α -Catulin CTN-1 is required for BK channel subcellular localization in *C. elegans* body-wall muscle cells

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The BK channel, a voltage- and Ca^{2+} -gated large-conductance potassium channel with many important functions, is often localized at specific subcellular domains. Although proper subcellular localization is likely a prerequisite for the channel to perform its physiological functions, little is known about the molecular basis of localization. Here, we show that CTN-1, a homologue of mammalian α -catulin, is required for subcellular localization of SLO-1, the *Caenorhabditis elegans* BK channel α -subunit, in body-wall muscle cells. CTN-1 was identified in a genetic screen for mutants that suppressed a lethargic phenotype caused by expressing a gain-of-function (*gf*) isoform of SLO-1. In body-wall muscle cells, CTN-1 coclusters with SLO-1 at regions of dense bodies, which are Z-disk analogs of mammalian skeletal muscle. In *ctn-1* loss-of-function (*lf*) mutants, SLO-1 was mislocalized in body-wall muscle but its transcription and protein level were unchanged. Targeted rescue of *ctn-1* (*lf*) in muscle was sufficient to reinstate the lethargic phenotype in *slo-1(gf);ctn-1(lf)*. These results suggest that CTN-1 plays an important role in BK channel function by mediating channel subcellular localization.

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Introduction

The BK channel (also known as Slo1, K_{Ca} or MaxiK channel) is almost ubiquitously expressed and performs many important physiological functions. Malfunction of the channel may cause a variety of disorders, including epilepsy (Brenner *et al*, 2005; Du *et al*, 2005), hypertension (Brenner *et al*, 2000b), progressive hearing loss (Ruttiger *et al*, 2004), cerebellar ataxia (Sausbier *et al*, 2004), overactive bladder (Meredith *et al*, 2004), penile erectile dysfunction (Werner *et al*, 2005), impaired renal glomerular filtration and potassium excretion

(Pluznick *et al*, 2003) and paroxysmal dyskinesia (Du *et al*, 2005). The central components of a BK channel are four α -subunits, which form the channel pore and contain voltage and calcium sensing domains (Adelman *et al*, 1992; Butler *et al*, 1993; Pallanck and Ganetzky, 1994). In addition, the α -subunits may interact with other proteins that modulate channel functional properties or expression, such as β -subunits and a MinK-related peptide in mammals (Knaus *et al*, 1994; Wallner *et al*, 1999; Xia *et al*, 1999, 2000; Uebele *et al*, 2000; Weiger *et al*, 2000; Brenner *et al*, 2000a; Levy *et al*, 2008), and Slob and dSLIP1 in *Drosophila* (Xia *et al*, 1998; Zhou *et al*, 1999).

BK channels are often localized/enriched at specific subcellular domains. For example, in neurons, BK channels are enriched at the presynaptic nerve terminal (Robitaille *et al*, 1993; Knaus *et al*, 1996; Zhou *et al*, 1999; Hu *et al*, 2001; Misonou *et al*, 2006), where they colocalize with voltage-gated Ca^{2+} channels (VGCCs) (Roberts *et al*, 1990; Robitaille *et al*, 1993; Issa and Hudspeth, 1994; Yazejian *et al*, 2000) to regulate neurotransmitter release (Robitaille *et al*, 1993; Hu *et al*, 2001; Wang *et al*, 2001; Raffaelli *et al*, 2004; Liu *et al*, 2007; Wang, 2008). In epithelial cells, BK channels are localized to the apical membrane, where they may regulate potassium secretion and cell volume (Segal and Reuss, 1990; Pacha *et al*, 1991; Takeuchi *et al*, 1992; Hirsch *et al*, 1993; James and Okada, 1994; Huang *et al*, 1999). In mouse inner hair cells of the cochlea, BK channels are localized to the apical membrane (Pyott *et al*, 2004) but their role in mammalian auditory function is unclear (Pyott *et al*, 2007). Proper subcellular localization of the channel is likely important to its physiological functions. However, this has not been experimentally demonstrated. The molecular basis of BK channel subcellular localization is also poorly understood. Although a recent study reported that ISLO-1, a protein with two putative membrane-spanning domains, contributes to SLO-1 subcellular localization in *Caenorhabditis elegans* (Kim *et al*, 2009), no homologues could be identified in mammals.

The *C. elegans* BK channel α -subunit SLO-1 is enriched at synaptic regions in the nervous system and clusters in the vicinity of dense bodies in body-wall muscle cells (Wang *et al*, 2001). In a genetic screen for suppressors of a lethargic phenotype caused by expressing a *slo-1(gf)* transgene, we identified CTN-1 as a protein indispensable for BK channel function in *C. elegans* body-wall muscle cells, presumably because of its function in channel subcellular localization. This finding may serve as a starting point for elucidating the molecular basis of BK channel subcellular localization in mammals.

Results

ctn-1 Mutants were isolated as suppressors of a lethargic phenotype caused by *slo-1(gf)*

To identify novel molecules related to BK channel function *in vivo*, we screened for mutants that suppressed the lethargic

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phenotype of a worm strain expressing SLO-1(*gf*) under the control of *slo-1* promoter (*Pslo-1*). SLO-1(*gf*) was created by mutating SLO-1 glutamate 350 to glutamine (E³⁵⁰Q). E³⁵⁰ is the same residue that was changed to lysine in a previously described *slo-1(gf)* mutant (Davies *et al*, 2003), and the equivalent of mouse Slo1 E³²¹, which contributes to one of the two negative rings at the entrance to the intracellular vestibule of the channel (Brelidze *et al*, 2003). Worms expressing *Pslo-1::SLO-1*(E³⁵⁰Q) exhibited distorted locomotion waveform and greatly decreased locomotion speed (Supplementary Movies 1 and 2). From a screening of 24 000 haploid genomes, we isolated 25 mutants. Twelve of the isolated mutants belong to one gene, which was mapped to a 107-kb interval on chromosome I (2562–2669 kb) through single-nucleotide polymorphism (SNP)-based mapping (Davis *et al*, 2005). We then tested whether cosmid or PCR-amplified genomic DNA fragments of predicted genes within this interval could reinstate the lethargic phenotype of *slo-1(gf)* when

they were expressed in one of the mutants that harboured the *slo-1(gf)* transgene. We found that two PCR-amplified overlapping genomic DNA fragments (total ~15 kb) corresponding to the predicted *ctn-1* gene (locus Y23H5A.5, <http://www.wormbase.org>) and 3 kb sequence upstream of its initiation site completely restored the lethargic phenotype of *slo-1(gf)* (not shown). The *ctn-1* encodes a homologue of mammalian α -catulin (Janssens *et al*, 1999). Although several splice variants of *ctn-1* have been identified (<http://www.wormbase.org>), we found that *ctn-1d* (Y23H5A.5d) is the predominant isoform based on reverse transcription PCR (RT-PCR). The predicted translational product of this isoform shares 41% identity with mammalian α -catulin (Janssens *et al*, 1999) in amino-acid sequence (Figure 1). Sequencing of five randomly picked *ctn-1* mutants revealed molecular lesions of this gene in all of them (Figure 1). The *ctn-1 (zwi)*, which is a putative null resulting from a premature stop codon, was used for all subsequent analyses.

Figure 1 The *ctn-1* encodes a homologue of mammalian α -catulin. Shown is the alignment between predicted amino-acid sequences of CTN-1 and human α -catulin (41% identity). The molecular lesions of five *ctn-1* alleles were determined. Four alleles have mutations leading to premature stop codon (marked with ‘*’) and one allele (marked with an arrow) disrupts a splice donor site leading to a frame shift after amino-acid Y92 and then a stop codon (CFNGOPIMCM STOP).

***ctn-1* was expressed in muscle cells and neurons**

To understand how CTN-1 contributes to SLO-1 function *in vivo*, we first analysed the expression pattern of *ctn-1* and compared it with that of *slo-1*. Two independent transgenic strains were created that expressed GFP under the control of the *ctn-1* promoter (*Pctn-1*) and *slo-1* promoter (*Pslo-1*), respectively. The expression pattern of *ctn-1* largely overlapped with that of *slo-1*. Specifically, both *ctn-1* and *slo-1* were expressed in many neurons and several types of muscles, including body-wall muscle, vulval muscle and stomatointestinal muscle. However, *slo-1* appeared to be expressed in more neurons in the head than *ctn-1*, whereas *ctn-1* was expressed in pharyngeal muscle cells and some other unidentified cells that did not express *slo-1* (Figure 2).

The identified *ctn-1* splice variants (<http://www.wormbase.org>) include 9 to 13 exons. All of the splice variants share the first 8 exons but each of them has a unique exon afterward. To determine where these splice variants might be expressed, we created transgenic strains expressing *ctn-1* genomic DNA with GFP-coding sequence inserted into each of the unique exons separately (Figure 3A). As GFP was fused to full-length CTN-1, GFP epifluorescence could reflect both the expression and subcellular localization patterns of the CTN-1 isoforms. We detected strong *ctn-1d* expression in both neurons and muscle cells, obvious *ctn-1c* expression in muscle cells but weak *ctn-1c* expression in neurons, and no *ctn-1a* or *ctn-1b* expression (Figure 3B). As CTN-1d::GFP appeared as puncta in neuronal processes but not in the soma, it is difficult to tell whether it is expressed in all or a subset of motoneurons in the ventral nerve cord. We then tested whether the expression of wild-type *ctn-1c* in a *ctn-1;slo-1(gf)* double mutant could reinstate the lethargic phenotype as *ctn-1d* did. However, such an effect of *ctn-1c* was not observed, suggesting CTN-1c is either unrelated or unimportant to SLO-1 function *in vivo*. Therefore, CTN-1d appeared to be the most important isoform with respect to SLO-1 function, and was used in subsequent experiments. CTN-1d is referred to as CTN-1 hereafter.

CTN-1 was required for SLO-1 function in body-wall muscle cells

As the expression patterns of *ctn-1* and *slo-1* appeared to overlap in body-wall muscle cells and some neurons, the suppression of *slo-1(gf)* lethargic phenotype by *ctn-1(lf)* could be due to CTN-1 deficiency in muscle cells, neurons or both. To answer this question, we tested whether targeted expression of wild-type CTN-1 in body-wall muscle cells or neurons could reinstate the lethargic phenotype in the *slo-1(gf);ctn-1(lf)* double mutant. Quantitative analyses of locomotion speed showed that *ctn-1(lf)* partially but significantly suppressed the inhibitory effect of *slo-1(gf)* on locomotion, which could be reversed by expressing wild-type CTN-1 under the control of the muscle-specific *myo-3* promoter (*Pmyo-3*) (Okkema *et al*, 1993) but not the pan-neuronal *rab-3* promoter (*Prab-3*) (Nonet *et al*, 1997) (Figure 4A; Supplementary Movies 3–7). These observations suggest that the suppression of *slo-1(gf)* phenotype by *ctn-1(lf)* resulted mainly from CTN-1 deficiency in muscle cells.

Both *slo-1(lf)* and *ctn-1(lf)* mutants appeared to be grossly distinct from the wild type in head movement behaviours. To further examine functional relationship between CTN-1 and SLO-1, we quantified the head-bending angle of the wild type and mutant animals using an automated tracking and

Figure 2 The expression pattern of *ctn-1* was similar to that of *slo-1*. The expression patterns were analysed by expressing GFP under the independent controls of *slo-1* promoter (*Pslo-1*, 5.2 kb) and *ctn-1* promoter (*Pctn-1*, 4.3 kb). Strong expression was observed in body-wall muscle (BM) (including head muscle, HM), vulval muscle (VM), stomatodeum (SIM), nerve ring (NR), and many neurons in the head (not labelled), ventral cord (VC) and tail (not labelled) with both transcriptional fusions. In addition, *Pslo-1::GFP* was expressed in more neurons in the head, and anal depressor muscle (ADM) in the tail, whereas *Pctn-1::GFP* was expressed in some pharyngeal muscles (PM) in the corpus and terminal bulb. Scale bar = 20 μ m. A full-colour version of this figure is available at *The EMBO Journal* Online.

analysis system. Consistent with a previous report (Kim *et al*, 2009), we observed a significant increase in head-bending angle in *slo-1(lf)*. This phenotype was shared by *ctn-1(lf)*, and that its severity was not additive in the *ctn-1(lf);slo-1(lf)* double mutant (Figure 4B and C), suggesting that CTN-1 and SLO-1 likely function together. The head-bending phenotype of *ctn-1(lf)* could be rescued by expressing wild-type CTN-1 in muscle cells but not in neurons (Figure 4B and C), suggesting that the mutant phenotype was mainly caused by CTN-1 dysfunction in muscle cells.

SLO-1 is an important negative regulator of neurotransmitter release at the *C. elegans* neuromuscular junction (NMJ) (Wang *et al*, 2001; Liu *et al*, 2007). The identification of *ctn-1* expression in ventral cord motoneurons (Figure 2) raised the possibility that CTN-1 might be needed for SLO-1 function in regulating neurotransmitter release. To investigate this possibility, we analysed the effect of *ctn-1(lf)* on miniature and evoked postsynaptic currents (mPSCs and ePSCs) recorded from body-wall muscle cells at two different extracellular Ca^{2+} concentrations (5 and 0.25 mM). The higher $[\text{Ca}^{2+}]$ is

Figure 3 Expression patterns of *ctn-1* splicing variants. (A) Intron–exon organization of *ctn-1* splicing variants. GFP was inserted into the unique exon of each splicing variant. The arrows indicate the locations of GFP insertion. (B) No expression was observed for CTN-1a and CTN-1b. Both CTN-1c and CTN-1d were strongly expressed in body-wall muscle cells. CTN-1d was also strongly expressed in neurons, as indicated by the bright fluorescence signal in the nerve ring (arrow), whereas CTN-1c appeared to be weakly expressed only in some neurons (arrow). A corresponding DIC image was shown below each fluorescent image. Scale bar = 20 μ m. A full-colour version of this figure is available at *The EMBO Journal* Online.

more suitable for determining whether *slo-1(gf)* reduces ePSC amplitude and whether this effect may be reversed by *ctn-1(lf)*, whereas the lower $[Ca^{2+}]_o$ is more suitable for testing whether *ctn-1(lf)* could increase ePSC amplitude as *slo-1(lf)* does (Liu *et al*, 2007).

ePSCs were evoked by photoactivation of motoneurons expressing channelrhodopsin-2 under the control of the *unc-17* promoter (Liewald *et al*, 2008; Liu *et al*, 2009). At 5 mM $[Ca^{2+}]_o$, *slo-1(gf)* significantly decreased ePSC amplitude and mPSC frequency without affecting mPSC amplitude compared with the wild type, and these effects of *slo-1(gf)* were not suppressed by *ctn-1(lf)* (Figure 5). At 0.25 mM $[Ca^{2+}]_o$, *slo-1(lf)* significantly increased ePSC amplitude and mPSC frequency without affecting mPSC amplitude, and these effects of *slo-1(lf)* were not shared by *ctn-1(lf)* (Figure 5). These observations suggest that CTN-1 is not required for the function of SLO-1 in regulating neurotransmitter release at the NMJ.

CTN-1 was required for SLO-1 subcellular localization in body-wall muscle cells

CTN-1 could contribute to SLO-1 function in body-wall muscle cells through several potential mechanisms. We first tested whether CTN-1 has a function in SLO-1 subcellular localization. We previously showed that SLO-1 is enriched at dense body areas in body-wall muscle cells and in the synapse-rich nerve ring in the nervous system, and that

these subcellular localization patterns may be recapitulated by an SLO-1::GFP fusion protein (Wang *et al*, 2001). We created two transgenic strains expressing integrated *Pslo-1::SLO-1::GFP* and *Pmyo-3::SLO-1::GFP* for analysing SLO-1 subcellular localization in the nerve ring and body-wall muscle cells, respectively. These two transgenes were then separately crossed into *ctn-1(lf)* mutant. Although SLO-1::GFP appeared as puncta at locations matching dense bodies in body-wall muscle cells of the wild type, the fluorescent puncta were almost absent in *ctn-1(lf)* (Figure 6A and B). In contrast, SLO-1::GFP localization in the nerve ring appeared indistinguishable between the wild-type and *ctn-1(lf)* mutant (Figure 6A). We then asked whether *ctn-1(lf)* would affect the subcellular localization of two other proteins in body-wall muscle cells, including INX-11 and vinculin. INX-11 is an innexin that may form gap junctions or hemichannels, whereas vinculin is a membrane-cytoskeletal protein. Both proteins are expressed in body-wall muscle cells and localized to dense body regions (Francis and Waterston, 1985; Barstead and Waterston, 1989; Altun *et al*, 2009). We found that both GFP-tagged INX-11 and native vinculin were normally localized in the *ctn-1(lf)* mutant (Supplementary Figure S2). These observations suggest that CTN-1 may be specifically required for SLO-1 subcellular localization in body-wall muscle cells.

The disappearance of SLO-1::GFP puncta in body-wall muscle cells of *ctn-1(lf)* mutants could be due to decreased

Figure 4 CTN-1 was required for SLO-1 function in body-wall muscle cells but not neurons. (A) The *ctn-1(lf)* mutant counteracted the inhibitory effect of *slo-1(gf)* on locomotion speed, and this effect could be reversed by expressing wild-type CTN-1 in body-wall muscle cells under the control of the *myo-3* promoter (*Pmyo-3*) but not in neurons under the control of the pan-neuronal *rab-3* promoter (*Prab-3*). (B, C) Quantification of the maximal head-bending angle (B) and root mean square (RMS) of head-bending angle (C). The *ctn-1(lf)* and *slo-1(lf)* mutants showed increased head-bending angle, and the severity of this phenotype was nonadditive in the *slo-1(lf);ctn-1(lf)* double mutant. The abnormal bending phenotype of the *ctn-1(lf)* mutant could be rescued by expressing wild-type CTN-1 in muscle cells using *Pmyo-3* but not in neurons using *Prab-3*. Data are shown as mean $\pm$ s.e. The asterisk (*) indicates a statistically significant difference ($P < 0.01$) for comparisons either between the specified groups (A), or between the wild type and a mutant strain (B, C). The number of worms in each group analysed was 20–30 for the data shown in (A) and 10 for the data shown in (B, C).

gene transcription or decreased protein synthesis/stability. To determine whether CTN-1 controls *slo-1* transcription in muscle cells, we compared the expression of a *Pslo-1::GFP* transcriptional fusion between the wild-type and *ctn-1(lf)* mutant. GFP expression in body-wall muscle cells was similar between the two groups (Figure 6C). To determine whether CTN-1 has an effect on SLO-1 protein level, we compared the total SLO-1::GFP protein level between the wild-type and *ctn-1(lf)* mutant by western blot using a GFP antibody but found no difference (Figure 6D). These observations suggest that the apparent SLO-1 mislocalization observed in *ctn-1(lf)* mutant did not result from a deficiency in *slo-1* transcription

Figure 5 The *ctn-1(lf)* mutant did not affect neurotransmitter release at the NMJs. (A) Representative traces of miniature postsynaptic currents (mPSCs) (top) and photo-evoked postsynaptic currents (ePSCs) (bottom) from the wild type, *slo-1(gf)*, and *slo-1(gf);ctn-1(lf)*, *slo-1(lf)*, and *ctn-1(lf)* recorded at either 5 or 0.25 mM $[Ca^{2+}]_o$. (B) Comparisons of mPSC frequency, mPSC amplitude and ePSC amplitude between the different groups. Data are shown as mean $\pm$ s.e. The asterisk (*) indicates $P < 0.01$ compared with the wild type. The number of samples analysed is indicated inside each column. One-way ANOVA (with Bonferroni *post hoc* test) was used for statistical comparisons of the means.

or SLO-1 protein synthesis/stability. We also asked whether CTN-1 regulates SLO-1 surface protein level by performing biotinylation assays with transfected HEK293 cells, and found that both the total and surface SLO-1 protein levels were comparable between cells transfected with SLO-1 alone and SLO-1 plus CTN-1 (Supplementary Figure S3), suggesting that CTN-1 probably does not regulate SLO-1 trafficking to the plasma membrane. However, given that HEK293 cells are different from *C. elegans* body-wall muscle cells in many ways, we cannot exclude a function of CTN-1 in SLO-1 membrane trafficking *in vivo* solely based on this observation.

CTN-1 physically interacted with SLO-1 both *in vivo* and *in vitro*

To determine whether CTN-1 mediates SLO-1 subcellular localization through a local effect, we analysed subcellular localization pattern of a CTN-1::EGFP fusion protein in body-wall muscle cells. The fusion protein was expected to recapitulate the subcellular localization pattern of wild-type CTN-1 because it reinstated the lethargic phenotype when expressed in the *slo-1(gf);ctn-1(lf)* double mutant (not

Figure 6 CTN-1 mediates SLO-1 subcellular localization in body-wall muscle cells. (A) SLO-1::GFP was mislocalized in body-wall muscle cells but not neurons of *ctn-1(lf)*, as indicated by the absence of fluorescent puncta (upper panel) in muscle cells but apparently normal epifluorescence in the nerve ring (indicated with arrow) (lower panel). (B) Quantification of the intensity of SLO-1::GFP puncta in body-wall muscle cells. The intensity in *ctn-1(lf)* was normalized to that in the wild type. Data are shown as mean $\pm$ s.e. Compared with the wild type, the intensity was significantly reduced in the *ctn-1(lf)* mutant ($P < 0.01$, unpaired *t*-test). The numbers of cells analysed were 24 and 22 for the wild-type and *ctn-1* mutant, respectively. (C) *slo-1* Transcription was unaltered in *ctn-1(lf)*. A *slo-1* promoter and GFP transcriptional fusion was expressed in wild-type animals and *ctn-1(lf)*. GFP epifluorescence in body-wall muscle cells, imaged under identical exposure conditions, was indistinguishable between the two groups. The body muscle (BM) and ventral cord (VC) are indicated (arrows). (D) Western blot shows that the level of total SLO-1::GFP protein was comparable between wild-type and *ctn-1(lf)* mutant worms. α -Tubulin was blotted to show equal loading of the protein samples. (E) SLO-1 and CTN-1 colocalized in dense body areas of body-wall muscle cells. GFP-tagged SLO-1 (green) and mStrawberry-tagged CTN-1 (red) were coexpressed in body-wall muscle cells under the control of the *myo-3* promoter. The merged picture shows colocalization of the two fusion proteins. Scale bar = 20 μ m.

shown). We found that CTN-1::EGFP was enriched at dense body regions (Supplementary Figure S4A), which was independent of SLO-1 (Supplementary Figure S4B). Furthermore, we found that CTN-1::mStrawberry, which was also fully functional, colocalized with SLO-1::GFP at dense body regions (Figure 6E). These observations suggest that CTN-1 likely mediates SLO-1 subcellular localization through a local effect.

The colocalization data shown in Figure 6E did not have enough resolution to suggest whether CTN-1 physically interacts with SLO-1. To address this question, we performed bimolecular fluorescence complementation (BiFC) assays (Chen *et al*, 2007; Shyu *et al*, 2008), which tells not only whether but also where two proteins interact *in vivo*. In this assay, the nonfluorescent amino- and carboxyl-terminal portions of yellow fluorescent protein (YFPa and YFPc) are fused separately to a pair of proteins of interest. The fluorophore of YFP may be reconstituted if these two proteins are physically very close (Shyu *et al*, 2008). We inserted YFPa into the linker region between the two RCK domains of SLO-1 and fused YFPc to the carboxyl terminus of CTN-1, and coexpressed these two fusion proteins in body-wall muscle cells under the control of *Pmyo-3*. Fluorescent puncta were observed in body-wall muscle cells coexpressing SLO-1::YFPa and CTN-1::YFPc (Figure 7A), suggesting that SLO-1 and CTN-1 are physically very close in the muscle cells. SLO-1 may be

divided into two major structural components, including the amino-terminal portion (1–352 aa) that contains seven membrane-spanning domains and the channel pore domain, and the cytoplasmic carboxyl terminal portion (353–1140) that contains two RCK domains (Wang *et al*, 2001; Jiang *et al*, 2002; Salkoff *et al*, 2006; Yusifov *et al*, 2008; Yuan *et al*, 2010). To determine which part of SLO-1 is important for the interaction with CTN-1, we tested whether the amino- or carboxyl-terminal portion of SLO-1 is required for BiFC with CTN-1. In these experiments, YFPa was either fused to the carboxyl terminus of SLO-1 (1–370) or inserted into the linker region between the two RCK domains of SLO-1 (371–1140). We found that SLO-1(371–1140) but not SLO-1(1–370) allowed BiFC with CTN-1 (Figure 7A). These observations suggest that CTN-1 is physically very close to SLO-1, and likely interacts with the carboxyl terminal portion of SLO-1 to mediate SLO-1 subcellular localization.

To obtain independent evidence showing the interaction between CTN-1 and SLO-1, we performed coimmunoprecipitation experiments with HA-tagged CTN-1, and Myc-tagged full-length SLO-1 or SLO-1 amino- and carboxyl-terminal portions. We found that either full-length SLO-1 or SLO-1(371–1140) coimmunoprecipitated with CTN-1 whereas SLO-1(1–370) did not (Figure 7B). These observations reinforced the notion that the cytoplasmic carboxyl terminal portion of SLO-1 is important to the interaction with CTN-1.

Figure 7 CTN-1 physically interacts with SLO-1. (A) CTN-1 and SLO-1 were physically very close. The nonfluorescent amino- and carboxyl-terminal portions of YFP (YFPa and YFPc) were fused to SLO-1 (or its variants) and CTN-1, respectively. Bimolecular fluorescence complementation (BiFC) assays were performed by coexpressing CTN-1::YFPc with each of the SLO-1 fusions (full-length SLO-1::YFPa, SLO-1(1-370)::YFPa and SLO-1(371-1140)::YFPa) in body-wall muscle cells under the control of the *myo-3* promoter. Fluorescent puncta were observed in dense body areas when CTN-1::YFPc was coexpressed with either full-length SLO-1::YFPa (upper panel) or SLO-1(371-1140)::YFPa (lower panel) but not with SLO-1(1-370)::YFPa (middle panel). The negative result with SLO-1(1-370)::YFPa was not due to poor expression of the fusion protein because SLO-1(1-370)::YFPa was able to reconstitute YFP epifluorescence when coexpressed with another protein (not shown). Scale bar = 20 μ m. (B) CTN-1 and SLO-1 coimmunoprecipitated from transfected HEK293 cells. CTN-1 coimmunoprecipitated with either full-length SLO-1 or SLO-1(371-1140) but not with SLO-1(1-370). CTN-1 was tagged with HA, whereas SLO-1 and its variants were tagged with Myc. Immunoprecipitation (IP) and immunoblot (IB) were performed with Myc and HA antibodies. All specific bands are indicated by their relative molecular sizes. The antibody heavy chain dimmer is indicated with an arrow. A full-colour version of this figure is available at *The EMBO Journal Online*.

CTN-1 did not modulate SLO-1 current properties

The physical closeness between CTN-1 and SLO-1, as revealed by the biochemical assays, suggested that CTN-1 might be able to directly modulate SLO-1 functional properties. We examined this possibility by coexpressing CTN-1 with SLO-1 in *Xenopus* oocytes, and analysing SLO-1 currents recorded from inside-out patches at three different cytoplasmic Ca^{2+} concentrations (10 μ M, 100 μ M and 1 mM). CTN-1 did not show a significant effect on SLO-1 conductance-voltage relationship (Supplementary Figure S5). SLO-1 current amplitude and kinetics also appeared to be unaltered by CTN-1 (not quantified). These observations indirectly suggest that proper subcellular localization of SLO-1 is critical to its function in body-wall muscle cells.

CTN-1 was normally localized in *dys-1* mutant

A recent study suggested that ISLO-1 contributes to SLO-1 subcellular localization in body-wall muscle cells by interacting with the dystrophin-associated protein complex (DAPC). SLO-1 is mislocalized in mutants of either *islo-1* or *dys-1* (dystrophin) (Kim *et al*, 2009). We were able to reproduce the reported SLO-1 mislocalization in the *dys-1(cx18)* mutant (Figure 8). To determine whether CTN-1 depends on DAPC for localization, we compared CTN-1::EGFP expression and subcellular localization in body-wall muscle cells between the wild-type and *dys-1(cx18)* mutant but found no difference (Figure 8), suggesting that dystrophin is not required for CTN-1 subcellular localization in body-wall muscle cells. To address whether mutation of CTN-1 would have an effect on

Figure 8 SLO-1 but not CTN-1 was mislocalized in *dys-1* mutant. SLO-1::GFP and CTN-1::EGFP fusions were expressed separately in body-wall muscle cells of the wild type and *dys-1(cx18)* under the control of the *myo-3* promoter. The mutation of *dys-1* mislocalized SLO-1::GFP but not CTN-1::EGFP. Scale bar = 10 μ m. A full-colour version of this figure is available at *The EMBO Journal Online*.

the assembly of the DAPC, we cloned the cDNA of α -dystrobrevin gene (*dyb-1*), which encodes a major component of the DAPC (Blake *et al*, 2002), and expressed mStrawberry-tagged DYB-1 (DYB-1::mStrawberry) in body-wall muscle cells of the wild-type and *ctn-1* mutant. We found

that DYB-1::mStrawberry was localized in dense body areas in both the wild-type and *ctn-1* mutant (Supplementary Figure S6). These results suggest that CTN-1 and DAPC might be required for SLO-1 localization independently.

Discussion

This study showed that CTN-1-mediated SLO-1 subcellular localization was important to SLO-1 function *in vivo*. This conclusion is supported by the disruption of SLO-1 localization in body-wall muscle cells of *ctn-1(lf)* and the suppression of *slo-1(gf)*-induced lethargy by *ctn-1(lf)*. Although there may be other proteins implicated in BK channel subcellular localization, the fact that 12 independent alleles of *ctn-1* were isolated in the genetic screen suggests that CTN-1 is an important player in SLO-1 function.

What might be the physiological significance of localizing SLO-1 to dense body regions by CTN-1? The function of body-wall muscle cells is to contract and relax in response to changes in $[Ca^{2+}]_i$. EGL-19, an L-type ($Ca_v1.1$) VGCC (Bargmann, 1998), is also localized to dense body regions (Kim *et al*, 2009) and is the predominant carrier of inward currents in *C. elegans* body-wall muscle cells (Jospin *et al*, 2002). It is well established that Ca^{2+} entry through VGCCs creates local high Ca^{2+} concentration domains known as Ca^{2+} microdomains at the inner openings of the channels, where $[Ca^{2+}]$ could be as high as over 100 μ M (Adler *et al*, 1991; Llinas *et al*, 1992; Yazejian *et al*, 2000). The high $[Ca^{2+}]$ may serve as a strong activator of colocalized SLO-1. Indeed, the activity of BK channels has been used as a measure of Ca^{2+} concentrations at the presynaptic terminal resulting from the opening of VGCCs (Yazejian *et al*, 2000). Thus, localization of SLO-1 to the vicinity of EGL-19 by CTN-1 potentially allows SLO-1 to be activated by Ca^{2+} entering through EGL-19, and SLO-1 could in turn down-regulate the activity of EGL-19 through its effect on the membrane potential.

The mammalian CTN-1 homologue α -catulin is almost ubiquitously expressed (Janssens *et al*, 1999; Park *et al*, 2002). However, there are only a few published studies on α -catulin (Janssens *et al*, 1999; Demacio and Ray, 2001; Park *et al*, 2002; Merdek *et al*, 2004; Wiesner *et al*, 2008). As α -catulin shares sequence homology with α -catenin, which binds a variety of cell adhesion or cytoskeletal proteins, such as α -catenin, β -catenin, zona occludence protein 1 (ZO-1) and α -actinin (Knudsen *et al*, 1995; Nieset *et al*, 1997; Muller *et al*, 2005; Nelson, 2008), CTN-1 may also bind such proteins, thus contributing to BK channel subcellular localization by serving as a cytoplasmic linker between the channel and cytoskeletons. Recently, ISLO-1 was suggested to mediate SLO-1 subcellular localization in *C. elegans* body-wall muscle by interacting with the DAPC (Kim *et al*, 2009), which raises the possibility that CTN-1 might also interact with the DAPC to localize SLO-1. However, mutation of dystrophin did not disrupt CTN-1 subcellular localization in body-wall muscle cells (Figure 8), and *ctn-1(lf)* did not affect the assembly of dystrophin complex (Supplementary Figure S6), suggesting that SLO-1 subcellular localization in body-wall muscle cells likely involves two independent mechanisms, and disrupting one of them is sufficient to mislocalize SLO-1.

Although *ctn-1* appeared to be also expressed in many neurons, we were unable to detect a significant effect of CTN-1 in the nervous system through analyses of locomotion behaviours and postsynaptic currents at the NMJ. These observations, however, do not exclude the possibility that CTN-1 has a function in the nervous system but was not detected in this study because our analyses were biased toward detecting defects in muscle cells, motoneurons or other neurons important to locomotion. Thus, the function of CTN-1 in the nervous system remains to be further investigated.

An elucidation of the molecular basis of BK channel subcellular localization could be a key to understanding how the channel performs its various physiological functions. Given that the primary sequences of both SLO-1 and CTN-1 share high level of homology with their mammalian counterparts, it would be interesting to know whether α -catulin has a similar function in localizing mammalian BK channels, and whether an analogous mechanism is used to localize the channel in other types of cells.

Materials and methods

Growth and culture of *C. elegans*

C. elegans hermaphrodites were grown on agar plates with a layer of OP50 *Escherichia coli* at room temperature (21–22°C) or inside an environmental chamber (21°C).

Mutant screening

An integrated transgenic strain expressing *Pslo-1::SLO-1(E³⁵⁰Q)* was used for mutant screen. Synchronized L4-stage *slo-1(gf)* worms were treated with the chemical mutagen ethyl methanesulfonate (50 mM) for 4 h at room temperature. The F2 progeny were screened for animals that moved better than the original *slo-1(gf)* animals. Isolated mutants were grouped through complementation tests.

Behavioural assay

Locomotion velocity was determined using a technique described previously (Liu *et al*, 2006), whereas the head-bending angle was quantified using a newly developed worm tracking and analysing system. Specifically, a single adult hermaphrodite was transferred to an agar plate with a thin layer of OP50 *E. coli*. After allowing ~30 s for recovery from the transfer, snapshots of the worm were taken at 15 frames per second for 30 s using a VGA FireWire camera (XCD-V60, Sony, Tokyo, Japan) mounted on a stereomicroscope (SMZ800, Nikon, Tokyo, Japan). The worm was constantly kept in the centre of the view field with a motorized microscope stage (OptiScan™ ES111, Prior Scientific, Inc., Rockland, MA). Both the camera and the motorized stage were controlled by a custom program running in MATLAB (The MathWorks, Inc., Natick, MA).

Cloning of *ctn-1*

ctn-1(zw1) was used for SNP-based genetic mapping (Davis *et al*, 2005). After mapping the mutation to a small interval, the candidate gene was identified by testing whether cosmid and PCR-amplified genomic DNA fragments covering this interval could reinstate the lethargic phenotype in *ctn-1(lf);slo-1(gf)*. Full-length cDNA of the candidate gene was obtained through RT-PCR. Molecular lesions of *ctn-1* were identified by sequencing the cDNA prepared from five randomly chosen mutant alleles.

Analysis of expression pattern and subcellular localization

The expression pattern of *ctn-1* was determined by expressing GFP under the control of 4.3 kb *ctn-1* promoter (*Pctn-1::GFP*, wp761), whereas that of *slo-1* by expressing EGFP under the control of 5.2-kb *slo-1* promoter (*Pslo-1::EGFP*, wp758). The plasmids were separately injected into the *lin-15(n765)* strain using a *lin-15* rescue plasmid as a transformation marker. Cells expressing the fluorescent protein were visualized and photographed with a Zeiss

Axiovert 200 M fluorescence microscope ($\times 40$ objective) with an apotome device (Zeiss) for optical sectioning.

To determine the expression patterns of different splicing variants of *ctn-1*, we cloned *ctn-1* genomic DNA including 4.3 kb upstream of the initiation site and 0.6 kb downstream of the last exon, and inserted GFP into a unique axon of each splicing variant. As the genomic DNA of *ctn-1* was too long (~ 16 kb) to be cloned into one plasmid, it was cloned into two plasmids as two separate fragments with 0.6 kb overlap. Homologous recombination *in vivo* would result in a full-length CTN-1::GFP translational fusion. After linearization, the plasmids were coinjected with a *lin-15* rescue plasmid into the *lin-15(n765)* strain. Transformed worms were identified as lacking a multivulval phenotype. Recombination of the two *ctn-1* gene fragments in transgenic worms was verified by PCR using a GFP primer and a primer specific to the plasmid that did not contain GFP-coding sequence. Epifluorescence of the fusion protein in transgenic animals was visualized and photographed with a Nikon TE2000-U inverted microscope ($\times 60$ objective) and a monochrome-cooled CCD camera (F-View II, Olympus).

Subcellular localization of CTN-1 was determined by fusing EGFP to its carboxyl terminus and expressing the fusion protein in body-wall muscle cells under the control of *Pmyo-3* (Okkema *et al*, 1993) (*Pmyo-3::CTN-1::EGFP*, wp771). To determine whether SLO-1 protein expression or subcellular localization was altered in *ctn-1(lf)* mutant, a transgenic strain expressing *Pmyo-3::SLO-1::GFP* (wp746) or *Pslo-1::SLO-1::GFP* (wp5) was integrated through γ -irradiation, backcrossed with wild-type worms three times, and crossed into *ctn-1(lf)*. To determine whether CTN-1 and SLO-1 are colocalized in muscle cells, CTN-1 was fused with mStrawberry (*Pmyo-3::CTN-1::mStrawberry*, wp804) and coexpressed with *Pmyo-3::SLO-1::GFP*. Epifluorescence of the fusion proteins in transgenic animals was visualized and photographed with a Nikon TE2000-U inverted microscope ($\times 60$ objective) and the F-view II digital camera.

BiFC assay

The DNA sequences encoding YFP amino and carboxyl terminals (YFPa and YFPc) were amplified by PCR from pCE-BiFC-VN173 and pCE-BiFC-VC155 vectors (Hiatt *et al*, 2008), respectively, to make the following plasmids: *Pmyo-3::SLO-1::YFPa* (wp805), *Pmyo-3::SLO-1(1–370)::YFPa* (wp913), *Pmyo-3::SLO-1(371–1140)::YFPa* (wp914) and *Pmyo-3::CTN-1::YFPc* (wp772). The plasmid encoding SLO-1::YFPa was first injected into *lin-15(n765)* to establish independent transgenic lines, with a *lin-15* plasmid coinjected to serve as a transformation marker. A representative transgenic line thus obtained was then injected with the plasmids encoding CTN-1::YFPc and the transformation marker *Pmyo-2::DsRED2* (wp568). Epifluorescence of transgenic worms was visualized and photographed as described above.

Coimmunoprecipitation and western blot

HA-tagged CTN-1 and Myc-tagged SLO-1 were cloned into the pIRES2-mCherry and pIRES2-EGFP vectors (Clontech), respectively, to generate the following plasmids: CTN-1::HA-IRES2-mCherry (wp847), SLO-1::Myc-IRES2-EGFP (wp857), SLO-1(1–370)::Myc-IRES2-EGFP (wp932) and SLO-1(371–1140)::Myc-IRES2-EGFP (wp933). HEK293 cells were cultured in DMEM with 10% FBS, and transiently transfected with Lipofectamine 2000 (Invitrogen). Cells were harvested 48 hours after transfection, and lysed in 1% CHAPS/150 mM NaCl/1 mM CaCl_2 /62.5 mM Tris, pH 6.8 plus protease inhibitor (Roche). The supernatants of cell lysates were incubated with a Myc antibody (Santa Cruz Biotechnology) for 3 h at 4°C, and immunoprecipitated with protein A/G PLUS agarose (Santa Cruz Biotechnology) for 2 h at 4°C. Immune complexes were separated on 4–12% SDS–PAGE gels and probed with a HA antibody (NeoMarkers).

To examine SLO-1::GFP protein level expressed in worms, worms of mixed stages were homogenized in a lysis buffer (2% SDS/100 mM NaCl/10% glycerol/50 mM Tris, pH 6.8). Soluble protein extracts were separated on 4–12% SDS–PAGE gels and probed with GFP (Molecular Probes) and α -tubulin (Santa Cruz Biotechnology) antibodies. Anti-mouse IgG HRP (Santa Cruz Biotechnology) was used as the secondary antibody for detection by enhanced chemiluminescence (Pierce).

Surface biotinylation

Biotinylation assays were performed in transiently transfected HEK293 cells using the Cell Surface Protein Isolation Kit (Pierce). Surface proteins were biotinylated 48 hours after the transfection, precipitated with neutrAvidin-agarose beads, and eluted with SDS sample buffer (1% SDS/50 mM DTT/10% glycerol/62.5 mM Tris, pH 6.8). Total lysate or biotinylated proteins were separated by 4–12% SDS–PAGE, and the blots were detected as described above.

Recording of postsynaptic currents

An integrated *Punc-17::ChR-2::mCherry* transgene in the genetic background of wild-type worms (Liu *et al*, 2009) was crossed into *slo-1(md1745)*, *ctn-1(zw1)*, *slo-1(gf)* and *slo-1(gf);ctn-1(zw1)* strains. Both the *Punc-17::ChR-2::mCherry* and *Pslo-1::SLO-1(gf)* transgenes appeared to be integrated into the X chromosome at close proximity because we were unable to identify a worm homozygous for both transgenes from several hundreds of cross progeny. Therefore, worms heterozygous for both transgenes were used for experiments involving comparisons with *slo-1(gf)* mutant (Figure 5), whereas worms homozygous for *Punc-17::ChR-2::mCherry* were used for experiments that did not involve comparisons with *slo-1(gf)* (Figure 5). Both mPSCs and ePSCs were recorded from body-wall muscle cells using the voltage-clamp technique with the membrane potential held at -60 mV, as described previously (Liu *et al*, 2005, 2007). ePSCs were evoked by applying a pulse (3 ms) of blue light using a 470 ± 20 nm excitation filter (59222, Chroma Technology Corp.), and a light source equipped with a shutter (Lambda XL with SmartShutter, Sutter Instrument). The recording pipette solution contained (in mM) 120 KCl, 20 KOH, 5 Tris, 0.25 CaCl_2 , 4 MgCl_2 , 36 sucrose, 5 EGTA and 4 Na_2ATP (pH 7.2). Two external solutions with different $[\text{Ca}^{2+}]$ (5 and 0.25 mM) were used. The external solution with the higher $[\text{Ca}^{2+}]$ contained (in mM) 140 NaCl, 5 KCl, 5 CaCl_2 , 5 MgCl_2 , 11 dextrose and 5 HEPES (pH 7.2). This solution was modified by reducing CaCl_2 to 0.25 mM and increasing NaCl to 145 mM to make the external solution with the lower $[\text{Ca}^{2+}]$.

Xenopus oocyte expression

Capped cRNAs were synthesized using the mMessage mMachine Kit (Ambion). Approximately 50 nl cRNA (1 ng/nl) was injected into each oocyte using a Drummond Nanoject II injector (Drummond Scientific). Inside-out patches were obtained from the oocyte 2–3 days after cRNA injection. Macroscopic currents induced by voltage steps (-80 to $+160$ mV in 20 mV increments, 50 ms duration) were amplified with a Multiclamp 700B amplifier (Molecular Devices), and acquired with the Clampex software (version 10.2, Molecular Devices). Data were sampled at 10 kHz after filtering at 2 kHz.

Compositions of the pipette solution included (in mM) 140 K^+ gluconate, 1 Mg^{2+} gluconate and 5 HEPES (pH 7.2). Three cytoplasmic solutions (pH 7.2) that differed in free $[\text{Ca}^{2+}]$ were used to perfuse the cytoplasmic side of the patches. All cytoplasmic solutions contained 140 mM K^+ gluconate and 10 mM HEPES. Besides, other components were added to adjust free $[\text{Ca}^{2+}]$ (1 mM Ca^{2+} gluconate for 1 mM $[\text{Ca}^{2+}]$, 0.1 mM Ca^{2+} gluconate for 100 μM $[\text{Ca}^{2+}]$ and 3.39 mM Ca^{2+} gluconate plus 5 mM HEDTA for 10 μM free $[\text{Ca}^{2+}]$). Free $[\text{Ca}^{2+}]$ was calculated using online software (<http://maxchelator.stanford.edu>).

Data analysis

Electrophysiological data. Amplitude and frequency of mPSCs were analysed using MiniAnalysis (Synaptosoft, Inc., Decatur, GA). A detection threshold of 10 pA was used in initial automatic analysis, followed by visual inspections to include missed smaller events (5 pA or larger) and to exclude false events resulting from baseline fluctuations. Amplitudes of ePSCs were measured with the Clampfit software (Molecular Devices). The average of the two largest ePSCs from each experiment was used for statistical analysis. Peak macroscopic currents from isolated oocyte patches were determined and used to plot the conductance–voltage relationship.

Behavioural data. The longest series of successive worm images showing continuous forward locomotion (10–20 s) was chosen from the 30-s recording of each worm. To find head-bending angles, a custom MATLAB program was used to detect the shape of the

worm, place 13 equally spaced markers along the midline, and distinguish the head and tail (Supplementary Figure S1A). The angle supplementary to the angle formed by two straight lines connecting the marker points 1 and 2, and the marker points 2 and 3 (Supplementary Figure S1B) was plotted over successive frames to produce a sinusoidal head-bending trace (Supplementary Figure S1D). From the head-bending trace, the maximal-bending angle was found as the full amplitude of bending between the ventral and dorsal sides (Supplementary Figure S1C). Although the maximal-bending angle metric provides an intuitive description of the data, it does not account for smaller and/or irregular oscillations. To mathematically quantify the magnitude of the head bending as a whole, the root mean square of the trace was also calculated.

Data graphing and statistical analyses. Graphing and statistical analyses were performed with Origin (version 8.0, OriginLab). One-way ANOVA (followed by Bonferroni's *post hoc* test) was used for statistical comparisons. $P < 0.01$ is considered to be statistically significant. All values are expressed as mean $\pm$ s.e. n is the number of patches or muscle cells analysed.

References

- Adelman JP, Shen KZ, Kavanaugh MP, Warren RA, Wu YN, Lagrutta A, Bond CT, North RA (1992) Calcium-activated potassium channels expressed from cloned complementary DNAs. *Neuron* **9**: 209–216
- Adler EM, Augustine GJ, Duffy SN, Charlton MP (1991) Alien intracellular calcium chelators attenuate neurotransmitter release at the squid giant synapse. *J Neurosci* **11**: 1496–1507
- Altun ZF, Chen B, Wang ZW, Hall DH (2009) High resolution map of Caenorhabditis elegans gap junction proteins. *Dev Dyn* **238**: 1936–1950
- Bargmann CI (1998) Neurobiology of the Caenorhabditis elegans genome. *Science* **282**: 2028–2033
- Barstead RJ, Waterston RH (1989) The basal component of the nematode dense-body is vinculin. *J Biol Chem* **264**: 10177–10185
- Blake DJ, Weir A, Newey SE, Davies KE (2002) Function and genetics of dystrophin and dystrophin-related proteins in muscle. *Physiol Rev* **82**: 291–329
- Brelidze TI, Niu X, Magleby KL (2003) A ring of eight conserved negatively charged amino acids doubles the conductance of BK channels and prevents inward rectification. *Proc Natl Acad Sci USA* **100**: 9017–9022
- Brenner R, Chen QH, Vilaythong A, Toney GM, Noebels JL, Aldrich RW (2005) BK channel beta4 subunit reduces dentate gyrus excitability and protects against temporal lobe seizures. *Nat Neurosci* **8**: 1752–1759
- Brenner R, Jegla TJ, Wickenden A, Liu Y, Aldrich RW (2000a) Cloning and functional characterization of novel large conductance calcium-activated potassium channel beta subunits, hKCNMB3 and hKCNMB4. *J Biol Chem* **275**: 6453–6461
- Brenner R, Perez GJ, Bonev AD, Eckman DM, Kosek JC, Wiler SW, Patterson AJ, Nelson MT, Aldrich RW (2000b) Vasoregulation by the beta1 subunit of the calcium-activated potassium channel. *Nature* **407**: 870–876
- Butler A, Tsunoda S, McCobb DP, Wei A, Salkoff L (1993) mSlo, a complex mouse gene encoding “maxi” calcium-activated potassium channels. *Science* **261**: 221–224
- Chen B, Liu Q, Ge Q, Xie J, Wang ZW (2007) UNC-1 regulates gap junctions important to locomotion in *C. elegans*. *Curr Biol* **17**: 1334–1339
- Davies AG, Pierce-Shimomura JT, Kim H, VanHoven MK, Thiele TR, Bonci A, Bargmann CI, McIntire SL (2003) A central role of the BK potassium channel in behavioral responses to ethanol in *C. elegans*. *Cell* **115**: 655–666
- Davis MW, Hammarlund M, Harrach T, Hullett P, Olsen S, Jorgensen EM (2005) Rapid single nucleotide polymorphism mapping in *C. elegans*. *BMC Genomics* **6**: 118
- Demacio PC, Ray PN (2001) Alpha-catulin maps to the familial dysautonomia region on 9q31. *Genome* **44**: 990–994
- Du W, Bautista JF, Yang H, Diez-Sampedro A, You SA, Wang L, Kotagal P, Luders HO, Shi J, Cui J, Richerson GB, Wang QK (2005)

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

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Conflict of interest

The authors declare that they have no conflict of interest.

- Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. *Nat Genet* **37**: 733–738
- Francis GR, Waterston RH (1985) Muscle organization in *Caenorhabditis elegans*: localization of proteins implicated in thin filament attachment and I-band organization. *J Cell Biol* **101**: 1532–1549
- Hiatt SM, Shyu YJ, Duren HM, Hu CD (2008) Bimolecular fluorescence complementation (BiFC) analysis of protein interactions in *Caenorhabditis elegans*. *Methods* **45**: 185–191
- Hirsch J, Leipziger J, Frobe U, Schlatter E (1993) Regulation and possible physiological role of the Ca^{2+} -dependent K^{+} channel of cortical collecting ducts of the rat. *Pflügers Arch* **422**: 492–498
- Hu H, Shao LR, Chavoshy S, Gu N, Trieb M, Behrens R, Laake P, Pongs O, Knaus HG, Ottersen OP, Storm JF (2001) Presynaptic Ca^{2+} -activated K^{+} channels in glutamatergic hippocampal terminals and their role in spike repolarization and regulation of transmitter release. *J Neurosci* **21**: 9585–9597
- Huang Y, Chung YW, Wong PY (1999) Potassium channel activity recorded from the apical membrane of freshly isolated epithelial cells in rat caudal epididymis. *Biol Reprod* **60**: 1509–1514
- Issa NP, Hudspeth AJ (1994) Clustering of Ca^{2+} channels and Ca^{2+} -activated K^{+} channels at fluorescently labeled presynaptic active zones of hair cells. *Proc Natl Acad Sci USA* **91**: 7578–7582
- James AF, Okada Y (1994) Maxi K^{+} channels from the apical membranes of rabbit oviduct epithelial cells. *J Membr Biol* **137**: 109–118
- Janssens B, Staes K, van Roy F (1999) Human alpha-catulin, a novel alpha-catenin-like molecule with conserved genomic structure, but deviating alternative splicing. *Biochim Biophys Acta* **1447**: 341–347
- Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R (2002) Crystal structure and mechanism of a calcium-gated potassium channel. *Nature* **417**: 515–522
- Jospin M, Jacquemond V, Mariol MC, Segalat L, Allard B (2002) The L-type voltage-dependent Ca^{2+} channel EGL-19 controls body wall muscle function in *Caenorhabditis elegans*. *J Cell Biol* **159**: 337–348
- Kim H, Pierce-Shimomura JT, Oh HJ, Johnson BE, Goodman MB, McIntire SL (2009) The dystrophin complex controls bk channel localization and muscle activity in *Caenorhabditis elegans*. *PLoS Genet* **5**: e1000780
- Knaus HG, Folander K, Garcia-Calvo M, Garcia ML, Kaczorowski GJ, Smith M, Swanson R (1994) Primary sequence and immunological characterization of beta-subunit of high conductance Ca^{2+} -activated K^{+} channel from smooth muscle. *J Biol Chem* **269**: 17274–17278
- Knaus HG, Schwarzer C, Koch RO, Eberhart A, Kaczorowski GJ, Glossmann H, Wunder F, Pongs O, Garcia ML, Sperk G (1996) Distribution of high-conductance Ca^{2+} -activated K^{+} channels in

- rat brain: targeting to axons and nerve terminals. *J Neurosci* **16**: 955–963
- Knudsen KA, Soler AP, Johnson KR, Wheelock MJ (1995) Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. *J Cell Biol* **130**: 67–77
- Levy DI, Wanderling S, Biemesderfer D, Goldstein SA (2008) MiRP3 acts as an accessory subunit with the BK potassium channel. *Am J Physiol Renal Physiol* **295**: F380–F387
- Liewald JF, Brauner M, Stephens GJ, Bouhours M, Schultheis C, Zhen M, Gottschalk A (2008) Optogenetic analysis of synaptic function. *Nat Methods* **5**: 895–902
- Liu Q, Chen B, Gaier E, Joshi J, Wang ZW (2006) Low conductance gap junctions mediate specific electrical coupling in body-wall muscle cells of *Caenorhabditis elegans*. *J Biol Chem* **281**: 7881–7889
- Liu Q, Chen B, Ge Q, Wang ZW (2007) Presynaptic Ca^{2+} /calmodulin-dependent protein kinase II modulates neurotransmitter release by activating BK channels at *Caenorhabditis elegans* neuromuscular junction. *J Neurosci* **27**: 10404–10413
- Liu Q, Chen B, Yankova M, Morest DK, Maryon E, Hand AR, Nonet ML, Wang ZW (2005) Presynaptic ryanodine receptors are required for normal quantal size at the *Caenorhabditis elegans* neuromuscular junction. *J Neurosci* **25**: 6745–6754
- Liu Q, Hollopeter G, Jorgensen EM (2009) Graded synaptic transmission at the *Caenorhabditis elegans* neuromuscular junction. *Proc Natl Acad Sci USA* **106**: 10823–10828
- Llinas R, Sugimori M, Silver RB (1992) Microdomains of high calcium concentration in a presynaptic terminal. *Science* **256**: 677–679
- Merdek KD, Nguyen NT, Toksoz D (2004) Distinct activities of the alpha-catenin family, alpha-catenin and alpha-catenin, on beta-catenin-mediated signaling. *Mol Cell Biol* **24**: 2410–2422
- Meredith AL, Thorneioe KS, Werner ME, Nelson MT, Aldrich RW (2004) Overactive bladder and incontinence in the absence of the BK large conductance Ca^{2+} -activated K^{+} channel. *J Biol Chem* **279**: 36746–36752
- Misonou H, Menegola M, Buchwalder L, Park EW, Meredith A, Rhodes KJ, Aldrich RW, Trimmer JS (2006) Immunolocalization of the Ca^{2+} -activated K^{+} channel Slo1 in axons and nerve terminals of mammalian brain and cultured neurons. *J Comp Neurol* **496**: 289–302
- Muller SL, Portwich M, Schmidt A, Utepbergenov DI, Huber O, Blasig IE, Krause G (2005) The tight junction protein occludin and the adherens junction protein alpha-catenin share a common interaction mechanism with ZO-1. *J Biol Chem* **280**: 3747–3756
- Nelson WJ (2008) Regulation of cell-cell adhesion by the cadherin-catenin complex. *Biochem Soc Trans* **36** (Part 2): 149–155
- Nieset JE, Redfield AR, Jin F, Knudsen KA, Johnson KR, Wheelock MJ (1997) Characterization of the interactions of alpha-catenin with alpha-actinin and beta-catenin/plakoglobin. *J Cell Sci* **110** (Part 8): 1013–1022
- Nonet ML, Staunton JE, Kilgard MP, Fergestad T, Hartwig E, Horvitz HR, Jorgensen EM, Meyer BJ (1997) *Caenorhabditis elegans* rab-3 mutant synapses exhibit impaired function and are partially depleted of vesicles. *J Neurosci* **17**: 8061–8073
- Okkema PG, Harrison SW, Plunger V, Aryana A, Fire A (1993) Sequence requirements for myosin gene expression and regulation in *Caenorhabditis elegans*. *Genetics* **135**: 385–404
- Pacha J, Frindt G, Sackin H, Palmer LG (1991) Apical maxi K channels in intercalated cells of CCT. *Am J Physiol* **261** (4 Part 2): F696–F705
- Pallanck L, Ganetzky B (1994) Cloning and characterization of human and mouse homologs of the *Drosophila* calcium-activated potassium channel gene, slowpoke. *Hum Mol Genet* **3**: 1239–1243
- Park B, Nguyen NT, Dutt P, Merdek KD, Bashir M, Sterpetti P, Tosolini A, Testa JR, Toksoz D (2002) Association of Lbc Rho guanine nucleotide exchange factor with alpha-catenin-related protein, alpha-catenin/CTNNA1, supports serum response factor activation. *J Biol Chem* **277**: 45361–45370
- Pluznick JL, Wei P, Carmines PK, Sansom SC (2003) Renal fluid and electrolyte handling in BKCa-beta1-/- mice. *Am J Physiol Renal Physiol* **284**: F1274–F1279
- Pyott SJ, Glowatzki E, Trimmer JS, Aldrich RW (2004) Extrasynaptic localization of inactivating calcium-activated potassium channels in mouse inner hair cells. *J Neurosci* **24**: 9469–9474
- Pyott SJ, Meredith AL, Fodor AA, Vazquez AE, Yamoah EN, Aldrich RW (2007) Cochlear function in mice lacking the BK channel alpha, beta1, or beta4 subunits. *J Biol Chem* **282**: 3312–3324
- Raffaelli G, Saviane C, Mohajerani MH, Pedarzani P, Cherubini E (2004) BK potassium channels control transmitter release at CA3-CA3 synapses in the rat hippocampus. *J Physiol* **557** (Part 1): 147–157
- Roberts WM, Jacobs RA, Hudspeth AJ (1990) Colocalization of ion channels involved in frequency selectivity and synaptic transmission at presynaptic active zones of hair cells. *J Neurosci* **10**: 3664–3684
- Robitaille R, Garcia ML, Kaczorowski GJ, Charlton MP (1993) Functional colocalization of calcium and calcium-gated potassium channels in control of transmitter release. *Neuron* **11**: 645–655
- Ruttiger L, Sausbier M, Zimmermann U, Winter H, Braig C, Engel J, Knirsch M, Arntz C, Langer P, Hirt B, Muller M, Kopschall I, Pfister M, Munkner S, Rohbock K, Pfaff I, Rusch A, Ruth P, Knipper M (2004) Deletion of the Ca^{2+} -activated potassium (BK) alpha-subunit but not the BKbeta1-subunit leads to progressive hearing loss. *Proc Natl Acad Sci USA* **101**: 12922–12927
- Salkoff L, Butler A, Ferreira G, Santi C, Wei A (2006) High-conductance potassium channels of the SLO family. *Nat Rev Neurosci* **7**: 921–931
- Sausbier M, Hu H, Arntz C, Feil S, Kamm S, Adelsberger H, Sausbier U, Sailer CA, Feil R, Hofmann F, Korth M, Shipston MJ, Knaus HG, Wolfer DP, Pedroarena CM, Storm JF, Ruth P (2004) Cerebellar ataxia and Purkinje cell dysfunction caused by Ca^{2+} -activated K^{+} channel deficiency. *Proc Natl Acad Sci USA* **101**: 9474–9478
- Segal Y, Reuss L (1990) Maxi K^{+} channels and their relationship to the apical membrane conductance in *Necturus* gallbladder epithelium. *J Gen Physiol* **95**: 791–818
- Shyu YJ, Hiatt SM, Duren HM, Ellis RE, Kerppola TK, Hu CD (2008) Visualization of protein interactions in living *Caenorhabditis elegans* using bimolecular fluorescence complementation analysis. *Nat Protoc* **3**: 588–596
- Takeuchi S, Marcus DC, Wangemann P (1992) Maxi K^{+} channel in apical membrane of vestibular dark cells. *Am J Physiol* **262** (6 Part 1): C1430–C1436
- Uebele VN, Lagrutta A, Wade T, Figueroa DJ, Liu Y, McKenna E, Austin CP, Bennett PB, Swanson R (2000) Cloning and functional expression of two families of beta-subunits of the large conductance calcium-activated K^{+} channel. *J Biol Chem* **275**: 23211–23218
- Wallner M, Meera P, Toro L (1999) Molecular basis of fast inactivation in voltage and Ca^{2+} -activated K^{+} channels: a transmembrane beta-subunit homolog. *Proc Natl Acad Sci USA* **96**: 4137–4142
- Wang ZW (2008) Regulation of synaptic transmission by presynaptic CaMKII and BK channels. *Mol Neurobiol* **38**: 153–166
- Wang ZW, Saifee O, Nonet ML, Salkoff L (2001) SLO-1 potassium channels control quantal content of neurotransmitter release at the *C. elegans* neuromuscular junction. *Neuron* **32**: 867–881
- Weiger TM, Holmqvist MH, Levitan IB, Clark FT, Sprague S, Huang WJ, Ge P, Wang C, Lawson D, Jurman ME, Glucksmann MA, Silos-Santiago I, DiStefano PS, Curtis R (2000) A novel nervous system beta subunit that downregulates human large conductance calcium-dependent potassium channels. *J Neurosci* **20**: 3563–3570
- Werner ME, Zvara P, Meredith AL, Aldrich RW, Nelson MT (2005) Erectile dysfunction in mice lacking the large-conductance calcium-activated potassium (BK) channel. *J Physiol* **567** (Part 2): 545–556
- Wiesner C, Winsauer G, Resch U, Hoeth M, Schmid JA, van Hengel J, van Roy F, Binder BR, de Martin R (2008) Alpha-catenin, a Rho signalling component, can regulate NF-kappaB through binding to IKK-beta, and confers resistance to apoptosis. *Oncogene* **27**: 2159–2169
- Xia X, Hirschberg B, Smolik S, Forte M, Adelman JP (1998) dSLo interacting protein 1, a novel protein that interacts with large-conductance calcium-activated potassium channels. *J Neurosci* **18**: 2360–2369
- Xia XM, Ding JP, Lingle CJ (1999) Molecular basis for the inactivation of Ca^{2+} - and voltage-dependent BK channels in adrenal chromaffin cells and rat insulinoma tumor cells. *J Neurosci* **19**: 5255–5264
- Xia XM, Ding JP, Zeng XH, Duan KL, Lingle CJ (2000) Rectification and rapid activation at low Ca^{2+} of Ca^{2+} -activated, voltage-

- dependent BK currents: consequences of rapid inactivation by a novel beta subunit. *J Neurosci* **20**: 4890–4903
- Yazejian B, Sun XP, Grinnell AD (2000) Tracking presynaptic Ca^{2+} dynamics during neurotransmitter release with Ca^{2+} -activated K^+ channels. *Nat Neurosci* **3**: 566–571
- Yuan P, Leonetti MD, Pico AR, Hsiung Y, Mackinnon R (2010) Structure of the human BK channel Ca^{2+} -activation apparatus at 3.0 Å resolution. *Science* **329**: 182–186
- Yusifov T, Savalli N, Gandhi CS, Ottolia M, Olcese R (2008) The RCK2 domain of the human BKCa channel is a calcium sensor. *Proc Natl Acad Sci USA* **105**: 376–381
- Zhou Y, Schopperle WM, Murrey H, Jaramillo A, Dagan D, Griffith LC, Levitan IB (1999) A dynamically regulated 14-3-3, Slob, and Slowpoke potassium channel complex in *Drosophila* presynaptic nerve terminals. *Neuron* **22**: 809–818