

5. The genetics of fat storage

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In mice and humans, a complex neuroendocrine pathway functions to balance energy intake with energy expenditure, ultimately to determine body weight. Energy is mostly stored as triglycerides in specialized fat droplets, the biogenesis and cell biology of which are poorly understood. Little is known about the determinants of body weight in *C. elegans*. As in mammals, a neuroendocrine system, including intact insulin and serotonergic signaling pathways, couple metabolism with food intake and behavior in worms.

We have used Nile Red to visualize fat droplets in the intestinal and hypodermal cells of living *C. elegans*. Nile Red is colorless in aqueous environments but becomes brightly fluorescent in hydrophobic environments. Nile Red staining largely recapitulates the known patterns of staining in various *C. elegans* mutants (e.g. *daf-2*, *daf-2:daf-16*), and conditions where body fat is known to be altered (e.g. dauer). We have performed screens on EMS-mutagenized worms to identify genetic components that may determine total body fat content, and/or fat droplet formation, size, and localization. In addition to screening mutagenized wild type (N2) worms, the screens were also conducted on *daf-16* (a forkhead transcription factor which is a key target of insulin signaling pathway), and *tph-1* (a mutation in tryptophan hydroxylase resulting in lack of serotonin biosynthesis) animals. These latter screens were aimed at identifying genetic components which may only be revealed in the absence of *daf-16* mediated insulin signaling or serotonin.

A total of 12 mutants from the three screens have been selected for further analysis (8 from the mutagenized N2, 2 from *daf-16*, and 2 from *tph-1* animals). Both of the mutants recovered in the *daf-16* background and one of the mutants recovered from the *tph-1* background have been back-crossed into otherwise wild type animals (N2). The 12 mutants fall into several general categories. They include: (i) increased Nile Red Staining, (ii) decreased staining, (iii) small, diffuse droplets, (iv) enlarged droplets, and (v) animals with altered color of stain (e.g. green instead of red).

The Nile Red patterns of staining of each of these mutants were fully recapitulated by feeding fluorescently labeled C12:0 and C5:0 fatty acids to the animals (Bodipy C12:0, and Bodipy C5:0). Similarly, most of the staining patterns were mimicked by Sudan Black B staining of the animals (a method of fat staining that requires fixation of the animals).

None of the mutants are defective in dauer formation or form dauers constitutively at 25°C or 27°C. Two of the mutants confer longer lifespans (up to 2.5 fold over wild type). We are currently mapping the mutations for molecular analysis.