

## Pre-mRNA Splicing Factors and a Novel Nuclear Protein Interact to Regulate Rubberband Unc Gene Activity

*Long Ma, H. Robert Horvitz*

MIT, Cambridge, MA 02139

*sup-9*, *sup-10* and *unc-93* encode components of a presumptive *C. elegans* two-pore domain K<sup>+</sup> channel complex. Rare gain-of-function mutants of each of these three genes have abnormal body muscle contraction and exhibit the rubberband phenotype: when prodded on the head, the animal contracts and relaxes along its entire body without moving backwards. Loss-of-function mutants of each of these genes have no obviously abnormal phenotype. The SUP-9 protein is similar to the mammalian Two-pore Acid Sensitive K<sup>+</sup> channels TASK-1 and TASK-3. *sup-10* encodes a novel single transmembrane protein. *unc-93* encodes a multiple transmembrane protein that defines a novel family of proteins conserved from *C. elegans* to mammals. A mammalian UNC-93 homolog, UNC-93b, has been shown to play important roles in the innate immune response.

To seek essential genes that might interact with *unc-93*, we screened ~10,000 EMS-mutagenized F1 *unc-93(e1500sd)* animals clonally for progeny with improved locomotion and identified three new suppressors, *n4562*, *n4564* and *n4588*. *n4588* and *n4564* cause recessive lethality at 25°C, while *n4562* causes recessive sterility at all temperatures. *n4588* is a missense mutation in the gene *uaf-1*, which encodes a *C. elegans* homolog of the splicing factor U2AF65 (U2 snRNP auxiliary factor large subunit). *n4562* is a nonsense mutation in the gene *sfa-1*, which encodes a *C. elegans* homolog of the splicing factor SF1. We found that *uaf-1(n4588) unc-93(e1500)* animals have an abnormally spliced *unc-93* transcript that encodes a truncated loss-of-function UNC-93 protein. This transcript is caused by the generation of a cryptic 3' splice site by the *e1500* missense mutation, so that this new 3' splice site, instead of the endogenous 3' splice site, is recognized. However, *sfa-1(n4562)* does not cause abnormal splicing of the *unc-93(e1500)* transcript at the cryptic 3' splice site. We think that abnormal splicing of *unc-93* is not the reason that *uaf-1* and *sfa-1* mutations suppress *unc-93(e1500)*. We have identified two missense mutations in the gene *F43G9.10* in *n4564* mutant animals. *F43G9.10* encodes a highly conserved nuclear protein of unknown function. *uaf-1* and *F43G9.10* interact genetically, and *F43G9.10* requires *uaf-1* to suppress *unc-93(e1500)*. We hypothesize that *uaf-1(n4588)*, *sfa-1(n4562)* and *F43G9.10(n4564)* all suppress *unc-93(e1500)* by affecting the splicing of one or more unidentified genes required for *unc-93* activity.

**Contact:** longma@mit.edu

**Lab:** Horvitz