Supporting methods and results

**Isolation of suppressors of unc-17(e245):**

We performed several genetic screens for mutations that suppress the growth and movement deficits of unc-17(G347R) mutants. As described previously, the e245 and p300 alleles are associated with the same G347R sequence alteration (Alfonso et al. 1993; Zhu et al. 2001). unc-17(e245) or unc-17(p300) dpy-13(e184) L4 homozygotes were mutagenized with 50 mM ethyl methanesulfonate (EMS) and plated onto 100 mm NGM plates. unc-17 mutant animals grow slowly and are highly uncoordinated; suppressed progeny were identified by their larger size and improved movement, and we identified several dominant extragenic suppressors. In particular, the e995 mutation (isolated against e245) and the md44 mutation (isolated against p300) both mapped to LGIII.

**Isolation of a putative sup-1(null) mutation:**

The e995e2636 allele was isolated by mutagenizing a strain with genotype sup-1(e995); unc-17(e245) him-8(e1489). Mutagenized males were then crossed with females with the genotype dpy-18(e499); unc-17(e245) fem(hc17); xol-1(y9). The only progeny of this cross are hermaphrodite cross-progeny, which are homozygous for unc-17(e245) and heterozygous for sup-1(e995). Therefore, the cross progeny are non-Unc, unless the sup-1(e995) mutation has been over-ridden, in which case the animal will be Unc. About 4000 progeny were screened for Unc progeny. Seven candidate Uncs were picked, of which six produced some wild-type self-progeny, suggesting that they were mosaic or carried a dominant Unc mutation. The seventh bred true, with a standard Unc-17 phenotype. Single Unc non-Dpy progeny were picked, of which 2/9 segregated no Dpy Uncs, and these two were therefore presumed to be homozygous with the genotype sup-1(e995e2636); unc-17(e245). One of these was used to establish the strain CB5265. This strain was then outcrossed six times to remove possible background mutations; the outcrossed strain was designated RM3571.

Further experiments suggested that e2636 is indeed a sup-1 null or severe loss of function allele, rather than a revertant to the wild-type allele. An outcrossed strain CB5285 with genotype sup-1(e995e2636); unc-17(e245) was mutagenized with EMS to select for suppressors. None was found, apart from a partial same-site revertant of unc-17(e245) itself, whereas a parallel control experiment with unc-17(e245) yielded numerous suppressors (0/16 experimental versus 16/16 control 100 mm plates). This result suggested that sup-1 locus had lost function, and therefore could no longer be mutated to create a dominant e245 suppressor. After the sup-1 locus was cloned (see below), the e2636 allele was sequenced, and was found to be associated with both the original G84E mutation and a nonsense mutation in exon 2 (W58*).
Candidate gene strategy to identify the sup-1 gene:

Mapping experiments indicated that sup-1 mapped to the right arm of chromosome III (12.09 ± 0.60 cM). This placed sup-1 (with 95% confidence) in a region between 11.49 and 12.69 cM, which corresponds to a physical distance of 149 kb and includes 24 protein-coding genes. Genes were excluded as candidates if the predicted protein did not include a transmembrane domain, if there were RNAi data suggesting that null alleles were lethal, or if there were data indicating that the protein was not expressed in the nervous system. Genomic DNA was isolated from the RM921 strain (dpy-18(e364) sup-1(e995); unc-17(e245)) and used as a template for the PCR amplification of 1-2 kb regions, which were then sequenced and compared to the canonical wild-type C. elegans genome. We identified a sequence alteration associated with the e995 allele in the Y41C4.13 open reading frame. We then sequenced the md44 and e995e2636 alleles, and identified sequence alterations in the same open reading frame. The identity of the sup-1 gene was confirmed by transformation experiments: microinjection of a genomic fragment from RM921 animals containing the Y41C4.13 open reading frame with the e995 mutation suppressed the unc-17(245) phenotype.

PCR-based method for identification of point-mutants:

For outcrossing and generation of double mutants, the presence of mutant and wild-type alleles was determined in parallel reactions using a PCR-based point-mutant detection method (Qiang et al. 2002). For each reaction, either the mutant-specific primer or the wild-type-specific primer was paired with the "common" primer as indicated.

\(\text{unc-17}(e245)\) or \(\text{unc-17}(p300)\)

- Mutant-Specific Primer: 5'-TGGTTGGGTTGGCTATGGAAA-3'
- WT-Specific Primer: 5'-TGGTTGGGTTGGCTATGGAAG-3'
- "Common" Primer: 5'-ATCACTACAGTAGCCCGACA-3'
- Annealing temperature: 65°C (mutant) or 66°C (wild type)
- Size: 1077 bp.

\(\text{sup-1}(e995)\) or \(\text{sup-1}(md44)\)

- Mutant-Specific Primer: 5'-GGTAACCCTCGCCTTGTTCAA-3'
- WT-Specific Primer: 5'-GGTAACCCTCGCCTTGTTCAG-3'
- "Common" Primer: 5'-TGGGCAGCAGCTAAAGTTAC-3'
- Annealing temperature: 57°C (mutant or wild type)
- Size: 721 bp.
sup-1(e2636)

Mutant-Specific Primer: 5’-CTCTAGCATCTTCCACTACTAA-3’
WT-Specific Primer: 5’-CTCTAGCATCTTCCACTACTAG-3’
"Common" Primer: 5’-CTTAGTGGCCTATACGTCTC-3’
Annealing temperature: 62°C (mutant or wild type)
Size: 624 bp.

sup-1 is not unc-123:

A previous report (Walthall et al. 1993) suggested that the unc-123 and sup-1 loci might correspond to the same gene. We sequenced the sup-1 region in unc-123(jd5) and unc-123(jd5jd10) mutants, and neither of the unc-123 strains had any changes in the sup-1 coding sequence or splice sites. We conclude that unc-123 and sup-1 are separate genes.

Transcriptional reporters and expression patterns of C. elegans SUP-1-like proteins:

F52A8.1: The reporter had 5082 bp of upstream sequence driving expression of NLS-CFP. We did not observe any expression in transgenic animals. Meissner et al. (2009) observed expression in muscles.

F58F12.4: The reporter had 2374 bp of upstream sequence driving expression of NLS-CFP. We observed bright fluorescence in the vulva, a few head neurons, and lateral lines down the body; these may be the lateral hypodermal (seam) cells.

F42C5.6: The reporter had 1020 bp of upstream sequence driving NLS-CFP expression. We observed fluorescence in two cell bodies with processes, at the level of the vulva. The posterior cell sends a process to the posterior, and another process anterior, terminating at the pharynx. The anterior cell body sends a process anterior.

C08H9.15: The reporter had 3405 bp of upstream sequence driving expression of NLS-CFP. We observed fluorescence in the intestine, hypodermis, and vulva.

R10E11.9: The reporter had 3466 bp of upstream sequence driving expression of NLS-CFP. We observed very faint fluorescence.

ZK353.2: The reporter had 2130 bp of upstream sequence driving expression of NLS-CFP. We observed fluorescence in the vulva and in the tail; the tail fluorescence may be the anal or intestinal muscles. In addition, we also observed fluorescence in a few cells in the head; these may be interlabial neurons, or sheath and socket cells.

K06B4.3: The reporter had 1122 bp of upstream sequence driving expression of NLS-CFP. We observed extremely faint fluorescence, including in cells around the pharynx; these may be pharyngeal cells or neurons.