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.