Article Addendum

Overexpression of caveolins in Caenorhabditis elegans induces changes in egg-laying and fecundity

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Caveolae are small plasma membrane-associated invaginations that are enriched in proteins of the caveolin family in addition to sphingolipids, glycosphingolipids and cholesterol. Caveolae have been implicated in several endocytic and trafficking mechanisms. Mutations in caveolins have been shown to cause disease and caveolae offer one site for pathogen entry. The Caenorhabditis elegans genome encodes two caveolins (cav-1 and cav-2); we have shown that these two proteins have distinct expression patterns. CAV-1 is found in the majority of cells in embryos and in the body-wall muscles, neurons and germ line of adult worms. CAV-2 is expressed in the intestine and is required for apical lipid trafficking. In the course of our studies, we generated several constructs to overexpress caveolins in C. elegans. Here we show that overexpression of cav-1 protects against the decrease in brood size associated with the effects of heat shock and the presence of extrachromosomal arrays in heat-shocked animals. Furthermore, we show that overexpression of cav-2 in the nervous system increases the rate of egg-laying and total number of eggs laid.

Cells utilize a range of different mechanisms to mediate trafficking between the plasma membrane and intracellular membranes. One such mechanism involves caveolae, which are small (50–100 nm) invaginations in the plasma membrane. These invaginations are unusually rich in caveolin, sphingolipids, glycosphingolipids, cholesterol and many signaling proteins. Caveolae require caveolin for their formation and most caveolin appears to traffic to the plasma membrane; however, we and others have identified intracellular caveolin-containing bodies. Caveolae have been implicated in the endocytosis of a range of molecules and pathogens and have also been implicated in a number of signaling processes. Mammals encode three caveolin proteins; caveolin-1 is widely expressed in many tissue types, the closely related caveolin-3 protein is restricted to myocytes, and caveolin-2 has been shown to require caveolin-1 for transport to the plasma membrane. Caveolins have been implicated in a wide range of processes, such as cancer, lung disease, lipid homeostasis and disease, liver regeneration and dystrophies.

We have used Caenorhabditis elegans as a model system to explore the roles of caveolins. C. elegans encodes two caveolin proteins. The C. elegans caveolin-2 protein (CAV-2) is localized to the apical membrane of intestinal cells. Ablation of cav-2 induces abnormal trafficking of yolk proteins and uptake of lipid markers; furthermore, cav-2 mutants suppress an intestinal phenotype induced by defective basolateral recycling in rne-1 and rab-10 mutants. In contrast, caveolin-1 (cav-1) is widely expressed in eggs, and in many tissues at early larval stages but with maturation, CAV-1 becomes restricted to the germ line, nervous system, body-wall muscles, and most likely, the post-synaptic side of the neuromuscular junction. Ablation of CAV-1 function, using dominant-negative constructs based on dystrophic mutations identified in humans, induces neurotransmission and locomotion defects that may offer insights into the pathology of certain muscular dystrophies.

Overexpression of Caveolin-1 Protects Against Reduced Fecundity Caused by Extrachromosomal Arrays and Heat Shock

We found that overexpression of caveolin proteins was able to protect against deleterious effects of transgenes and heat shock in worms. In experiments designed to identify the effects of caveolin overexpression we used a heat-shock inducible promoter, hsp16-2 (pHS), which drives gene expression in a wide variety of tissues, to express the caveolin genes. pHS::cav constructs were introduced into C. elegans using standard microinjection techniques, which result in transgenes which are present on extrachromosomal arrays.
Overexpression of caveolins in C. elegans

In a related set of experiments we overexpressed *cav-1* and *cav-2* in the nervous system of worms. We reasoned that overexpression of *cav-1* in the nervous system, one of the main tissues expressing *cav-1*, might induce phenotypes indicative of its neuronal function and related to those we observed in animals carrying *cav-1* dominant-negative constructs.\(^{18}\) To this end, we cloned the *cav-1* gene downstream from the neuron-specific promoter from the *unc-119* gene.\(^{24}\) To ensure that *cav-1* was being expressed in neurons, we also made a *cav-1::gfp* construct. As a control, we made the same constructs previously described, but used the *cav-2* gene. Surprisingly, we found no obvious phenotypes in animals overexpressing *cav-1*; however, we found that *cav-2* overexpression induced an increase in the total number of eggs laid, and an increase in the rate of egg laying (data not shown). This phenotype was most obvious at 50 hours after the initiation of egg laying (*cav-1* overexpression, mean = 173, sd = 18; *cav-2* overexpression, mean = 307, sd = 37; N2 wild-type, mean = 174, sd = 14). Thus overexpression of *cav-2* from the *unc-119* promoter appears to improve fecundity in worms.

Although *cav-1* is not solely expressed in neurons, it was surprising that its overexpression there produced no detectable, neuronally-controlled, phenotypes. In contrast, overexpression of *cav-2*, a gene expressed in the intestine, did generate a phenotype when overexpressed in the nervous system. Egg laying in *C. elegans* is under neuronal control thus changes in egg laying may be the result of changes in neuronal function brought about by the presence of ectopic CAV-2 protein. The ability of CAV-2 to form caveolae has not been tested,\(^{23}\) however, bioinformatic analysis
suggests that it is more likely to form caveolae than CAV-1. Thus, CAV-2 may alter neuronal function by changing the trafficking in neurons or by changing the behavior of the membrane. The ability of cav-2 to alter neuronal function in contrast to cav-1 and the differential effects of cav-1 and cav-2 following heat shock further support the notion that the two proteins play different roles in cell function in C. elegans.

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