Two Rab2 Interactors Regulate Dense-Core Vesicle Maturation

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SUMMARY

Peptide neuromodulators are released from a unique organelle: the dense-core vesicle. Dense-core vesicles are generated at the trans-Golgi and then sort cargo during maturation before being secreted. To identify proteins that act in this pathway, we performed a genetic screen in Caenorhabditis elegans for mutants defective in dense-core vesicle function. We identified two conserved Rab2-binding proteins: RUND-1, a RUN domain protein, and CCCP-1, a coiled-coil protein. RUND-1 and CCCP-1 colocalize with RAB-2 at the Golgi, and rab-2, rund-1, and cccp-1 mutants have similar defects in sorting soluble and transmembrane dense-core vesicle cargos. RUND-1 also interacts with the Rab2 GAP protein TBC-8 and the BAR domain protein RIC-19, a RAB-2 effector. In summary, a pathway of conserved proteins controls the maturation of dense-core vesicles at the trans-Golgi network.

INTRODUCTION

The nervous system has two modes of chemical signaling at synapses. Fast signaling occurs by the release of small-molecule neurotransmitters that activate ligand-gated channels. Slow signaling occurs by the release of neuromodulators, such as neuropeptides or monoamines, that activate G protein-coupled receptors. Fast neurotransmitters are released from synaptic vesicles, whereas many neuromodulators are released from dense-core vesicles (DCVs). A wide variety of biological processes are regulated by DCV secretion, including cell survival, neuronal development, synaptic plasticity, and excitability.

The pathway for the generation of DCVs in neurons is likely to be related to the generation of secretory vesicles in neuroendocrine cells such as chromaffin cells or the insulin-secreting cells of the pancreas (Borgonovo et al., 2006; Kim et al., 2006; Park and Loh, 2008; Tooze et al., 2001). The secretory granules are generated at the trans-Golgi and undergo several maturation steps, including the homotypic fusion of immature secretory granules, and removal of some soluble and transmembrane cargo (Ahras et al., 2006; Dittie et al., 1996; Kakhlon et al., 2006; Klumperman et al., 1998; Tooze et al., 1991; Urbé et al., 1998; Wendler et al., 2001). Cargo sorting relies on poorly defined motifs that probably interact with multiple sorting receptors (Dikeakos and Reudelhuber, 2007; Dikeakos et al., 2009). It is likely that DCVs in neurons undergo similar maturation processes at the Golgi, but some mechanisms may differ since these cells have a very different architecture.

The rich variety of small Rab GTPase proteins provides vesicles in all trafficking pathways with identity. Rabs orchestrate numerous aspects of vesicle trafficking including vesicle budding, transport, tethering, uncoating, and fusion (Stenmark, 2009; Zerial and McBride, 2001). There are about 60 different Rabs in humans and 26 in C. elegans (Lundquist, 2006; Pereira-Leal and Seabra, 2001). Rabs are localized to different intracellular compartments and help provide specificity to vesicular trafficking (Chavrier et al., 1990). In C. elegans, Rab2 (RAB-2) localizes to the Golgi (Sumakovic et al., 2009) and rab-2 mutants (also known as unc-108) have defects in sorting soluble and transmembrane DCV cargos (Edwards et al., 2009; Sumakovic et al., 2009). Thus, there are likely to be Rab2 effectors involved in DCV cargo sorting.

Here we use a genetic screen to identify proteins involved in DCV function. We identify two proteins that are Rab2 interactors: RUND-1 and CCCP-1. RUND-1 and CCCP-1 are conserved proteins that colocalize with RAB-2 at the trans-Golgi. RUND-1 and CCCP-1 bind to activated RAB-2, and like rab-2 mutants, rund-1 and cccp-1 mutants exhibit defects in sorting soluble and transmembrane DCV cargo. We conclude that RUND-1 and CCCP-1 may function as RAB-2 effectors in DCV maturation at the trans-Golgi.

RESULTS

A Genetic Screen for Regulators of Dense-Core Vesicle Function

In C. elegans, loss-of-function mutants in the trimeric G protein Gαq (encoded by egl-30) have a straight posture and are almost immobile on food (Brundage et al., 1996). Activated Gαq mutants
exhibit the opposite phenotype; these mutants are hyperactive and exhibit tightly coiled body bends (Bastiani et al., 2003). Mutants in the DCV secretion protein CAPS (encoded by the unc-31 gene) have a straight posture and are almost immobile on food (Avery et al., 1993), similar to egl-30(lf) mutants. Mutations in CAPS suppress activated alleles of Gaq (Charlie et al., 2006), suggesting that these proteins act in a pathway. Thus, mutations in other genes involved in DCV biogenesis and secretion should also suppress activated alleles of egl-30. We screened for genetic suppressors of the activating Gq mutation egl-30(tg26) (Doi and Iwasaki, 2002). egl-30(tg26) mutants have hyperactive, loopy locomotion and are smaller and slower growing than the wild-type (Movies S1 and S2 available online). We searched for less hyperactive animals among the F2 progeny of ENU-mutagenized egl-30(tg26). We isolated six independent alleles of unc-31, validating the strategy of the screen. In addition, we isolated recessive mutations in six complementation groups with locomotion phenotypes reminiscent of unc-31, though somewhat weaker. These mutants exhibit a novel locomotion phenotype, which we call “unmotivated.” When separated from the activated Gq mutation, the unmotivated mutants have a fairly normal posture but exhibit little spontaneous movement on food (Movie S3). However, they are capable of coordinated locomotion when stimulated. Conditions that stimulate movement include starvation, harsh touch, and UV light (Movies S3 and S4). Thus, these genes appear to function in the regulation of movement rather than the execution of coordinated movements.

We mapped and characterized in detail two of the six strong unmotivated mutants, rund-1 and cccp-1. Two mutations (ox281 and ox328) define the gene rund-1. Mutations at a second locus, conserved coiled-coil protein-1 (cccp-1), caused a similar phenotype. The mutation cccp-1(ox334) was isolated in the screen, and we found that it failed to complement an existing locomotion mutant e1122 (previously known as unc-81).

**rund-1 and cccp-1 Encode Conserved Proteins**

We cloned rund-1 and cccp-1 by standard methods (Experimental Procedures). The rund-1 phenotype is fully rescued by the single gene T19D7.4. This open reading frame encodes a protein of 549 amino acids that has two coiled-coil (CC) domains and a RUN domain. ox281 is a missense mutation in the second coiled-coil domain, ox281 is a splice site mutation. The tm3622 deletion is marked, but because this deletion starts in an intron (A), its effect on the protein is unknown. Cccp-1b has two transcripts. (B) Domain structures of RUND-1 and CCCP-1. RUND-1 is a 549 amino acid protein with two coiled-coil (CC) domains and a RUN domain. The position of the ox328 T237P mutation is shown by an asterisk.

(B) Domain structures of RUND-1 and CCCP-1. RUND-1 is a 549 amino acid protein with two coiled-coil (CC) domains and a RUN domain. Cccp-1b has two transcripts. (B) Domain structures of RUND-1 and CCCP-1. RUND-1 is a 549 amino acid protein with two coiled-coil (CC) domains and a RUN domain. The position of the ox328 T237P mutation is shown by an asterisk.

(C) Alignment of the second coiled-coil domain of RUND-1 and its orthologs from Trichoplax adhaerens (Trichoplax), C. elegans (worm), Drosophila melanogaster (fly), and Homo sapiens (human). The position of the ox328 T237P mutation is shown by an asterisk.

(D) Alignment of the RUN domain of RUND-1. The six conserved blocks A–F of the RUN domain (Callebaut et al., 2001) are marked with black bars. The tm3622 deletion removes the A block but ends before the beginning of the B block. See also Figures S1 and S2.
humans and Drosophila, and even the primitive metazoan Trichoplax adhaerens (Figure S1). RUND-1 shows conservation throughout the length of the protein; the second coiled-coil domain and the RUN domain are especially well conserved (Figures 1C and 1D).

RUND-1 and its human ortholog RUNDC1 are about 32% identical (Figure S1). We found that human RUNDC1 could rescue the worm rund-1 mutants (Table S1; two-tailed p < 0.0001 for both strains, Fisher’s exact test). RUNDC1 was tagged with tagRFP and exhibited a cellular localization pattern similar to the native RUND-1 (data not shown). Thus, the function and localization of RUND-1 are conserved in the human ortholog RUNDC1.

cccp-1 corresponds to the open reading frame Y49E10.23. cccp-1 has two alternatively spliced transcripts, cccp-1a and cccp-1b, which encode proteins of 734 or 743 amino acids with multiple coiled-coil domains (Figures 1A and 1B). Like RUND-1, CCCC-1 has a single conserved ortholog in other metazoans including flies and humans and is not found outside metazoans (Figure S2).

rund-1 Mutants Exhibit Unmotivated Locomotion

The unmotivated phenotype is typified by inactivity on food (Movie S3). We used a computer-tracking system to characterize the movement of animals over a 30 min period. The speed of wild-type animals is highest immediately after a harsh mechanical stimulation and then decays over approximately the next 20 to 30 min to a stable baseline rate (Figure 2A).

rund-1 Mutants Exhibit Unmotivated Locomotion, but Respond to Stimulation

(A and B) Locomotion of wild-type (A) and rund-1 mutants (B). The graphs plot mean speed during the 30 min after transfer to a new plate. Wild-type locomotion is stimulated by transfer and decays to baseline in 20 min. rund-1 mutants are stimulated by transfer, but less than wild-type, and have a reduced baseline locomotion rate. n = 21–24 animals.

(C) Left: mean crawling speed during the first 2 min after transfer to a new plate. All three rund-1 mutants exhibited reduced speed (**p < 0.001 versus wild-type) and the rundown-1(tm3622) mutant is fully rescued by a rundown-1(+ single-copy transgene. Right: expression of rundown-1(+) in the nervous system rescues the rund-1 mutant. The rundown-1 cDNA was expressed in either the nervous system (Prab-3) or the intestine (Pvha-6). Expression in the nervous system rescued (**p < 0.01), but expression in the intestine did not (p > 0.05). Error bars, SEM; n = 21–27 (left) and n = 9 (right).

(D) The rundown-1 locomotion defect is rescued by expression of a rundown-1(+) transgene in mature animals. The frequency of body bends was measured during the first 2 min after transfer to a new plate. Heatshock-induced expression of rundown-1(+) fully rescued the rundown-1 mutant defect (**p < 0.001 versus no heatshock). Heatshock did not affect rundown-1 (p > 0.05). +hs, heatshock; –hs, no heatshock. Error bars, SEM; n = 10.

(E) rundown-1 and rab-2 act in the same genetic pathway to regulate locomotion. Mean speed was measured for 2 min after transfer to a new plate. The rab-2 rundown-1 double mutant is similar to the rundown-1 single mutant. Error bars, SEM; n = 24–31.

(F) rundown-1 and cccp-1 act in the same genetic pathway to regulate locomotion. Frequency of body bends ("thrashes") in liquid was reduced for rundown-1(ox281), cccp-1(ox334) and rundown-1(ox281); cccp-1(ox334) mutants compared to wild-type (p < 0.001). The cccp-1 rundown-1 double mutant is similar to the single mutants (p > 0.05). Error bars, SEM; n = 8–9.

(G) rundown-1 and ric-19 act in parallel to regulate locomotion. Frequency of body bends was measured for two minutes after transfer to a new plate. ric-19(pk690); rundown-1(tm3622) animals show a more severe defect than either single mutant (***p < 0.001; **p < 0.01). ric-19 is not significantly different from wild-type (p > 0.05).

The wild-type and rundown-1 data are identical to (D); these experiments were performed together. Error bars, SEM; n = 10.

See also Figures S3 and S7 and Table S2.
wild-type (Figures 2B and 2C; Figures S3B–S3D) and the animals become mostly inactive after 15 min; the phenotype is fully rescued by a single-copy insertion of the wild-type rund-1(+) gene (Figure 2C; Figure S3E).

The unmotivated phenotype is reminiscent of the locomotion of unc-31/CAPS mutants. CAPS is required for DCV fusion (Ann et al., 1997; Gracheva et al., 2007; Speese et al., 2007; Walent et al., 1992; Zhou et al., 2007) and may also regulate synaptic vesicle exocytosis (Gracheva et al., 2007; Jockusch et al., 2007). Mutants lacking CAPS/UNC-31 are paralyzed on food but not off food (Avery et al., 1993; Speese et al., 2007); rundown-1 mutants also exhibit increased speed off food. rundown-1 and unc-31 mutants also have defects in egg laying and defecation and enter the dauer diapause stage at high temperature (Alion et al., 1999; Avery et al., 1993; Speese et al., 2007; data not shown). Also, both unc-31 and rundown-1 mutants can be suppressed by an activating mutation in the Gαs ortholog gsa-1 (Charlie et al., 2006; data not shown). Thus, rundown-1 probably regulates a DCV pathway shared by CAPS.

Although the unmotivated phenotype resembles a worm behavior called “quiescence,” the resemblance is superficial (Van Buskirk and Sternberg, 2007; Gaglia and Kenyon, 2009; Singh et al., 2011; You et al., 2008). First, unlike rundown-1 mutants, quiescent animals do not forage for food or eat. Second, quiescence can be suppressed by mutations in the cyclic GMP-dependent protein kinase egl-4, but rundown-1 mutants are not suppressed by egl-4 (data not shown). Third, quiescence depends on function of the ALA neuron and is suppressed by mutations that affect ALA development such as ceh-17. rundown-1 mutants, however, are not suppressed by ceh-17 (data not shown). We conclude that unmotivated locomotion is a novel worm phenotype.

**rund-1 Acts in Mature Neurons to Regulate Locomotion**

We determined the cellular expression patterns of the rundown-1 and cccp-1 genes by fusing their promoters to GFP. Prund-1::GFP was expressed in most or all neurons, the pharynx, the intestine, the spermatheca, and the uterus (Figures S4A and S4B) but was not seen in skin or muscle cells. Pcccp-1::GFP was also expressed in most or all neurons, the intestine, and the spermatheca but was not observed in the pharynx, skin, or muscle (Figure S4C).

To determine the cellular focus of the locomotory phenotype, we expressed rundown-1 and cccp-1 under the control of tissue-specific promoters. Expression of rundown-1(+) under the neuronal specific rab-3 promoter rescued rundown-1 mutant locomotion, but expression of rundown-1 in the intestine using the vha-6 promoter did not rescue (Figure 2C; Figures S3G–S3I). Under its own promoter, the tagged cccp-1 cDNA rescued the cccp-1 mutant locomotion defect (data not shown). This transgene was primarily expressed in the nervous system but at low levels. Interestingly, the cccp-1 gene caused defects in locomotion in wild-type worms when expressed under the more highly expressed rab-3 promoter (data not shown). Thus, both reduced expression and overexpression of cccp-1 in neurons cause defects in locomotion.

To determine whether rundown-1 affects the development of neurons, we first examined neuronal architecture using the GABA neuron marker Punc-47::GFP (McIntire et al., 1997). No defects were seen in the rundown-1 mutant, indicating that rundown-1 is not generally required for axon guidance or maintenance (data not shown). Second, we examined the distribution of synaptic varicosities by imaging fluorescently tagged synaptobrevin, a synaptic vesicle protein (Punc-129::mCherry::SNB-1). No defects were observed in rundown-1 mutants, indicating that rundown-1 is not required for synapse development (Figures S5A and S5B).

The lack of a role in development was confirmed by rescuing the phenotype postdevelopmentally. We expressed the rundown-1 cDNA fused to tagRFP under a heat shock promoter. rundown-1 mutant animals carrying this construct were heat shocked at various stages of larval development. Twenty-four hours after heat-shock, adults were fully rescued for locomotion on food or in liquid (Figure 2D and data not shown). However, 5 hr after heat-shock, animals exhibited no discernible rescue, even though RFP expression was detected in neurons. Thus, rundown-1 acts in mature neurons but in a rather slow process.

**RUND-1 and CCCP-1 Act in the Same Genetic Pathway as RAB-2**

The presence of a RUN domain suggested that rundown-1 may interact with a small GTPase in either the Rab or Rap family. RAB-2 is a Golgi Rab that functions in DCV maturation in *C. elegans* (Edwards et al., 2009; Sumakovic et al., 2009). Mutants lacking the rab-2 gene (also called unc-108) exhibit similar but somewhat stronger locomotion defects than rundown-1 (Chun et al., 2008; Edwards et al., 2009; Sumakovic et al., 2009). Like rundown-1, rab-2 mutants exhibit little spontaneous movement on food but move normally when stimulated by UV light (Edwards et al., 2009). Unlike rundown-1, rab-2 mutants cannot be stimulated by a harsh mechanical stimulus (Figure 5A).

The phenotypic similarity between rundown-1, cccp-1, and rab-2 mutants suggests that they may act in the same pathway, and analysis of double mutants supports this conclusion. First, rab-2, rundown-1, and cccp-1 mutations all suppress the hyperactive locomotion of the activated Gq mutant egl-30(tg26) (data not shown). Second, double mutants among rab-2, rundown-1, and cccp-1 do not show enhanced locomotion defects (Figures 2E and 2F; Figure 5A; data not shown). These data suggest that RAB-2, RUND-1, and CCCP-1 function together in DCV maturation.

DCV maturation involves the sorting of neuropeptide precursors into vesicles; the vesicles are then acidified, which activates the endopeptidases that cleave the precursors into mature neuropeptides. In *C. elegans*, there are four proprotein convertases that process neuropeptides. Most peptides in neurons are processed by the convertase EGL-3 (Husson et al., 2006; Kass et al., 2001), though other convertases can contribute to the processing of peptides and some peptides are not processed at all (Leinwand and Chalasani, 2013; Pierce et al., 2001). Interestingly, egl-3 mutants have a weaker locomotion defect than the unmotivated mutants rab-2, rundown-1, and cccp-1. However, when egl-3 is combined with rab-2, rundown-1, or cccp-1, the double mutants are essentially paralyzed (Figures 5B and 5C; data not shown), indicating that egl-3 acts in parallel to these genes. The double mutants are both qualitatively and quantitatively similar to unc-31/CAPS mutants (Edwards et al., 2009;...
Sumakovic et al., 2009; Figures S7B–S7D and data not shown). These data support a model in which the unmotivated class of proteins, RAB-2, RUND-1, and CCCP-1, act in parallel to EGL-3 to regulate locomotion, perhaps via different DCV cargos; both pathways may require UNC-31/CAPS for secretion.

**RUND-1 and CCCP-1 Are Required to Sort Dense-Core Vesicle Cargo**

To test whether *rund-1* affects synaptic or dense-core vesicle transport, we examined the ultrastructure of *rund-1(tm3622)* using electron microscopy. *rund-1* does not exhibit defects in synaptic vesicle number (Figure 3) or localization of synaptic vesicles (Figures S5C–S5G). Also, DCVs of a *C. elegans* cargo trafficking. IDA-1 is the ventral and dorsal nerve cords of the *rund-1(tm3622)* mutants (Edwards et al., 2009). However, in both *rab-2 egl-3* and *rund-1 egl-3* double mutants, there is still a reduction in axonal fluorescence compared to *egl-3* single mutants (Edwards et al., 2009; Sumakovic et al., 2009; data not shown), suggesting that there is also reduced trafficking of unprocessed cargo in *rab-2* and *rund-1* mutants.

To assay the release of DCV cargo, we measured the accumulation of Venus fluorescence in coelomocytes, scavenger cells residing in the body cavity of the worm (Sieburth et al., 2007; Speese et al., 2007). *cccp-1* mutants have reduced fluorescence in coelomocytes, suggesting that missorted cargo is not released in *cccp-1* mutants. *rund-1* mutants lack fluorescence in axons but accumulate fluorescence in the coelomocytes at wild-type levels (Figure 4D), suggesting that an alternative release pathway in the cell body is still intact in *rund-1* mutants. This may be due to missorting of cargo to the constitutive secretory pathway.

**RUND-1 and CCCP-1 Are Localized to the trans-Golgi in Neuronal Cell Bodies**

The *rund-1* gene was tagged at its C terminus with GFP, tdRFP, or tdEos and inserted as a single copy in the genome. These constructs fully rescued the *rund-1* mutant locomotion defect of fluorescence in the dendrites (Figure 4D). Expression of the *rund-1* cDNA in the same cells fully rescued the *rund-1* trafficking defect (Figure 4C), indicating that RUND-1 acts cell autonomously.

**Figure 3. RUND-1 Is Not Required to Make DCVs**

(A) Electron microscopy of synapses in the ventral nerve cord of wild-type and *rund-1(tm3622)*. White arrowheads point to DCVs. Black arrowheads point to the presynaptic density. Scale bar, 200 nm.

(B) Quantification of synaptic vesicles (SVs) and dense-core vesicles (DCVs) at synapses in the ventral cord (V) or dorsal cord (D). Values are represented as mean ± SEM.
RUND-1 localized mainly to two or three perinuclear puncta in neuronal cell bodies; only extremely faint fluorescence was observed in axons (Figure 5; Figure S8A). We examined colocalization of RUND-1::tagRFP with GFP-tagged markers for various compartments (Figure 5A). All markers were expressed as single-copy insertions since overexpression of some markers can change the size of the corresponding compartment (Bucci et al., 1992, 2000). RUND-1 did not localize to compartments that communicate with the Golgi, including the ER (TRAM-1), the early endosome (RAB-5), recycling endosome (RAB-11), late endosome (RAB-7), or with the general endosome marker syntaxin-13 (SYN-13). RUND-1 showed partial overlap with a cis-Golgi marker (the COP I vesicle marker ε-COP) and more overlap with a medial-Golgi marker (AMAN-2, mannosidase II). In neuronal cell bodies, RUND-1 showed almost perfect colocalization with the trans-Golgi markers RAB-6.2 and syntaxin 6 (SYX-6). RAB-6.2 is localized predominantly to the trans-Golgi and is also found on exocytotic vesicles leaving the Golgi (Goud et al., 1990; Grigoriev et al., 2007); syntaxin 6 is a trans-Golgi SNARE also found on immature secretory granules in neuroendocrine cells (Bock et al., 1997; Klumperman et al., 1998). We conclude that RUND-1 localizes to the trans-Golgi or to vesicles closely associated with the trans-Golgi. Localization of RAB-6.2 and syntaxin 6 were not altered in a rund-1 mutant background (data not shown), suggesting that rund-1 is not required for normal Golgi structure.

To confirm that RUND-1 localized to the Golgi, we performed correlative nanometer-resolution fluorescence electron microscopy (Watanabe et al., 2011). Fluorescence from the tdEos-tagged RUND-1 was localized using superresolution PALM imaging and superimposed on scanning electron micrographs. RUND-1 was localized to the Golgi in neuronal cell bodies (Figure S8B) and in pharyngeal cells (data not shown).
To determine which part of RUND-1 mediates its localization, we split RUND-1 into two pieces and tagged each with tagRFP. The coiled-coil domain of RUND-1 was spread diffusely throughout the cell body and axons (Figure 5D). In contrast, the RUN domain of RUND-1 was sufficient for localization to perinuclear puncta (Figure 5D). Neither truncation rescued a rundown-1 mutant, suggesting that both domains are required for normal function but that the RUN domain is sufficient for localization.

We also tagged CCCP-1 with GFP and expressed it using the Prab-3 neuronal promoter. CCCP-1 is localized mainly to perinuclear puncta in neuronal cell bodies (Figure 5C) but unlike RUND-1 is also more diffusely spread throughout cell bodies and axons. This pattern resembles that of RAB-2 (Edwards et al., 2009; Sumakovic et al., 2009). To confirm the broader localization of RAB-2, we inserted a GFP::RAB-2 single-copy transgene in the genome (Figure S8A). CCCP-1 is still punctate when coexpressed with RAB-2(GDP), which is diffusely expressed.

(D) The RUN domain of RUND-1 mediates its localization. Full-length RUND-1, the coiled-coil domain, and the RUN domain were tagged at their C termini with tagRFP-T and integrated in the genome. The truncated proteins were expressed at lower levels. See also Figure S8.

Figure 5. RUND-1 Colocalizes with RAB-2 at the trans-Golgi Network

(A) RUND-1 colocalizes with RAB-2 and trans-Golgi markers. Each panel shows a single slice of a confocal image of motor neuron cell bodies in the ventral cord of young adult animals. The top boxes show the localization of a single-copy rescuing RUND-1::tagRFP-T fusion protein (oxIs590). RUND-1 localizes almost exclusively to two or three perinuclear puncta per cell. The middle boxes show the localization of single-copy GFP-tagged compartment markers. The bottom boxes show the merged images. Scale bar, 5 μm, applies to all panels. RUND-1 shows tightest colocalization with RAB-6.2, a trans-Golgi Rab protein, and with SYX-6, the ortholog of the trans-Golgi SNARE syntaxin 6. RUND-1 puncta also colocalize well with RAB-2 puncta. RUND-1 partially overlaps with the medial-Golgi marker mannosidase II (AMAN-2) and the cis-Golgi marker εCOP. RUND-1 is not colocalized with the rough ER marker TRAM-1 or with the endosomal markers RAB-5, RAB-11.1, RAB-7, and SYN-13.

(B) RUND-1 localizes to the Golgi. Left: a backscatter scanning electron micrograph of the cell body of a neuron in the nerve ring. Right: the same image overlaid with the corresponding fluorescence PALM image of RUND-1::tdEos. Scale bar, 1 μm.

(C) CCCP-1 colocalizes with RUND-1 and RAB-2. CCCP-1 colocalizes with RUND-1 and RAB-2(GTP). CCCP-1 is still punctate when coexpressed with RAB-2(GDP), which is diffusely expressed.

(D) The RUN domain of RUND-1 mediates its localization. Full-length RUND-1, the coiled-coil domain, and the RUN domain were tagged at their C termini with tagRFP-T and integrated in the genome. The truncated proteins were expressed at lower levels. See also Figure S8.
RUND-1 and CCCP-1 Bind RAB-2 Specifically

Using yeast two-hybrid assays, we examined the interactions of full-length RUND-1 and CCCP-1 with three different forms of RAB-2: (1) the constitutively active GTP-bound form RAB-2(GTP), (2) the constitutively inactive GDP-bound form RAB-2(GDP), and (3) the wild-type RAB-2. RUND-1 interacted with GTP-bound RAB-2 but not with the GDP-bound RAB-2 or the wild-type RAB-2 (Figure 6A). Thus, like other RUN-domain proteins, the interaction of RUND-1 with RAB-2 is specific to the activated GTP-bound form of the Rab. CCCP-1 interacted with both the GTP-bound and wild-type RAB-2 but not with the GDP-bound RAB-2 (Figure 6A). This suggests a stronger interaction of RAB-2 and CCCP-1 that is still GTP dependent, given that some of the wild-type RAB-2 is expected to be GTP bound. RUND-1 and CCCP-1 did not interact with each other in two-hybrid assays (data not shown).

To test for specificity of these interactions, we performed yeast two-hybrid assays with RUND-1 or CCCP-1 and GTP-binding versions of other members of the Rab, Rap, Ras, and Ral families of small GTPases. RUND-1 and CCCP-1 showed an interaction only with RAB-2 (Figure 6B; data not shown). Thus, RUND-1 and CCCP-1 are specific RAB-2 interactors.

We split RUND-1 into two pieces and assayed binding to RAB-2. The coiled-coil domain fragment did not interact with GTP-bound RAB-2. The RUN domain fragment interacted robustly with GTP-bound RAB-2 (Figure 6C). This construct carries the entire RUN domain and a putative upstream α-helix that is needed for proper folding of other RUN domains (Kukimoto-Niino et al., 2006; Recacha et al., 2009). A shorter construct lacking the upstream α-helix and the beginning of the conserved A block of the RUN domain did not show an interaction with RAB-2 (data not shown). Thus, it seems likely that the RUN domain is required for the interaction of RUND-1 with RAB-2.

RUND-1 Binds RIC-19 and TBC-8

RAB-2 binds TBC-8, a putative RAB-2 GAP, and RIC-19, a BAR domain protein (Hannemann et al., 2012; Sumakovic et al., 2009). RUND-1 also interacts with TBC-8 in yeast two-hybrid assays and with both TBC-8 and RIC-19 when coexpressed in HEK293 cells (Figures 6D and 6E). In contrast, CCCP-1 did not interact with TBC-8 or RIC-19 in two-hybrid assays (data not shown). Thus, RUND-1, but not CCCP-1, independently binds RAB-2 and other RAB-2 partners including RIC-19 and TBC-8.

TBC-8 consists of an N-terminal RUN domain and a C-terminal TBC domain responsible for the GAP activity. A fragment of TBC-8 carrying its RUN domain binds RUND-1, but fragments carrying only the TBC GAP domain do not bind (Figures 6E and 6F). The TBC-8 RUN domain also binds RIC-19 (Hannemann et al., 2012). The RUND-1 coiled-coil domains do not interact with TBC-8, but the RUND-1 RUN domain exhibited a weak interaction with TBC-8 (data not shown). Thus, RUND-1 and TBC-8 interact via regions containing their RUN domains.

To determine whether ric-19 and tbc-8 exhibit genetic interactions with rund-1, we built double mutants and assayed locomotion and DCV cargo trafficking. Although ric-19 had little effect on locomotion on its own (Sumakovic et al., 2009), it significantly enhanced the locomotion defect of rund-1 (Figure 2G). Similarly, ric-19 enhanced the NLP-21::Venus trafficking defect of rund-1 (Figure 6B). These data indicate that RIC-19 acts in parallel to RUND-1, possibly as two distinct effectors of RAB-2. tbc-8 mutants also have defects in sorting vesicle cargo (Hannemann et al., 2012). However the sorting defects in rund-1 tbc-8 mutants were not enhanced in rund-1 tbc-8 double mutants (Figure 6B), indicating that tbc-8 and rund-1 act in the same pathway.

DISCUSSION

We performed a genetic screen for mutants defective in dense-core vesicle function and identified two proteins, RUND-1 and CCCP-1. RUND-1 and CCCP-1 are required for proper sorting of DCV cargo but are not required for DCV morphology or transport. Genetic and biochemical data show that these proteins are probably Rab2 interactors at the trans-Golgi. RUND-1 also interacts with the BAR domain protein RIC-19 and the RAB-2 GAP TBC-8, both of which individually interact with RAB-2. These results identify a set of interacting proteins that function in the trafficking of DCV cargos.

RUND-1 and CCCP-1 Interact with RAB-2 at the Golgi

The function of a Rab protein is defined by its localization to a specific cellular compartment and by its effector proteins. Rab2 was originally localized to the ER-Golgi intermediate compartment (Chavrier et al., 1990) and was thought to function in ER-to-Golgi anterograde trafficking (Tisdale et al., 1992), but Rab2 is distributed more broadly across the Golgi and may have additional roles (Chun et al., 2008; Sumakovic et al., 2009). Known effectors of Rab2 include several coiled-coil proteins associated with the Golgi (Hayes et al., 2009; Short et al., 2001; Sinka et al., 2008) and the BAR domain protein ICA69/RIC-19 (Buffa et al., 2008; Sumakovic et al., 2009).

Here we demonstrate that the RUN domain protein RUND-1 and the conserved coiled-coil protein CCCP-1 are possible novel effectors of RAB-2. Five lines of evidence support this conclusion. First, rundown-1, cccp-1, andrab-2 mutants exhibit a similar locomotory phenotype. Second, rundown-1, cccp-1, andrab-2 mutants exhibit similar defects in trafficking DCV cargo. Third, rundown-1, cccp-1, andrab-2 act in the same genetic pathway. Fourth, RUND-1, CCCP-1, and RAB-2 colocalize at the trans-Golgi network. Fifth, RUND-1 and CCCP-1 bind to the active GTP-bound form of RAB-2 but not the inactive GDP-bound form.

Classical Rab effectors are recruited to a compartment by binding to the active GTP-bound Rab. For example, RIC-19 is recruited to the Golgi by GTP-RAB-2 and dispersed into the cytoplasm by GDP-RAB-2 (Sumakovic et al., 2009). However, neither RUND-1 nor CCCP-1 localization is dependent on RAB-2. RUND-1 localization is mediated by its RUN domain but independently of RAB-2 binding. Recently, the RAB-10 effector
EHBP-1 has been shown instead to mediate localization of its cognate Rab (Shi et al., 2010). However, RAB-2 localization does not depend on either RUND-1 or CCCP-1. Nor are RUND-1 and CCCP-1 required for each other’s localization. Thus, RUND-1, CCCP-1, and RAB-2 all appear to localize independently or have multiple binding partners at the Golgi.

RUND-1 is a Member of the RUN Domain Protein Family

RUND-1, CCCP-1, and RAB-2 are all members of the RUN domain protein family. RUN domain proteins were originally proposed to be potential Rab and Rap effectors (Callebaut et al., 2001). Clear physical interactions with Rab and Rap proteins have been shown for some of these proteins (Bayer et al., 2005; Cormont et al., 2001; Fouraux et al., 2004; Janoueix-Lerosey et al., 1995,

Figure 6. RUND-1 and CCCP-1 Interact Physically with Activated RAB-2

(A) RUND-1 interacts specifically with GTP-bound RAB-2 (RAB-2 GTP) in a yeast two-hybrid assay. RUND-1 did not show an interaction with wild-type RAB-2 (RAB-2 WT) or inactive GDP-bound RAB-2 (RAB-2 GDP). CCCP-1 interacts with RAB-2 GTP and RAB-2 WT, but not with RAB-2 GDP. Growth without histidine (− his) indicates a physical interaction.

(B) The RUND-1 and CCCP-1 interactions with RAB-2 are specific. The interactions of RUND-1 and CCCP-1 with C. elegans RAB proteins were examined by yeast two hybrid. Numbers indicate the number of the RAB gene in C. elegans (e.g., 1 = RAB-1). RUND-1 and CCCP-1 interact only with RAB-2. RAB-27 could not be tested because of self-activation.

(C) RUND-1 interacts with RAB-2 via the RUN domain. Two truncations of RUND-1 were used: RUND-1 (CC) consists of amino acids 1–261 and RUND-1 (RUN) consists of amino acids 262–549.

(D) RUND-1 interacts with RIC-19 and TBC-8. V5-tagged RUND-1 was coexpressed with GFP, GFP::RIC-19, or GFP::TBC-8 in HEK293 cells. Immunoprecipitation of GFP::RIC-19 or GFP::TBC-8 pulled down RUND-1. Immunoprecipitation of untagged GFP did not pull down RUND-1. IN, input; IP, immunoprecipitation; IB, immunoblotting.

(E) RUND-1 interacts with TBC-8 outside of its TBC domain. Truncations of TBC-8 were examined for interactions with RUND-1 by yeast two hybrid.

(F) RUND-1 interacts with TBC-8 outside of its TBC domain. V5-tagged TBC-8 (1–597 aa) was coexpressed with GFP or GFP::RUND-1 in HEK293 cells. Immunoprecipitation of GFP::RUND-1 pulled down TBC-8 (1–597). IN, input; IP, immunoprecipitation; IB, immunoblotting.
We demonstrate that the RUN domain of RUND-1 is sufficient to interact with RAB-2. Like for other RUN domains, this interaction is specific to GTP-bound RAB-2. Moreover, RAB-2 is probably the only small GTPase that interacts with RUND-1. Most RUN domain proteins studied interact with at most a single Rab protein, although this has not been exhaustively analyzed (Bayer et al., 2005; Fukuda et al., 2011; Miserey-Lenkei et al., 2007; Recacha et al., 2009). A recent genome-wide analysis detected Rab interactors for only six of 19 mammalian RUN domain proteins by yeast two-hybrid assays (Fukuda et al., 2011). However, this study did not detect an interaction between the mammalian orthologs of RUND-1 and RAB-2, so it is possible that weaker interactions were missed.

In addition to the RUN domain, most RUN domain proteins have other interaction domains (Callebaut et al., 2001). RUND-1 has two N-terminal coiled-coil domains. Coiled-coil domains typically mediate protein-protein interactions and are especially common in RUN domain proteins (Cormont et al., 2001; Fouraux et al., 2004; Janoueix-Lerosey et al., 1998; Lan et al., 2005; Matsunaga et al., 2009; Mori et al., 2007; Yang et al., 2002; Zhong et al., 2009). Unlike the coiled-coil domains of some Rab effectors such as the golgins (Burguete et al., 2008; Hayes et al., 2009; Short et al., 2001; Sinka et al., 2008), the coiled-coil domains of RUND-1 are not required for binding to RAB-2. However, the coiled-coil domains of RUND-1 are conserved, and the ox328 missense mutation in the second coiled-coil domain is a severe allele. It seems likely that the coiled-coil domains of RUND-1 mediate important interactions with other proteins acting in the RAB-2 pathway.

**Function of RAB-2 and Its Interactors**

RAB-2 appears to have at least three interacting proteins in the DCV maturation pathway: RUND-1, CCCP-1, and RIC-19. These proteins could act separately as independent interactors of RAB-2, or they could act together in a single complex. The data are consistent with the possibility that RAB-2 forms a single complex with RUND-1, RIC-19, and TBC-8 since these proteins exhibit direct pairwise interactions and are colocalized at the Golgi (Figure 7A). In contrast, CCCP-1 interacts only with RAB-2 and may act as an independent effector. Interestingly, the locomotion and DCV cargo trafficking phenotypes ofrab-2, rund-1, and cccp-1 are similar but not identical, indicating that there are distinct functions for each protein. The locomotion and trafficking defects of rundown and ccckp-1 mutants are generally weaker than therab-2 mutant defects, indicating that RUND-1 and CCCP-1 may participate in only certain aspects of RAB-2 function or to only a partial degree. Also, rundown mutants do not impair cargo secretion as assayed by coelomocyte uptake, suggesting a different final destination for missorted cargos in rundown mutants versus ccckp-1 and rab-2 mutants (Sumakovici et al., 2009). However, the overt phenotypic similarities of these mutants as well as the genetic interactions suggest that these proteins act in the same process during cargo sorting into DCVs.

The primary cellular defect that we observe in these mutants is the reduced trafficking of fluorescent proteins targeted to DCVs, with no major effects on the morphology or transport of vesicles themselves. It is not clear which class of cargo accounts for the behavioral defects. The fact that the locomotion defects ofrab-2,
A Dense-Core Vesicle Cargo Trafficking Pathway

Table 1. Mutants Isolated as Gq Suppressors

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein Function</th>
<th>Number of Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>egl-30</td>
<td>Gq-alpha GTPase</td>
<td>6</td>
</tr>
<tr>
<td>ric-8</td>
<td>synembryn</td>
<td>1</td>
</tr>
<tr>
<td>egl-8</td>
<td>PLCbeta Gq effector, DAG synthesis</td>
<td>4</td>
</tr>
<tr>
<td>unc-13</td>
<td>Munc13 DAG binding, MUN domain, SV + DCV exocytosis</td>
<td>1</td>
</tr>
<tr>
<td>unc-31</td>
<td>CAPS MUN domain, DCV exocytosis</td>
<td>6</td>
</tr>
<tr>
<td>pck-1</td>
<td>Protein kinase C</td>
<td>1</td>
</tr>
<tr>
<td>unc-73</td>
<td>Trio Gq-alpha effector, Rho GEF</td>
<td>2</td>
</tr>
<tr>
<td>rund-1</td>
<td>Rundc1 Golgi Rab2 effector</td>
<td>2</td>
</tr>
<tr>
<td>cccp-1</td>
<td>C10orf118 Golgi Rab2 effector</td>
<td>1</td>
</tr>
<tr>
<td>13 others</td>
<td>–</td>
<td>15</td>
</tr>
</tbody>
</table>

rund-1, and cccp-1 mutants are enhanced by mutations in the proprotein convertase egl-3 indicates that the behaviorally relevant missorted cargos are not processed by EGL-3 but rather may be peptides processed by other convertases or cargos that do not require processing at all.

What are the functions of RAB-2, RUND-1, and CCCP-1 at the Golgi during maturation of DCVs? There are several possibilities, not all mutually exclusive, such as specifying vesicle identity, tethering, budding, or cargo sorting. First, vesicle identity is often conveyed by Rab proteins (Stenmark, 2009), and RAB-2 and its interactors could provide identity to maturing DCVs. In the absence of markers of identity, the vesicles may become endosomal in character and fuse with the endosomal system, thereby losing some of their cargos (Figure 7B). Second, RUND-1 and CCCP-1 may tether vesicles via the “tentacular matrix” that moves vesicles processively through the Golgi (Munro, 2011; Sinka et al., 2008; Yu and Hughson, 2010). The tentacular matrix is posited to be formed by the golgins, long coiled-coil proteins that localize to the Golgi and capture vesicles by binding Rab proteins. Third, RUND-1 may act directly in the formation of vesicles. RUND-1 and RAB-2 interact with RIC-19, which contains a crescent-shaped BAR domain that may induce membrane curvature (Frost et al., 2009). Finally, RAB-2 and its interactors could be involved more directly in sorting. Since none of the proteins have a transmembrane domain, they are unlikely to serve as a sorting receptor per se but may be involved in the retrograde retrieval of a sorting receptor.

The possible RUND-1 complex may consist not only of the RAB-2 interactors RIC-19 and RUND-1 but the RAB-2 GAP protein TBC-8, which should turn off the Rab. The combination of positive and negative factors suggests that the complex may be quite dynamic. The binding of interactors to RAB-2 may simultaneously recruit the GAP that will hydrolyze GTP and cause dissolution of the complex. Such fine temporal control of RAB-2 activation may help deliver vesicles to their trans-Golgi acceptor and then release them from the acceptor complex as maturation proceeds.

The human ortholog of RUND-1 is RUNDC1, which is highly conserved in both the coiled-coil and RUN domains. When RUNDC1 is expressed in rundown-1 mutants, the human protein is localized to the Golgi and rescues the mutant phenotype, indicating a strong conservation of function. Human RUNDC1 has been identified as an inhibitor of the tumor suppressor p53 (Llanos et al., 2006), and RUNDC1 expression is used as a prognostic marker of metastasis in breast cancer tumors (van ‘t Veer et al., 2002). Thus, RUNDC1 is a possible oncoogene. RUNDC1 has not been reported to have any function in the nervous system but maps to chromosomal position 17q21, in the same region of a likely autism gene (Cantor et al., 2005). An individual with autism has also been found to carry a de novo nonsense mutation in Rab2A, the human ortholog of RAB-2 (Sanders et al., 2012). Because Rundc1 and Rab2A are expressed in the mouse brain (Allen Brain Atlas), it is possible that RUND-1 and its interactors function in DCV cargo trafficking in the vertebrate brain as well as in nematodes.

EXPERIMENTAL PROCEDURES

Strains

Worm strains were cultured and maintained using standard methods (Brenner, 1974). A complete list of strains and mutations used is provided in the Supplemental Experimental Procedures.

Screen for Suppressors of Activated Gq

egl-30(tg26) encodes an activating mutation in Gq. We mutagenized egl-30(tg26) worms with 0.5 mM ENU (N-ethyl-N-nitosourea) for 4 hr as described in De Stasio and Dorman (2001). We screened the Fl generation for suppression of the hyperactive locomotion phenotype of egl-30(tg26). From screens of ~18,000 mutagenized haploid genomes, we isolated 43 mutants. Most of these mutants suppressed not only the hyperactivity of egl-30(tg26) but also the small size and slower growth rate, indicating that they specifically suppress Gq and do not affect locomotory function indirectly. To more generally test for the specificity of suppression, we built double mutants between egl-30(tg26) and a number of mutations that impair movement by affecting function of the neuromuscular junction: the N-type calcium channel unc-2, the synaptic vesicle docking/priming protein unc-18, the synaptic vesicle kinesin unc-104, the ryanodine receptor unc-68, the proprotein convertase egl-3, and the acetylcholine receptor assembly factors unc-50 and unc-74. In all cases, the double mutants exhibited phenotypes that resembled a combination of the two individual mutants, indicating additive effects with egl-30(tg26). Locomotion rates were reduced, but double mutants were uncoordinated, small, and slower growing than either of the parents. Thus, general disruptions of neuronal or muscular function do not nonspecifically suppress egl-30(tg26).

We mapped 39 of the suppressors and assigned them to 22 different complementation groups (Table 1). Several were known to act in the Gq pathway, including loss-of-function mutations in egl-30/Gqα, ric-8/Gqα, Gef, unc-73/Ric-8, egl-30/Phospholipase Cβ3, and the DAG-binding synaptic protein UNC-13. Others included genes thought to be involved in DCV secretion, including unc-31/CAPS, and pck-1/protein kinase C (Sieburth et al., 2007).

The remaining 18 unidentified mutants comprised 15 complementation groups with one or two alleles each. Mutants in six genes had a strong unmotivated locomotion phenotype. We characterized two of these in detail, rundown-1 and cccp-1.

Mapping and Cloning rundown-1 and cccp-1

The rundown-1(ox281) mutation was mapped to the left arm of chromosome X using SNPs in the Hawaiian strain CB4856 (Davis et al., 2005). Fine mapping, facilitated by picking recombinants between rundown-1(ox281) and dpy-3, narrowed the rundown-1 interval to a 60 kb region with nine predicted genes, most on cosmid T19D7. Injection of cosmid T19D7 into rundown-1(ox281) rescued the mutant phenotype. RNAi of the gene T19D7.4 caused a delecation defect reminiscent of rundown-1 mutants. We sequenced the T19D7.4 gene in the rundown-1 mutants ox281 and ox328. rundown-1(ox281) is a T to A transition in the splice acceptor of the sixth intron. rundown-1(ox328) is an A to C transversion.

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that causes a T237P missense mutation in the second coiled-coil domain. We rescued rundown-1 mutants with a transgene containing only T19D7.4, confirming the gene identification.

The ccpp-1 (ox334) mutation was mapped to a 126 kb region on the left arm of chromosome III with 16 predicted genes. Because no C. elegans cosmids covered this region, we obtained two C. briggsae BACs (from CHORI) carrying orthologs of the genes in this region and injected them into ccpp-1 (ox334) mutants. The BAC RPCI94_09N13 rescued the ccpp-1 locomotion defect, while the BAC RPCI94_26P21 did not. RPCI94_09N13 carries orthologs of two C. elegans genes in this region, Y49E10.23 and Y49E10.24. Both ccpp-1 alleles introduce early stop mutations in Y49E10.23: ccpp-1 (ox334) carries a T to A transversion in exon 7 and ccpp-1(e1122) carries a C to T transition in exon 9, leading to stop mutations at L343 and 448, respectively. We subsequently rescued ccpp-1 (ox334) with a transgene containing only Y49E10.23.

**Locomotion Assays**

We performed tracking assays by videotaping worms and processing the movies using a custom-made plugin for ImageJ (White et al., 2007). Assays were initiated by transferring three to five well-fed worms with a platinum worm pick to a tracking plate with a spot of food. Movies were taken for 30 min. For the rare periods in which a worm left the food, the data were omitted. For all experiments except the tissue-specific rescue of rundown-1, data were collected on 2 different days for each strain and combined. There was no significant day-to-day variation.

To measure stimulated locomotion on food, body bends were counted in the first 2 min after placing animals on thin lawns of bacteria. A body bend was defined as the movement of the tail from maximum to minimum amplitude of the sine wave (Miller et al., 1999). For heatshock expression, animals were exposed to 34°C for 1 hr and assayed 24 hr later as adults. To measure locomotion in liquid, we assayed thrashing as described in Hobson et al. (2011). We placed animals in a drop of M9 on an unseeded plate, waited for 1 min, and then counted thrashes for 90 s. A thrash was defined as a change in the direction of the bend in the middle of the animal. All body bending and thrashing assays were performed on at least 2 different days for each set of strains, but each graph shows the data from a single representative experiment.

**Imaging and Image Analysis**

Worms were mounted on 2% agarose pads and anesthetized with 50 mM sodium azide. Images were obtained using a Zeiss Pascal confocal microscope, a Leica SP2 confocal microscope, or a Nikon 80i wide-field compound microscope. To image the dorsal or ventral nerve cords, we oriented young adult animals with dorsal or ventral side up by exposure to the anesthetic for 10 min on the slide before placing the coverslip. For quantitative imaging of dorsal cord fluorescence, all strains in a given experiment were imaged on the same days and all microscope settings were kept constant. The same section of the dorsal cord posterior of the vulva was imaged in all worms. Maximum intensity projections were quantified using ImageJ software, measuring the total fluorescence in a region of interest encapsulating the cord and subtracting the background fluorescence of a region of identical size adjacent to the cord. For colocalization images, Pearson’s correlation coefficients were calculated using Nikon Elements software by selecting a circular region of interest around individual cell bodies in the ventral cord.

**Statistics**

p values were determined using InStat 3.1a and GraphPad Prism 5.0d (GraphPad Software). Data sets were analyzed by a one-way ANOVA to test for differences among the set followed by a Bonferroni posthoc test to examine selected comparisons, or by the Kruskal-Wallis nonparametric ANOVA to test for differences among the set followed by Dunn’s test to examine selected comparisons. For categorical data, we calculated a two-sided p value using Fisher’s exact test.

For other methods see Supplemental Experimental Procedures.

**ACCESSION NUMBERS**

The GenBank accession numbers for the full-length sequences of the rundown-1 and ccpp-1b transcripts reported in this paper are JN986879 and JN988880.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, eight figures, two tables, and four movies and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2014.02.017.

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**REFERENCES**


Two Rab2 Interactors Regulate Dense-Core Vesicle Maturation

Michael Ailion, Mandy Hannemann, Susan Dalton, Andrea Pappas, Shigeki Watanabe, Jan Hegermann, Qiang Liu, Hsiao-Fen Han, Mingyu Gu, Morgan Q. Goulding, Nikhil Sasidharan, Kim Schuske, Patrick Hullett, Stefan Eimer, and Erik M. Jorgensen
Ailion et al.

Figure S7

**A**

*rab-2 rand-1 double mutant analysis*

- **Wild type**
- **rab-2(nu415)**
- **rand-1(tm3622)**
- **rab-2(nu415); rand-1(tm3622)**

**B**

*rab-2 egl-3 double mutant analysis*

- **rab-2(nu415)**
- **egl-3(ok979)**
- **rab-2(nu415); egl-3(ok979)**

**C**

*rand-1 egl-3 double mutant analysis*

- **rand-1(tm3622)**
- **egl-3(ok979)**
- **egl-3(ok979); rand-1(tm3622)**

**D**

*unc-31 crawling speed*

- **unc-31(e928)**
Figure S8

A

RUND-1::RFP

WT  rab-2  cccp-1

B

CCCP-1::GFP

WT  rab-2  rund-1

C

RAB-2::GFP

WT  rund-1  cccp-1

D

RUND-1::RFP

GFP markers

RAB-6.2  RAB-5  RAB-7

Merge

rab-2 mutant

E

Supplemental Information

Supplemental Figure and Movie Legends

Figure S1. Alignment of the full-length RUND-1 protein. (related to Figure 1)
Alignment of C. elegans RUND-1 (worm, accession # JN986879) and its orthologs from Trichoplax adhaerens (Trichoplax, hypothetical protein TRIADDRAFT_52054, accession # XP_002108010.1), Drosophila melanogaster (fly, CG3703, accession # NP_569874.1) and Homo sapiens (human, RUNDC1, accession # AAH39247.1). Identical residues are shaded in darker red and similar residues are shaded in lighter red. The coiled-coil domains (from SMART, using the worm protein; http://smart.embl-heidelberg.de/) are marked with double black bars. The six conserved blocks A-F of the RUN domain (Callebaut et al., 2001) are marked with single black bars. Alignment was made with MUSCLE (Edgar, 2004) using default parameters and exhibited with Boxshade 3.21 (http://www.ch.embnet.org/software/BOX_form.html).

Figure S2. Alignment of the full-length CCCP-1b protein. (related to Figure 1)
Alignment of C. elegans CCCP-1b (worm, accession # JN986880) and its orthologs from Drosophila melanogaster (fly, CG4925, accession # NP_648879.1) and Homo sapiens (human, C10orf118, accession # AAI03500.1). Identical residues are shaded in darker yellow and similar residues are shaded in lighter yellow. The coiled-coil domains (from SMART, using the worm protein; http://smart.embl-heidelberg.de/) are marked with double black bars. The positions of the ox334 and e1122 stop mutations are marked with asterisks. Alignment was made with MUSCLE (Edgar, 2004) using default parameters and exhibited with Boxshade 3.21 (http://www.ch.embnet.org/software/BOX_form.html).

Figure S3. Locomotion data. (related to Figure 2)
(A-E) Each graph shows the mean speed of 21 to 27 animals during the 30 minute period immediately after being transferred to a new plate. These graphs show the complete tracking data associated with the left side of Figure 2C.
(F-I) Each graph shows the mean speed of 9 animals during the 30 minute period immediately after being transferred to a new plate. These graphs show the complete tracking data associated with the right side of Figure 2C.

Figure S4. rund-1 and cccp-1 are expressed widely in neurons and other tissues.
Expression of rund-1 and cccp-1 promoter::GFP fusions are shown in transgenic animals carrying extrachromosomal arrays.
(A) An L4 stage late-larval animal is shown. Prund-1::GFP is expressed throughout the nervous system, including head and tail neurons and motor neurons in the ventral cord. Expression is also observed in the pharynx and intestine, but not in skin or muscle cells. This animal has intestinal expression in only the posterior intestine due to mosaicism of the extrachromosomal array. Scale bar: 50 µm.
(B) Expression of Prund-1::GFP in the spermatheca, uterus and ventral nerve cord (arrowheads) of a gravid adult. GFP and DIC images are superimposed. Scale bar: 10 µm.
(C) An L4 stage late-larval animal is shown. Pcccp-1::GFP is expressed throughout the nervous system, including head and tail neurons and motor neurons in the ventral cord. Expression is also seen in the intestine, but is not seen in pharynx, skin or muscle cells. This animal has intestinal expression in only the posterior intestine due to mosaicism of the extrachromosomal array. Scale bar: 50 µm.
Figure S5. rundown-1 and cccp-1 mutants do not have defects in development or function of synapses. (A) rundown-1(ox281) shows normal synaptic development as visualized by Punc-129::mCherry::snb-1 localization in the dorsal nerve cord of young adult animals. Scale bar: 5 μm. (B) Quantification of mCherry::SNB-1 fluorescence levels in the dorsal cord. The mean fluorescence intensity per μm is given in arbitrary units. The rundown-1(ox281) mutant has normal levels of mCherry::SNB-1 in the dorsal cord (P=0.55, two-tailed unpaired t test). Error bars = SEM; n = 6 animals each genotype. (C) Representative traces of endogenous currents (minis) in wild-type, rundown-1(tm3622) and cccp-1(ox334). (D,E) rundown-1 and cccp-1 mutants have normal mini frequency and amplitude (P>0.05, paired t test). Error bars = SEM; n = 8 animals each genotype. (F) Representative traces of electrically evoked currents in wild-type, rundown-1(tm3622) and cccp-1(ox334). (G) rundown-1 and cccp-1 mutants have normal evoked currents (P>0.05, paired t test). Error bars = SEM; n = 6-8 animals each genotype.

Figure S6. Dense-core vesicle trafficking phenotypes. (A) The graph shows quantification of INS-22::Venus fluorescence levels in the dorsal nerve cord. The images show representative examples of the data. rab-2, rundown-1, and cccp-1 mutants all have decreased trafficking of INS-22::Venus to the dorsal nerve cord (**, P<0.001 compared to wild type; **, P<0.01). Error bars = SEM; n = 18-21 animals each genotype. (B) rundown-1 and ric-19 act in parallel. The graph shows quantification of NLP-21::Venus fluorescence levels in the dorsal nerve cord. A ric-19; rundown-1 double mutant has a stronger defect than either a ric-19 or rundown-1 single mutant (***, P<0.001 for both comparisons). A tbc-8; rundown-1 double mutant does not have a stronger defect than the rundown-1 single mutant (P>0.05). Error bars = SEM; n = 14-70 animals each genotype.

Figure S7. Locomotion data. (related to Figure 2) Each graph shows the mean speed of 24 to 31 animals during a 30 minute period immediately after being transferred to a new plate. The graphs in panel A show the complete tracking data associated with Figure 2E. Graphs of rab-2, rundown-1, and egl-3 are repeated in panels A-C for ease of comparison. Though rab-2 mutants are similar to rundown-1 and cccp-1 mutants at steady state, rab-2 mutants are not stimulated by harsh touch like rundown-1, cccp-1, and even unc-31/CAPS mutants. The additional lack of stimulated locomotion in rab-2 mutants may be due to a RAB-2 function that does not require CAPS and thus is likely to be unrelated to dense-core vesicle function.

Figure S8. RUND-1, RAB-2 and CCCP-1 are not required for each other’s localization (related to Figure 5) Each panel shows a single slice of a confocal (A-C) or wide-field (D) image of motor neuron cell bodies in the ventral nerve cord of young adult animals. (A) RUND-1::RFP is still localized in rab-2(nu415) and cccp-1(ox334) mutants. The figure shows the expression of the single-copy transgene otxIs590. (B) CCCP-1::GFP is still localized in rab-2(nu415) and rundown-1(tm3622) mutants. The figure shows the expression of the extrachromosomal array oxEx1366[Pcccp-1::cccp-1(+)] cDNA::GFP. (C) GFP::RAB-2 is still localized in rundown-1(tm3622) and cccp-1(ox334) mutants. The figure shows the expression of the single-copy transgene oxiS314. GFP is concentrated in puncta in the cell body, but is also seen more diffusely throughout the cell body and the axons. (D) In a rab-2(nu415) mutant, RUND-1::RFP still tightly colocalizes with RAB-6.2, but not with RAB-5 or RAB-7. (E) RUND-1::RFP tightly colocalizes with RAB-6.2, but not with RAB-5 or RAB-7. The graph shows quantification of colocalization data using the Pearson’s correlation coefficient. RUND-1 is
significantly more colocalized with RAB-6.2 than with RAB-5 or RAB-7 (P<0.0001, two-tailed unpaired t tests), but no changes in colocalization are seen in a rab-2 mutant (P>0.1 for all three comparisons). Error bars = SEM; n = 10-18 cells each genotype.

Movie S1. Locomotion of the wild-type strain N2, showing normal sinusoidal movement.

Movie S2. Locomotion of the activated Gq mutant egl-30(tg26). egl-30(tg26) mutant worms are smaller than wild-type and have hyperactive locomotion with deeper body bends and more frequent reversals.

Movie S3. rund-1 mutants have unmotivated spontaneous locomotion but respond to touch. The movie shows a field of rund-1(ox328) mutant larval and adult animals on a bacterial lawn. The worms show normal foraging behavior, but little spontaneous locomotion. Beginning at ten seconds into the video, several worms are prodded with a platinum wire worm pick. After being touched, the stimulated worms move away, exhibiting slow but coordinated locomotion.

Movie S4. rund-1 mutants are stimulated by UV light. The movie shows a close-up of a single rund-1(ox328) adult on a bacterial lawn. It exhibits normal foraging and feeding behavior, but little spontaneous locomotion. At four seconds into the video, UV light is turned on. After a few seconds delay, the worm stops feeding and moves away, exhibiting coordinated locomotion.
Table S1. Rescue of *rund-1* mutants by the human ortholog RUNDC1.

<table>
<thead>
<tr>
<th>Strain genotype</th>
<th>Animals picked</th>
<th>Presence of array</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>rund-1</em>(ox281); <em>Prund-1::RUNDC1(+)::tagRFP</em></td>
<td>Unc 0 10</td>
<td>+ -</td>
</tr>
<tr>
<td></td>
<td>Non-Unc 10 1</td>
<td></td>
</tr>
<tr>
<td><em>rund-1</em>(tm3622); <em>Prund-1::RUNDC1(+)::tagRFP</em></td>
<td>Unc 1 9</td>
<td>+ -</td>
</tr>
<tr>
<td></td>
<td>Non-Unc 11 0</td>
<td></td>
</tr>
</tbody>
</table>

To examine rescue of *rund-1* mutant locomotion by transgenic expression of its human ortholog RUNDC1, approximately ten putative Unc (non-rescued) and Non-Unc (rescued) adult animals were selected from a plate of each strain under a dissecting microscope and subsequently examined for the presence of the extrachromosomal array as scored by fluorescence. The experimenter was blind to the presence of the array at the time the animals were picked. There is strong correlation of the locomotion phenotype to the presence of the array in both strains, indicating that they are rescued (Fisher’s Exact Test, two-tailed P value, P<0.0001 for both strains).
<table>
<thead>
<tr>
<th>Comparison</th>
<th>Test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2 vs. rund-1(tm3622)</td>
<td>Kruskal-Wallis/Dunn</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N2 vs. rund-1(ox281)</td>
<td>Kruskal-Wallis/Dunn</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N2 vs. rund-1(ox328)</td>
<td>Kruskal-Wallis/Dunn</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N2 vs. oxIs590[rund-1(+)]; rund-1(tm3622)</td>
<td>Kruskal-Wallis/Dunn</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>rund-1(tm3622) vs. rund-1(ox281)</td>
<td>Kruskal-Wallis/Dunn</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>rund-1(tm3622) vs. rund-1(ox328)</td>
<td>Kruskal-Wallis/Dunn</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>rund-1(ox281) vs. rund-1(ox328)</td>
<td>Kruskal-Wallis/Dunn</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>N2 vs. rund-1(ox281); oxEx1197 [Prab-3::rund-1(+)]</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.05</td>
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<tr>
<td>rund-1(ox281) vs. rund-1(ox281); oxEx1197 [Prab-3::rund-1(+)]</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.01</td>
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<td>&gt;0.05</td>
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<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.001</td>
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<tr>
<td>rab-2(nu415) vs. rab-2(nu415); rund-1(tm3622)</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>rab-2(nu415) vs. unc-31(e928)</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>rab-2(nu415) vs. egl-3(ok979); rab-2(nu415)</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rund-1(tm3622) vs. rab-2(nu415); rund-1(tm3622)</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.001</td>
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<tr>
<td>rund-1(tm3622) vs. egl-3(ok979); rund-1(tm3622)</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.001</td>
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<td>egl-3(ok979) vs. unc-31(e928)</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.001</td>
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<td>One-way ANOVA/Bonferroni</td>
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<tr>
<td>egl-3(ok979) vs. egl-3(ok979); rund-1(tm3622)</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>unc-31(e928) vs. egl-3(ok979); rab-2(nu415)</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.001</td>
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<tr>
<td>unc-31(e928) vs. egl-3(ok979); rund-1(tm3622)</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>egl-3(ok979); rab-2(nu415) vs. egl-3(ok979); rund-1(tm3622)</td>
<td>One-way ANOVA/Bonferroni</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Extended Experimental Procedures

Analysis of *rund-1* and *cccp-1* cDNAs

We obtained the *rund-1* cDNA from the ORFeome library, and three predicted full-length SL1 trans-spliced *rund-1* cDNAs from Yuji Kohara. Restriction digests and partial sequencing indicated that all four cDNAs had the same splicing pattern (Figure 1A), with several differences from the gene structure predicted on Wormbase (exon 6 was 174 bp shorter than predicted on Wormbase, and Wormbase exon 7 was not present). The cDNAs *yk772b6*, *yk814f3* and the ORFeome cDNA contained mutations. *yk471g7* was sequenced completely and shown to be mutation free. This cDNA was cloned into a Gateway entry vector and used for rescue experiments.

We obtained three *cccp-1* cDNAs from Yuji Kohara. Sequencing revealed two alternatively spliced transcripts, *cccp-1a* (*yk812f4*) and *cccp-1b* (*yk1517a6* and *yk530g8*), differing in the inclusion of exon 12 (Figure 1A). The existence of both isoforms is supported by additional EST sequences on Wormbase. *cccp-1* is trans-spliced to SL1. cDNAs *yk812f4* and *yk1517a6* contained mutations. *yk530g8* was mutation-free and cloned into a Gateway entry vector for rescue experiments.

The full-length sequences of the *rund-1* and *cccp-1b* transcripts were deposited in GenBank under accession numbers JN986879 and JN986880.

Transgenes

A complete list of constructs, including sizes of promoter regions, is provided below. Most of the constructs were made using the three slot multisite Gateway system (Invitrogen). Typically, a promoter, a coding sequence (genomic DNA or cDNA), and an N- or C-terminal fluorescent tag (eGFP or tagRFP-T) were cloned along with a 3’UTR into either pCFJ150 or pCFJ201, destination vectors used for Mos1-mediated single copy insertion (MosSCI) on chromosome II at *ttTi5605* and chromosome IV at *cxTi10882*, respectively (Frøkjaer-Jensen et al., 2008). All insertions were made by the direct injection MosSCI method. For most constructs, we isolated multiple independent insertions that behaved similarly. Extrachromosomal arrays were made by standard transformation methods (Mello et al., 1991).

Yeast two-hybrid assays

The Matchmaker yeast two-hybrid assay was performed according to the manufacturer’s protocol (Clontech). *C. elegans rab*, *rap*, *ras*, and *ral* gene cDNAs were cloned into the bait vector pGBKT7, and the *rund-1* and *cccp-1b* cDNAs were cloned into the prey vector pGADT7. The appropriate plasmid combinations were transformed into the yeast strain AH109 and spread onto growth media lacking leucine and tryptophan for plasmid selection. Protein interactions were tested as follows: several clones of transformants were mixed, diluted to an OD$_{600}$ of 0.2 and spotted onto selective plates lacking leucine, tryptophan and histidine. Interactions were identified by growth after three days. All interacting proteins were tested for self-activation by transforming the interacting plasmid with the appropriate empty vector pGBKT7 or pGADT7. Both RUND-1 and RIC-19 self-activated when expressed in the DNA binding domain vector pGBKTK7 and thus could not be tested against each other.

Coimmunoprecipitation and immunoblotting

HEK293 cells were grown in high glucose (4.5 g/l) DMEM supplemented with 10% FBS, 110 mg/l sodium pyruvate, 2 mM glutamine, 100 U/ml penicillin, and 10 µg/ml streptomycin in a 5% CO$_2$ incubator at 37°C.

For coimmunoprecipitation, 4x10$^6$ HEK293 cells were plated onto two 10 cm petri dishes. Twenty-four hours later, cells were cotransfected with V5 tagged-RUND-1 and either GFP, GFP::RIC-19 or GFP::TBC-8 using TurboFect *in vitro* Transfection Reagent according to the manufacturer’s protocol (Fermentas). After 24 hours, cells were washed with PBS and harvested in lysis buffer (50
mM Tris pH 7.5, 150 mM NaCl, 1% Triton X100, 0.5 mM EDTA, 10% glycerol, Complete Mini Protease inhibitor (Roche)) for 30 min at 4°C. Lysates were pre-cleared by centrifugation at 4°C and the supernatant was incubated with 2 μg monoclonal anti-GFP antibody (clone 3E6, Invitrogen) for three hours at 4°C. Protein G Plus-sepharose beads (Pierce) were added. After incubating for two hours, the beads were washed three times with washing buffer (50 mM Tris pH 7.5, 500 mM NaCl, 0.1% Triton X100, 0.5 mM EDTA, 10% glycerol, Complete Mini Protease inhibitor (Roche)) and resuspended in Laemmli loading buffer. Samples were resolved on 10% SDS-polyacrylamide gels and blotted onto a nitrocellulose membrane. To detect coprecipitated proteins, we added a mixture of two mouse monoclonal anti-GFP antibodies (1:1000, clones 7.1 and 13.1, Roche) and monoclonal anti-V5 antibody (1:5000, Invitrogen) followed by goat anti-mouse horseradish peroxidase-conjugated secondary antibody (1:10,000, Jackson Laboratory). A FujiFilm LAS 3000 processor was used to develop images, which were then edited using ImageJ software (National Institutes of Health).

**Fluorescence electron microscopy (fEM)**

Correlative fEM was performed as previously described (Watanabe et al., 2011) with a slight modification in the protocol. In brief, transgenic animals expressing tdEos were high-pressure frozen and freeze-substituted in 0.1% potassium permanganate (EMS) + 0.001% osmium tetroxide (EMS) in 95% acetone (EMS). The freeze-substitution protocol was as follows: -90°C for 30 hours, 5°C/hour to -50°C, -50°C for 2 hours, and 5°C/hour to -30°C. The fixatives were removed at -50°C, and a solution containing 0.1% uranyl acetate (Polysciences) was added to the specimens. The uranyl acetate solution was removed when the temperature reached -30°C. The animals were then embedded into GMA plastic (SPI). Eighty nm thick sections were sliced and mounted onto a pre-cleaned coverslip. The PALM imaging was performed using the Zeiss PAL-M (Zeiss, Prototype Serial No. 2701000005) following the application of gold fiduciary markers (100nm; microspheres-nanospheres.com). The same sections were imaged using a backscatter electron detector on scanning electron microscope (FEI Nova Nano). The PALM and electron micrographs were aligned based on the fiduciary markers in Photoshop (Adobe Photoshop CS5). For the purpose of presentation, we applied a gradient transparency to the PALM image - only the background black pixels are transparent.

**Electron microscopy of synaptic and dense-core vesicles**

High pressure freeze electron microscopy and analysis of synaptic profiles were performed as described (Rostaing et al., 2004; Sumakovic et al., 2009).

**Electrophysiology**

Young adult hermaphrodites were used for electrophysiological analysis as described (Liu et al., 2009). In brief, animals were immobilized on a Sylgard-coated glass coverslip by applying a cyanoacrylate adhesive along the dorsal side. A longitudinal incision was made in the dorsolateral region. The cuticle flap was folded back and glued to the coverslip, exposing the ventral nerve cord and two adjacent muscle quadrants. An upright microscope (Axioskop; Carl Zeiss, Inc.) equipped with a 40x water immersion lens and 15x eyepieces was used for viewing the preparation. All experiments were performed with the bath at room temperature using single electrode (borosilicate glass, R ~ 5 MΩ) voltage clamp (Heka, EPC-10) with two stage capacitive compensation optimized at rest, and series resistance compensated to 50%. Electrically evoked responses were elicited using an electrode with a tip resistance of approximately 3–5 MΩ positioned along the ventral nerve cord approximately one muscle cell body away from the patched muscle. A square wave depolarizing current of 0.5 ms at 25 V was delivered from an SIU5 stimulation isolation unit driven by an S48 stimulator (Grass Technologies). The standard pipette solution was (all concentrations in mM) [KCl 120; KOH 20; MgCl₂ 4; TES 5; CaCl₂ 0.25; EGTA 5; Na₂ATP 4; sucrose 36] and the standard extracellular solution was [NaCl 150; KCl 5; CaCl₂ 5; MgCl₂ 1; sucrose 5; HEPES 15; glucose 10]. Experiments were controlled using PatchMaster software (Heka). Analog data were digitized at 10 kHz and filtered at 2 kHz.
List of strains

CB4856 Hawaiian wild-isolate
EG281 rund-1(ox281) X
EG328 rund-1(ox328) X
EG334 cccp-1(ox334) III
EG3404 unc-31(e928) IV
EG3654 egl-30(tg26) I ; cccp-1(ox334) III
EG3738 gsa-1(ce81) I ; unc-31(e928) IV
EG3741 rund-1(ox281) X dpy-3(e27) X
EG3765 egl-30(tg26) I ; rund-1(ox281) X
EG3773 egl-30(tg26) I ; unc-2(e55) X
EG3774 egl-30(tg26) I ; unc-18(md299) X
EG3775 egl-30(tg26) I ; unc-68(e540) V
EG3782 egl-30(tg26) I ; egl-3(ok979) V
EG3797 egl-30(tg26) I ; rund-1(ox328) X
EG4033 egl-30(tg26) I ; unc-104(e1265) II
EG4044 egl-30(tg26) I ; unc-68(r1162) V
EG4045 unc-31(e928) IV ; rund-1(ox281) X
EG4167 rund-1(ox281) X ; oxEx779[T19D7, Pmyo-2::gfp] X
EG4258 cccp-1(ox334) III ; oxEx1113[RPCI94_09N13, Pmyo-2::gfp, lin-15(+)]
EG4532 egl-30(tg26) I
EG4780 cccp-1(e1122) III
EG4781 egl-30(tg26) I ; cccp-1(e1122) III
EG4815 gsa-1(ce81) I ; rund-1(ox281) X
EG4816 gsa-1(ce81) I ; rund-1(ox328) X
EG4923 lin-15(n765ts) X ; oxEx1134[Prund-1::GFP, lin-15(+)] X
EG4937 rab-2(n501) I ; rund-1(ox281) X
EG4938 rab-2(n777) I ; rund-1(ox281) X
EG4939 egl-30(tg26) I rab-2(n501) I
EG4940 egl-30(tg26) I rab-2(n777) I
EG4941 rab-2(n501) I
EG5003 unc-119(ed9) III ; cxTi10882 IV
EG5039 rab-2(n3263) I ; rund-1(ox281) X
EG5102 egl-4(ks62) IV ; rund-1(ox281) X
EG5103 egl-4(ks62) IV ; rund-1(ox328) X
EG5108 ceh-17(np1) I ; rund-1(ox281) X
EG5109 ceh-17(np1) I ; rund-1(ox328) X
EG5111 rab-2(n3263) I ; rund-1(ox328) X
EG5112 rab-2(n3263) I ; egl-4(ks62) IV
EG5170 egl-4(ks62) IV
EG5228 nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III ; rund-1(ox281) X
EG5231 nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III : rund-1(ox328) X
EG5232 rab-2(n3263) I ; nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III
EG5258 nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III cccp-1(ox334) III
EG5260 cels61[Punc-129::fip-3::venus, Punc-129::mCherry-snbl-1, Pttx-3::mCherry] II ; rund-1(ox281) X
EG5261 cels61[Punc-129::fip-3::venus, Punc-129::mCherry-snbl-1, Pttx-3::mCherry] II ; rund-1(ox328) X
EG5334 cels61[Punc-129::fip-3::venus, Punc-129::mCherry-snbl-1, Pttx-3::mCherry] II ; cccp-1(ox334) III
EG5340 rab-2(nu415) I ; rund-1(ox281) X
EG5341 rab-2(nu415) I ; rund-1(ox328) X
EG5348 cccp-1(ox334) III ; rund-1(ox281) X
EG5349 cccp-1(ox334) III ; rund-1(ox328) X
EG5505 rund-1(tm3622) X
EG5606 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; unc-119(ed9) III
EG5608 oxls592[Cb unc-119(+), Prund-1::rund-1(+):eGFP] II ; unc-119(ed9) III
EG5609 eg1-30(tg26) I ; rund-1(tm3622) X
EG5610 eg1-30(tg26) I rab-2(nu415) I
EG5627 rab-2(nu415) I
EG5631 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; rund-1(tm3622) X
EG5633 oxls592[Cb unc-119(+), Prund-1::rund-1(+):eGFP] II ; rund-1(tm3622) X
EG5635 cels72[Punc-129::flip-3::venus, Punc-129::mCherry-snb-1, Pttx-3::mCherry] II ; rund-1(tm3622) X
EG5633 cels72[Punc-129::flip-3::venus, Punc-129::mCherry-snb-1, Pttx-3::mCherry] II ; eg1-3(ox979) V ; rund-1(tm3622) X
EG5644 cccp-1(ox334) III ; eg1-3(ox979) V
EG5645 eg1-3(ox979) V ; rund-1(tm3622) X
EG5647 rab-2(nu415) I ; eg1-3(ox979) V
EG5648 rab-2(nu415) I ; rund-1(tm3622) X
EG5649 rab-2(nu415) I ; cccp-1(ox334) III
EG5674 nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III ; rund-1(tm3622) X
EG5745 unc-119(ed9) III ; oxSi13[Prund-1::aman-2::eGFP, Cbunc-119] IV
EG5748 unc-119(ed9) III ; oxSi59[Prund-1::eGFP::tram-1, Cbunc-119] IV
EG5805 oxSi95[Prund-1::rund-1(+):tdEos, Cb-unc-119] II ; unc-119(ed9) III
EG5849 oxSi95[Prund-1::rund-1(+):tdEos, Cb-unc-119] II ; rund-1(tm3622) X
EG5857 eg1-30(tg26) I ; unc-50(e306) III
EG5858 eg1-30(tg26) I unc-74(ox78) I
EG5859 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; oxSi13[Prund-1::aman-2::eGFP, Cbunc-119] IV
EG5860 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; oxSi59[Prund-1::eGFP::tram-1, Cbunc-119] IV
EG5912 cccp-1(ox334) III ; nuls195[Punc-129::ins-22::venus, Pmyo-2::gfp]
EG5913 nuls195[Punc-129::ins-22::venus, Pmyo-2::gfp] ; rund-1(tm3622) X
EG5914 cccp-1(ox334) III ; cels72[Punc-129::ida-1::GFP, Pttx-3::mCherry]
EG5915 cels72[Punc-129::ida-1::GFP, Pttx-3::mCherry] ; rund-1(tm3622) X
EG5936 rab-2(nu415) I ; cels72[Punc-129::ida-1::GFP, Pttx-3::mCherry]
EG5938 rab-2(nu415) I ; nuls195[Punc-129::ins-22::venus, Pmyo-2::gfp]
EG6010 ric-19(pk690) I ; rund-1(tm3622) X
EG6193 unc-119(ed9) III ; oxSi266[Prund-1::eGFP::rab-5, Cb unc-119] IV
EG6244 nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III ; eg1-3(ox979) V ; rund-1(tm3622) X
EG6286 rab-2(nu415) I ; nuls183[NLP-21-Venus, Pmyo-2::GFP] III ; rund-1(tm3622) X
EG6359 unc-119(ed9) III ; oxSi308[Prund-1::eGFP::rab-6,2, Cb-unc-119] IV
EG6361 unc-119(ed9) III ; oxSi310[Prund-1::eGFP::rab-7, Cb-unc-119] IV
EG6362 unc-119(ed9) III ; oxSi311[Prund-1::eGFP::rab-11.1, Cb-unc-119] IV
EG6363 unc-119(ed9) III ; oxSi312[Prund-1::eGFP::e-COP, Cb-unc-119] IV
EG6364 unc-119(ed9) III ; oxSi313[Prund-1::eGFP::syn-13, Cb-unc-119] IV
EG6365 unc-119(ed9) III ; oxSi314[Prad-2::eGFP::rab-2, Cb-unc-119] IV
EG6366 unc-119(ed9) III ; oxSi315[Prund-1::eGFP::syx-6, Cb-unc-119] IV
EG6368 rab-2(nu415) I ; oxSi314[Prad-2::eGFP::rab-2, Cb-unc-119] IV
EG6369 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; oxSi266[Prund-1::eGFP::rab-5, Cb unc-119] IV
EG6371 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; oxSi308[Prund-1::eGFP::rab-6,2, Cb-unc-119] IV
EG6373 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; oxSi310[Prund-1::eGFP::rab-7, Cb-unc-119] IV
EG6374 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; oxSi311[Prund-1::eGFP::rab-11.1, Cb-unc-119] IV
EG6375 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; oxSi312[Prund-1::eGFP::e-COP, Cb-unc-119] IV
EG6376 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; oxSi313[Prund-1::eGFP::syn-13, Cb-unc-119] IV
EG6377 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; oxSi314[Prab-2::eGFP::rab-2, Cb-unc-119] IV
EG6378 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; oxSi315[Prund-1::eGFP::syx-6, Cb-unc-119] IV
EG6383 oxSi314[Prab-2::eGFP::rab-2, Cb-unc-119] IV ; rund-1(ox281) X
EG6384 oxSi314[Prab-2::eGFP::rab-2, Cb-unc-119] IV ; rund-1(tm3622) X
EG6388 nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III ; rund-1(tm3622) X ; oxEx1520[Punc-129::rund-1::tagRFP, Pmyo-2::mCherry]
EG6389 rund-1(tm3622) X ; oxEx1521[Prund-1::RUNDC1 cDNA::tagRFP, Pmyo-2::gfp]
EG6390 rund-1(ox281) X ; oxEx1522[Prund-1::RUNDC1 cDNA::tagRFP, Pmyo-2::gfp]
EG6391 rund-1(tm3622) X ; oxEx1523[Phsp16.2::rund-1 cDNA::tagRFP, Pmyo-2::gfp]
EG6652 rund-1(tm3622) X ; oxEx1575[Prund-1::rund-1(+):tdEos, Pmyo-2::gfp]
EG6917 oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II ; cccp-1(ox334) III
EG6920 cccp-1(ox334) III ; oxSi314[Prab-2::eGFP::rab-2, Cb-unc-119] IV
EG6922 oxSi315[Prund-1::eGFP::syx-6, Cb-unc-119] IV ; rund-1(tm3622) X
EG6923 oxSi308[Prund-1::eGFP::rab-6.2, Cb-unc-119] IV ; rund-1(tm3622) X
EG6927 rab-2(nu415) I ; oxls590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II
EG6929 oxSi503[Prund-1::rund-1 CC::tagRFP, Cb-unc-119] II ; unc-119(ed9) III
EG6933 rund-1(tm3622) X ; oxEx1366[Cb-unc-119(+) cccp-1::eGFP, Pmyo-2::mCherry, Pmyo-3::mCherry, Prab-3::mCherry]
EG6934 rab-2(nu415) I ; oxEx1366[Cb-unc-119(+) cccp-1::eGFP, Pmyo-2::mCherry, Pmyo-3::mCherry, Prab-3::mCherry]
EG6941 oxSi503[Prund-1::rund-1 CC::tagRFP, Cb-unc-119] II ; rund-1(tm3622) X
EG6943 oxSi505[Prund-1::rund-1 RUN::tagRFP, Cb-unc-119] II ; unc-119(ed9) III
EG6945 oxSi505[Prund-1::rund-1 RUN::tagRFP, Cb-unc-119] II ; rund-1(tm3622) X
EG6949 cccp-1(ox334) III ; oxEx1622[Prab-3::cccp-1::gfp, Prund-1::rund-1::tagRFP]
EG6951 cccp-1(ox334) III ; oxEx1624[Prab-3::cccp-1::gfp, Prab-3::tagRFP::rab-2(DA)]
EG6953 cccp-1(ox334) III ; oxEx1626[Prab-3::cccp-1::gfp, Prab-3::tagRFP::rab-2(DN)]
EG6982 cccp-1(ox334) III ; oxEx1628[Prab-3::cccp-1::gfp, Punc-122::gfp]
EG7187 unc-119(ed9) III ; oxEx1366[Cb-unc-119(+) cccp-1::eGFP, Pmyo-2::mCherry, Pmyo-3::mCherry, Prab-3::mCherry]
EG7227 lin-15(n765ts) X ; oxEx1251[Pcccp-1::gfp, lin-15(+)]
EG7242 rund-1(ox281) X lin-15(n765ts) X ; oxEx1197[Prab-3::rund-1 cDNA::mCherry, lin-15(+)]
EG7244 rund-1(ox281) X lin-15(n765ts) X ; oxEx1260[Pvha-6::rund-1 cDNA::mCherry, lin-15(+)]
EG7249 eri-1(tm366) IV ; lin-15(n744) X
FK234 egl-4(ks62) IV
IB16 ceh-17(np1) I
GQ640 ric-19(ok833) I ; nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III
GQ641 tbc-8(tm3802) III nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III
GQ693 nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III ; rund-1(tm3622) X
GQ698 ric-19(ok833) I ; nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III ; rund-1(tm3622) X
GQ6999 tbc-8(tm3802) III nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III ; rund-1(tm3622) X
KG421 gsa-1(ce81) I
KG1395 nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III
KG1475 rab-2(ce365) I ; nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III
KG1624 nuls195[Punc-129::ins-22::venus, Pmyo-2::gfp]
KG1645 cels61[Punc-129::flip-3::venus, Punc-129::mCherry-snb-1, Pttx-3::mCherry] II
KG1655 rab-2(ce365) I ; cels61[Punc-129::flip-3::venus, Punc-129::mCherry-snb-1, Pttx-3::mCherry] II
KG1852 cels72[Punc-129::ida-1::GFP, Pttx-3::mCherry]
MT1093 rab-2(n501) I
MT1656 rab-2(n777) I
N2 Bristol wild-isolate, standard lab wild type
NL2003 ric-19(pk690) I
VC671 eg1-3(ok979) V
XZ1022 rab-2(nu415) I; oxis590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II; oxiS266[Prund-1::eGFP::rund-5, Cb unc-119] IV
XZ1023 rab-2(nu415) I; oxis590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II; oxiS308[Prund-1::eGFP::rund-6.2, Cb-unc-119] IV
XZ1024 rab-2(nu415) I; oxis590[Cb unc-119(+), Prund-1::rund-1(+):tagRFP] II; oxiS310[Prund-1::eGFP::rund-7, Cb-unc-119] IV
XZ1026 rab-2(nu415) I; nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III
XZ1027 nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III cccp-1(ox334) III; rund-1(tm3622)
XZ1028 rab-2(nu415); nuls183[Punc-129::NLP-21-Venus, Pmyo-2::GFP] III cccp-1(ox334) III
ZH382 rab-2(n3263) I

List of plasmids

Miscellaneous plasmids
RPC194_09N13 BAC carrying the C. briggae ortholog of cccp-1, used to make oxEx1113 (10 ng/µl)
T19D7 cosmid carrying the rund-1 gene T19D7.4, used to make oxEx779 (10 ng/µl)
yk471g7 rund-1 full length cDNA
yk530g8 cccp-1b full length cDNA
Prab-3::tagRFP::rab-2(DA) used to make oxEx1624 (5 ng/µl)
Prab-3::tagRFP::rab-2(DN) used to make oxEx1626 (5 ng/µl)

Gateway destination vectors
pCFJ150 Gateway destination vector for insertion at chromosome II Mos site ttTi5605
pCFJ201 Gateway destination vector for insertion at chromosome IV Mos site cxTi10882
dEST-R4-R3 Gateway destination vector

Gateway entry clones
pCM1.56 Phsp-16.2 [4-1] (493 bp of the hsp-16.2 promoter upstream of the ATG)
pCR110 GFP [1-2]
pENTR[4-1] P[rab-3] Prab-3 [4-1] (1224 bp of the rab-3 promoter upstream of and including the ATG)
pGH107 tagRFP::let-858 3'UTR [2-3]
pGH112 eGFP::let-858 3'UTR [2-3]
pGH115 eGFP [1-2]
pGH271 tdEos::let-858 3'UTR [2-3]
pMA15 Pcccp-1 [4-1] (1696 bp of the cccp-1 promoter upstream of and including the ATG)
pMA18 cccp-1b cDNA [1-2] (from yk530g8)
pMA20 rund-1 cDNA [1-2] (from yk471g7)
pMA108 rab-5 cDNA::let-858 3'UTR [2-3]
pMA115 rab-7 cDNA::let-858 3'UTR [2-3]
pMA116 rab-11.1 cDNA::let-858 3'UTR [2-3]
pMA132 rab-6.2 cDNA::let-858 3'UTR [2-3]
pMA145 syx-6 cDNA::let-858 3'UTR [2-3]
pMA157 RUNDC1 cDNA [1-2]
pMA165 rund-1 coiled-coil domain [1-2] (aa 1-261)
pMA166 rund-1 RUN domain [1-2] (aa 262-549)
pPM1 aman-2 [1-2]
pPM2 tram-1 [2-3]
pSD11 Prab-2 [4-1] (3237 bp of the rab-2 promoter upstream of the ATG)
pSD12 Punc-129 [4-1] (2645 bp of the unc-129 promoter upstream of the ATG)
pSD16 rab-2(+) gene with introns and rab-2 3'UTR [2-3]
pSD25 syn-13 with let-858 3'UTR [2-3]
pSD26 ε-COP with let-858 3'UTR [2-3]
pT19D7.4 [4-1] Prund-1 [4-1] (2733 bp of the rund-1 promoter upstream of and including the ATG)
pT19D7.4 [1-2]  
run-1(+) gene with introns [1-2] 
p_VW02B12L.1_93  
*Pvha-6* [4-1] (881 bp of the *vha*-6 promoter upstream of and including the ATG)

**Gateway expression constructs**

<table>
<thead>
<tr>
<th>Construct</th>
<th>Description</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAP4 Prund-1::gfp</td>
<td>Gateway expression constructs</td>
<td>used to make oxEx1134 (10 ng/µl)</td>
</tr>
<tr>
<td>pMA17 Pcccp-1::gfp</td>
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<td>used to make oxEx1251 (10 ng/µl)</td>
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<tr>
<td>pMA24 Prab-3::run-1 cDNA::mCherry</td>
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<td>used to make oxEx1197 (10 ng/µl)</td>
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<tr>
<td>pMA38 Pvha-6::run-1 cDNA::mCherry</td>
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<td>used to make oxEx1260 (10 ng/µl)</td>
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<tr>
<td>pMA56 Prund-1::run-1(+)::tagRFP</td>
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<td>used to make oxIs590 and oxEx1622 (10 ng/µl)</td>
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<tr>
<td>pMA57 Prund-1::run-1(+)::eGFP</td>
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<td>used to make oxIs592</td>
</tr>
<tr>
<td>pMA58 Pcccp-1::cccp-1 cDNA::eGFP</td>
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<td>used to make oxEx1366 (50 ng/ml)</td>
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<tr>
<td>pMA74 Prund-1::aman-2::eGFP</td>
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<td>used to make oxSi13</td>
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<tr>
<td>pMA75 Prund-1::eGFP::tram-1</td>
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<td>used to make oxSi59</td>
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<tr>
<td>pMA90 Prund-1::run-1(+)::tdEos</td>
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<td>used to make oxEx1575 (10 ng/µl) &amp; oxSi95</td>
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<td>pMA112 Prund-1::eGFP::rab-5</td>
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<td>used to make oxSi266</td>
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<td>pMA118 Prund-1::eGFP::rab-7</td>
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<td>used to make oxSi310</td>
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<tr>
<td>pMA119 Prund-1::eGFP::rab-11.1</td>
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<td>used to make oxSi311</td>
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<tr>
<td>pMA138 Prund-1::eGFP::rab-6.2</td>
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<td>used to make oxSi308</td>
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<tr>
<td>pMA147 Prund-1::eGFP::syx-6</td>
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<td>used to make oxSi315</td>
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<tr>
<td>pMA150 Phsp16.2::run-1 cDNA::tagRFP</td>
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<td>used to make oxEx1523 (10 ng/µl)</td>
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<tr>
<td>pMA152 Punc-129::run-1 cDNA::tagRFP</td>
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<td>used to make oxEx1520 (10 ng/µl)</td>
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<tr>
<td>pMA159 Prab-3::cccp-1 cDNA::eGFP</td>
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<td>used to make oxEx1622, oxEx1624, oxEx1626, oxEx1628 (10 ng/µl)</td>
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<tr>
<td>pMA161 Prund-1::RUNDC1 cDNA::tagRFP</td>
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<td>used to make oxEx1521 &amp; oxEx1522 (10 ng/µl)</td>
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<tr>
<td>pMA172 Prund-1::run-1 CC::tag RFP</td>
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<td>used to make oxSi503</td>
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<tr>
<td>pMA173 Prund-1::run-1 RUN::tagRFP</td>
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<td>used to make oxSi505</td>
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<tr>
<td>pSD18 Prab-2::eGFP::rab-2(+)</td>
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<td>used to make oxSi314</td>
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<tr>
<td>pSD30 Prund-1::eGFP::syn-13</td>
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<td>used to make oxSi313</td>
</tr>
<tr>
<td>pSD31 Prund-1::eGFP::ε-COP</td>
<td></td>
<td>used to make oxSi312</td>
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Supplemental References


