53. *C. elegans* CREB mutants show defects in dauer formation and in behaviors that are coupled to food sensation

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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. *C. elegans* has a single *CREB*-homologous gene, *crh-1*. We characterized the *C. elegans* and *C. briggsae crh-1* genes and found that the *crh-1* gene contains four alternative promoters that give rise to four different CRH-1 isoforms. All CRH-1 isoforms contain a C-terminal DNA-binding bZIP domain; two isoforms contain a N-terminal cAMP-dependent kinase site. The bZIP domain and cAMP-dependent kinase site share high similarity with the respective domains in the *Aplysia*, *Drosophila* and mammalian CREB family members. 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*. Immunohistochemistry with an antibody that recognizes all four CRH-1 isoforms shows that CRH-1 is localized to nuclei and is ubiquitously expressed throughout development.

To determine *crh-1* function, we isolated three *crh-1* deletion. Western blot analysis shows that two deletion alleles, *crh-1(n3450)* and *crh-1(n3451)*, eliminate the expression of the two CRH-1 isoforms that contain the cAMP-dependent kinase site. A third deletion allele, *crh-1(n3315)*, eliminates the expression of all CRH-1 isoforms indicating that *crh-1(n3315)* is a null allele.

All *crh-1* mutants are viable and show no obvious abnormalities in brood size, locomotion, mechanosensation, chemotaxis or thermotaxis. However, *crh-1* mutants tend to form clumps of animals and burrow into the agar, reminiscent of wild-type worms after food has been exhausted. In addition, we found that *crh-1* mutants are dauer-constitutive (Daf-c) at 27˚C but not at 25˚C. Many mutants that have a similar Daf-c phenotype at 27˚C, like *unc-3*, *unc-31*, *unc-64*, have a synthetic Daf-c phenotype at 25˚C in double mutant combinations[1]. Double mutants between *crh-1* and either *unc-31* or *unc-64*, but not *unc-3*, show a strongly enhanced Daf-c phenotype at 25˚C. This observation suggests that *crh-1* and *unc-3* affect similar aspects of dauer formation. The decision to undergo dauer development is made in part by regulating the expression of the transforming growth factor (TGF)-beta homolog DAF-7 in the ASI chemosensory neurons in response to dauer pheromone, food availability, and temperature. *unc-3* encodes a transcription factor that is expressed in the ASI neurons and has been suggested to regulate the expression of *daf-7*[2]. The expression of a *daf-7::gfp* reporter is strongly reduced in *crh-1* mutants, indicating that *crh-1* is part of a sensory signal transduction cascade that regulates *daf-7* expression. Interestingly, *daf-7::gfp* expression is also strongly reduced in *tph-1* mutants which are defective in serotonin biosynthesis[3]. Serotonin modulates many behaviors of the worm in response to food. Our data suggest that *crh-1* mutants have defects in food sensation, and we hypothesize that *crh-1* functions downstream of serotonin in the long-term assessment of food availability.

1. Ailion and Thomas (2000). Genetics **156**, 1047-1067.
2. Ren, Qian, McCron, and Riddle (1998). Midwest Worm Meeting abstract, p. 30.
3. Sze, Victor, Loer, Shi, and Ruvkun (2000). Nature **403**, 560-564.