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A *C. elegans* CREB PROTEIN MODULATES TGF-BETA SIGNALING

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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. Analysis of the genome sequence showed that *C. elegans* has a single *CREB*-homologous gene (*crh-1*). We characterized the *C. elegans* and *C. briggsae crh-1* genes and found that they have a similar gene structure and that their 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 crh-1* gene has several alternatively-spliced isoforms. A *crh-1::gfp* transgene is ubiquitously expressed during early embryogenesis and is specifically expressed in several neurons from the L1 stage to adulthood. CRH-1 can bind to cyclic AMP-response element (CRE) sites and can be phosophorylated by cAMP-dependent protein kinase (PKA) and Calmodulin-dependent protein kinase II (CaMKII) *in vitro*.

To determine the function of *crh-1*, we isolated three *crh-1* deletion alleles from a deletion library. Two deletions remove the second exon and are predicted to cause early truncations; the third deletes part of the bZIP domain. No CRH-1 protein is detected by western blot analyses of these three mutant strains, suggesting that all three are null alleles.

crh-1 mutants are viable and show no obvious abnormalities in brood size, locomotion, mechanosensation, chemotaxis or thermotaxis. We found that mutations in *crh-1* confer a dauer-constitutive phenotype (Daf-c). However, like *unc-3, unc-31,* and *unc-64*1 mutants, *crh-1* mutants form dauers at 27C but not at 25C. In addition, like the Daf-c mutants *daf-1, daf-7, daf-8, and daf-14*, *crh-1* mutants tend to accumulate at the edge of a bacterial lawn (bordering) and form clumps of animals. Mutations in many genes that cause a Daf-c phenotype at 25C have been isolated. Characterization of these mutants has shown that an insulin-like and a DAF-7 transforming growth factor (TGF)-beta signaling pathway act in parallel to regulate dauer formation. Dauer pheromone, temperature, and food signals modulate the dauer decision in part by regulating the expression of the TGF-beta homolog, DAF-7 in the ASI chemosensory neurons.

Although *unc-3, unc-31, unc-64* 1, and *crh-1* single mutants do not have strong defects in dauer formation at lower temperatures, double mutant combinations of *unc-3, unc-31,* and *unc-64*1 are strongly Daf-c at 25C. Double mutants between *crh-1* and either *unc-31* or *unc-64,* but not *unc-3,* show a strongly enhanced Daf-c phenotype at 25C. This observation suggests that *crh-1* and *unc-3* affect similar aspects of dauer formation. *unc-3* encodes a transcription factor that is expressed in the ASI neurons and may regulate the expression of *daf-7* 2. Consistent with this notion we found that the expression of a *daf-7::gfp* reporter is strongly reduced in *crh-1* mutants. Our data suggest that *crh-1* mutants incorrectly integrate environmental cues that induce dauer formation and that *crh-1* is part of a chemosensory cascade that regulates TGF-beta signaling.

1. Ailion and Thomas. (2000) Genetics **156**, 1047-1067.

2. Ren, Qian, McCron, and Riddle. (1998) Midwest Worm Meeting abstract, p. 30.