phogrin, as well as FMRFamide-like peptides, and tyramine (Li et al., 1999; Zahn et al., 2001; Kim and Li, 2004; Alkema et al., 2005). UNC-31 expression was also detected in the vulval muscles, which have been shown to express the FMRFamide neuropeptide FLP-10 (FMRF-like peptide-10) (Kim and Li, 2004). These results are consistent with a function for UNC-31 in dense-core vesicle release.

Previous characterization of the unc-31 mutant phenotype in C. elegans identified multiple aspects of nervous system dysfunction beyond the uncoordinated locomotion phenotype. These included constitutive pharyngeal pumping, defective egg-laying, reduced recovery from the dauer state (Avery et al., 1993), defects in defecation (Miller et al., 1996), and prolonged lifespan (Allion et al., 1999). Our studies indicated that the unc-31 movement defects do not result from defects in neuronal differentiation, axon outgrowth, or synaptogenesis. Moreover, the acute rescue of defecation cycle defects by heat shock-induced expression of UNC-31 indicated that the mutant phenotype arises from defects in neuron function in the mature animal rather than developmental defects. A similar lack of involvement in neural development was found in dCAPS nulls in D. melanogaster (Renden et al., 2001) and in CAPS1−/−/CAPS2−/− mice (Speidel et al., 2005), whereas deletion of CAPS2 from mice caused developmental alterations in the cerebellum attributable to deficiencies in neurotrophin secretion (Sadakata et al., 2007a).

Studies of CAPS1 function in vertebrate cells indicate that CAPS1 is required for regulated dense-core vesicle exocytosis (Ann et al., 1997; Berwin et al., 1998; Tangdon et al., 1998; Elhamdani et al., 1999; Rupnik et al., 2000; Grishanin et al., 2002, 2004; Speidel et al., 2003, 2005), but genetic studies to assess CAPS1 function are complicated by redundancy with CAPS2. We assessed the requirement for UNC-31 in dense-core vesicle release in C. elegans by targeting ANF–GFP to dense-core vesicles. This ANF–GFP reporter has been used to study dense-core vesicle transport and exocytosis in vertebrate and invertebrate cells (Burke et al., 1997; Ng et al., 2002; Shakiryanova et al., 2005, 2006). Transgenic worms expressing ANF–GFP in neurons packaged this protein into organelles with the properties of dense-core vesicles. In wild-type worms, ANF–GFP was secreted into the pseudocoelomic space as monitored by its accumulation by coelomocytes, whereas little ANF–GFP secretion was detected in unc-31 mutants. In contrast, unc-13(s69) mutants exhibited wild-type characteristics, whereas unc-13(e51) mutants exhibited decreased ANF–GFP secretion in this in vivo assay. Previous molecular characterization of these two unc-13 alleles revealed that unc-13(s69) disrupts both left, right (LR) and middle, right transcripts, whereas unc-13(e51) only disrupts the LR transcript (Kohn et al., 2000). These two alleles display different electrophysiological phenotypes, with unc-13(s69) exhibiting essentially no transmitter release whereas some release persists in unc-13(e51) mutants (Richmond et al., 1999). We interpret the effect of unc-13(s69) on coelomocyte ANF–GFP uptake to indicate that synaptic activity can regulate peptide secretion. To more directly assess the role of UNC-31 in dense-core vesicle exocytosis, we used an isolated cell culture system. Depolarization of cultured neurons from worms carrying an integrated ANF–GFP
array in Ba\(^{2+}\) elicited extensive release of ANF–GFP, whereas unc-31(e928) worms were deficient for the evoked release of dense-core vesicle contents. From these results, we conclude that regulated dense-core vesicle exocytosis is defective in unc-31 mutants.

Consistent with this conclusion, studies in P30 adrenal chromaffin cells derived from a heterozygous CAPS\(^1/-\)/mouse, which lack CAPS2, showed that a \(\approx 35\%\) reduction in CAPS1 was associated with a \(\approx 30–35\%\) reduction in the readily releasable dense-core vesicle pool (Speidel et al., 2005). However, embryonic day 18 adrenal chromaffin cells derived from a homozygous CAPS\(^1/-\)/mouse, which contain CAPS2, exhibited normal exocytosis of dense-core vesicles (Speidel et al., 2005). Although this was likely attributable to CAPS2 expression and functional redundancy, the authors (Speidel et al., 2005) also reported defects in catecholamine secretion and concluded that CAPS1 was essential for proper catecholamine uptake or retention by dense-core vesicles. Catecholamine loading or storage is unlikely to be a primary function for UNC-31 in C. elegans. First, null mutants in the C. elegans vesicular monoamine transporter (VMAT), encoded by cat-1 (abnormal catecholamine distribution family member) are not paralyzed on food like unc-31 (Duerr et al., 1999). Second, VMAT and catecholamines (dopamine, serotonin, octopamine, and tyramine) are restricted to a small (~30) subset of neurons in C. elegans (Sulston et al., 1975; Horvitz et al., 1982; Desai et al., 1988; Sawin, 1996; Rand and Nonet 1997; Duerr et al., 1999; Alkema et al., 2005), whereas UNC-31 is expressed in many if not all neurons. Third, the movement defects in an unc-31 null mutant can be rescued to near wild-type levels by expressing an unc-31 transgene solely in a subset of the ventral cord cholinergic neurons (Charlie et al., 2006) that do not express catecholamines or VMAT (Sulston et al., 1975; Horvitz et al., 1982; Desai et al., 1988; Duerr et al., 1999; Alkema et al., 2005). Fourth, as shown here, unc-31 mutants exhibit profound defects in evoked peptide secretion, indicating that CAPS/UNC-31 functions broadly in dense-core vesicle exocytosis and is not restricted to catecholamine secretion.

CAPS/UNC-31 shares a large domain with the Unc13 family of proteins called the MUN domain. C. elegans UNC-13, Drosophila Dunc13, and the vertebrate Munc13 are essential for a premature priming step in synaptic vesicle exocytosis (Aravamudan et al., 1999; Augustin et al., 1999; Richmond et al., 1999). This sequence homology and the fact that CAPS mutants in C. elegans (Miller et al., 1996) and D. melanogaster (Renden et al., 2001) display mild defects in synaptic transmission in vivo could indicate that CAPS also functions in the exocytosis of synaptic vesicles. Alternatively, mild deficiencies in synaptic transmission in vivo could be caused by defects in modulatory transmitter secretion as was suggested for the non-cell-autonomous function of dCAPS in D. melanogaster (Renden et al., 2001). To directly address the possible role of CAPS/UNC-31 in synaptic vesicle exocytosis, we used cultured neurons that are free of neuronal circuitry and diffusible mediators. We found that stimulation-dependent synaptic uptake of FM4-64 in unc-31(-928) cultured neurons was indistinguishable from that of wild-type neurons. These results indicate that UNC-31 is not required for evoked synaptic vesicle recycling, although we cannot eliminate the possibility that a small subset of synaptic vesicles was not detected in the FM4-64 dye-loading studies. In contrast, neurons from unc-13(-s69) mutants were entirely deficient for stimulated FM4-64 uptake as would be anticipated from previous results documenting strong deficiencies in synaptic transmission (Aravamudan et al., 1999; Augustin et al., 1999; Richmond et al., 1999). Overall, the evidence indicates that CAPS/UNC-31 does not play a direct role in synaptic vesicle exocytosis at least in the dominant population of cholinergic and glutamatergic neurons present in cultures of C. elegans neurons.

Although CAPS/UNC-31 functioned selectively in dense-core vesicle exocytosis, the possibility exists that UNC-13 might also function in this pathway. However, we found that evoked ANF–GFP release in unc-13(-s69) neurons was indistinguishable from that in wild-type neurons. Thus, despite the sequence homology to CAPS/UNC-31, UNC-13 did not appear to be essential for dense-core vesicle release in cultured C. elegans neurons. This finding is consistent with the observation that evoked dense-core vesicle exocytosis is unaltered in chromaffin cells from Munc13-1 knock-out mice (Stevens et al., 2005) but contradictory to reported deficits in stimulated insulin secretion from pancreatic \(\beta\) cells from the same mice (Kang et al., 2006; Kwan et al., 2006). This may indicate that the multiple CAPS and Munc13 isoforms in vertebrates function in parallel in some secretory processes. Overall, our results suggest a nonoverlapping functional complementarity between UNC-31 and UNC-13 for dense-core vesicle and synaptic vesicle exocytosis, respectively, in the C. elegans nervous system.

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