CRB-1 MAY FUNCTION IN SYNAPTIC TRANSMISSION AND DAUER FORMATION

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The cyclic AMP-response element binding protein CREB plays a central role in long-term memory in *Aplysia*, *Drosophila* and mice. We characterized the *C. elegans* and *C. briggsae* CREB genes (*crb-1*) and found that the encoded proteins are 85% identical. The DNA-binding bZIP domain and cAMP-dependent kinase site, as defined in the mammalian and *Drosophila* CREB family members, are highly conserved in the nematode proteins. The *C. elegans crb-1* gene has two alternatively-spliced isoforms. Our immunohistochemical studies indicate that CRB-1 is ubiquitously expressed throughout development and in adults. CRB-1 can bind to cyclic AMP-response element (CRE) sites and can be phosophorylated by cAMP-dependent protein kinase (PKA) *in vitro*.

To determine the function of *crb-1* in the worm, we isolated three deletion alleles of *crb-1* from a chemical deletion library. Two are predicted to cause early truncations, and the third deletes part of the bZIP domain. No CRB-1 protein is detected by western blot analysis of these three mutant strains, suggesting that all three are null alleles.

crb-1 mutants are viable and show no obvious abnormalities in brood size, locomotion, mechanosensation, chemotaxis or thermotaxis. However,*crb-1* mutants tend to accumulate at the edge of the bacterial lawn (bordering) and form clumps of animals. In addition, mutations in *crb-1* confer a dauer-constitutive phenotype (Daf-c) at 27C but not at 25C. The Daf-c phenotype of *crb-1* animals is suppressed by mutations in *daf-16* and *daf-12* but not by mutations in *daf-5*. This finding suggests that *crb-1* acts in the DAF-2 insulin receptor-like signaling pathway. A similar role has been suggested for *unc-31* and *unc-64*,[1] which are implicated in synaptic vesicle release. Double mutants between *crb-1* and either *unc-31* or *unc-64* show a strongly enhanced Daf-c phenotype at 25C as has been shown for *unc-31; unc-64* double mutants.[1] This synergy suggests that mutations in *crb-1*, like mutations in *unc-31* and *unc-64*, impair synaptic function. Unlike *unc-31* or *unc-64* mutants, *crb-1* mutants do not have an increased life span. We are further investigating the role of *crb-1* in synaptic transmission, dauer formation, and behavior.

1. Ailion, M., Inoue, T., Weaver, C.I., Holdcraft, R.W. and J.H. Thomas, (1999) PNAS **96**: 7394-7397.