GÖTEBORGS UNIVERSITET

Regulators of Membrane Fluidity

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Abstract

Caenorhabditis elegans PAQR-2 (a homolog of the mammalian AdipoR1 and AdipoR2 proteins) and IGLR-2 (homolog of the mammalian LRIG proteins) form a complex at the plasma membrane that regulates fatty acid desaturation to protect against saturated fatty acid-induced membrane rigidification. Maintenance of membrane homeostasis is fundamental for most cellular processes and, given its importance, robust regulatory mechanisms must exist that adjust lipid composition to compensate for dietary variation. To better understand this phenomenon, we performed forward genetic screens in *C. elegans* and isolated mutants that improve tolerance to dietary saturated fatty acids. These include eight new loss of function alleles of the novel gene *fld-1,* one loss of function allele of *acs-13* and one gain of function allele of *paqr-1. fld-1* encodes a homolog of the human TLCD1/2 transmembrane proteins. The FLD-1 protein is localized on plasma membranes and mutations in the *fld-1* gene help to suppress the phenotypes of *paqr-2* mutant worms, including its characteristic membrane fluidity defects. The wild-type *C. elegans* FLD-1 and human TLCD1/2 proteins do not regulate the synthesis of long-chain polyunsaturated fatty acids but rather limit their incorporation into phospholipids.

C. elegans acs-13 encodes a homolog of the human acyl-CoA synthetase ACSL1. The ACS-13 protein is localized to mitochondrial membranes where it likely activates and channels long chain fatty acids for import. In human cells, ACSL1 activity potentiates lipotoxicity by the saturated fatty acid palmitate (16:0) because it depletes the cells of membrane-fluidizing unsaturated fatty acids. Echoing our findings in *C. elegans*, knockdown of ASCL1 in human cells using siRNA also protects against the membrane-rigidifying effects of palmitate and acts as a suppressor of AdipoR2 knockdown.

A novel gain-of-function allele of PAQR-1, a paralog of PAQR-2, takes over the role of PAQR-2 for downstream effectors. Through genetic interaction studies and domain swapping experiments we showed that the transmembrane domains of PAQR-2 are responsible for its functional requirement for IGLR-2. Conversely, PAQR-1 itself does not require IGLR-2 for its function. The less conserved N-terminal cytoplasmic domains of PAQR-1 and PAQR-2 likely regulate the activity of these proteins, speculatively via a “ball and chain” mechanism similar to that found in certain voltage-gated channels.

We conclude that inhibition of membrane fluidity regulators, such as *fld-1* or *acs-13,* or a gain-of-function allele of *paqr-1* can suppress *paqr-2* mutant phenotypes through different mechanisms, which suggests that *paqr-2* regulates membrane fluidity in more than one way. Despite acting differently, the effects of these three mutations converge into lowering SFA levels while increasing the PUFA levels within phospholipids, and show that membrane homeostasis is likely essential for our ability to tolerate dietary saturated fats.

Key words: PAQR, LRIG, membrane fluidity, domain swapping, lipotoxicity, long chain polyunsaturated fatty acids