47. THE PUTATIVE REGULATORY SUBUNITS SUP-10 AND SUP-18 REGULATE THE SUP-9 TWO-PORE POTASSIUM CHANNEL THROUGH A MECHANISM THAT IS DISTINCT FROM THAT OF THE UNC-93 SUBUNIT

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Rare altered-function mutations in the genes *unc-93*, *sup-9*, and *sup-10* result in the abnormal regulation of muscle contraction. These mutants move sluggishly and are unable to lay eggs. Loss-of-function mutations in any of these three genes completely suppress the phenotypes caused by any of the altered-function mutations, suggesting that all three genes act at the same step, possibly by encoding subunits of a protein complex. We have shown that *sup-9* encodes a two-pore K^+ channel subunit with similarity to the mammalian Two-pore Acid Sensitive K^+ channels TASK-1 and TASK-3. *unc-93* and *sup-10* encode novel putative transmembrane proteins that likely serve as regulatory subunits of SUP-9.

sup-18 may encode a positive regulator of the *sup-9*/*sup-10*/*unc-93* channel complex. *sup-18(lf)* mutations fully suppress the Unc and Egl defects of *sup-10(gf)* mutants, while only partially suppressing those of *unc-93(gf)* or *sup-9(gf)* mutants, suggesting that *sup-18* may be preferentially required for the *sup-10(gf)* activity. In addition, we have found that *sup-18(lf)* mutations also only partially suppress the weaker defects of partial *sup-10(lf)* double mutants with *unc-93(gf)* mutations, indicating that the partial suppression of *unc-93(gf)* defects by *sup-18(lf)* is not caused by the greater severity of *unc-93(gf)* defects. We previously cloned *sup-18* and found that it encodes a type-one transmembrane protein, the cytoplasmic domain of which contains a nitroreductase domain. We found that the strong loss-of-function allele *sup-18(n1010)* mutates a highly conserved serine to an asparagine within the nitroreductase domain. The equivalent serine in a nitroreductase from *Thermus thermophilus* (*Ttnox*) contacts a tightly-bound FMN cofactor. We found that recombinant TtNOX carrying a serine-to-asparagine mutation at this site had severely reduced NADH oxidase activity *in vitro,* consistent with the hypothesis that SUP-18 has an enzymatic activity *in vivo*.

To explore the mechanisms by which *sup-10(gf)* and *unc-93(gf)* mutations activate *sup-9*, we have analyzed an unusual *sup-9* mutant. *sup-9(n1435)* suppresses fully the Unc and Egl defects of *sup-10(gf)* mutants but only weakly those of *unc-93(gf)* mutants, unlike null mutations in *sup-9* which fully suppress defects in both mutants. We hypothesized that *sup-9(n1435)* may be insensitive to *sup-18* and therefore displays the same differential suppression as *sup-18(lf)* mutations. Consistent with this model, the partial suppressive effects of *sup-9(n1435)* and *sup-18(lf)* towards *unc-93(gf)* defects were not additive in the triple mutant. The *sup-9(n1435)* mutation leads to a serine-to-phenylalanine substitution in the presumptive C-terminal cytoplasmic domain of *sup-9*. Using site-directed mutagenesis, we identified another residue in this domain that is required for *sup-18*- but not for *unc-93*-dependent activation. In addition, we have found that overexpression of *sup-18* in a *sup-10(gf)* but not in an *unc-93(gf)* mutant enhances the severity of the Unc defects. Together, these results support a model in which the *sup-10(gf)* mutation acts with the *sup-18* nitroreductase to activate the *sup-9* channel through a mechanism that is distinct from that of *unc-93(gf)* mutations.