Background mutation in strain RB2126 affects the locomotion behavior of flp-1 mutants

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Abstract

The FLP-1 neuropeptide is involved in locomotion, mechanosensation, and reproduction. Strain RB2126 is reported to contain the flp-1(ok2811) mutation, a prospective null allele. Here we report that strain RB2126, in addition to flp-1(ok2811), also contains two additional background mutations. One of the mutations gives the animals a constitutive dauer phenotype (Daf-c), and complementation testing confirms that it is in the Daf-c gene daf-11. The second mutation is isolated based on the fact that it partially suppresses the elevated body curvature of flp-1(ok2811) mutants, but it remains uncloned. The updated genotype of RB2126 strain is flp-1(ok2811); daf-11(yak155); yak156.

Figure 1. Strain RB2126 contains background mutation(s) that affect the locomotion behavior of flp-1(ok2811) mutants: (A) Strain RB2126 (flp-1(ok2811); daf-11(yak155); yak156) contains a background mutation in a constitutive...
dauer (gene). Representative photos of dauers from the RB2126 strain (Upper panel) and the daf-11(sa195) mutant strain JT195 (Lower panel) grown at room temperature (23°C). (B) Strain RB2126 has background mutation(s) that reduce the deep body bends of flp-1(ok2811) mutants. Photos of worm tracks from wild type (WT), XZ2314 flp-1(ok2811), RB2126 flp-1(ok2811); daf-11(yak155); yak156, XZ2380 flp-1(ok2811); daf-11(sa195), XZ2406 flp-1(ok2811); daf-11(yak155), and XZ2394 flp-1(ok2811); yak156 strains. (C) Representative photos of young (~L3) XZ2314 flp-1(ok2811), XZ2380 flp-1(ok2811); daf-11(sa195), and XZ2406 flp-1(ok2811); daf-11(yak155) strains showing the characteristic "B"-like body posture. (D), (E), (F), (G) Quantification of the track waveform of the strains shown in Figure 1B (see Methods). The wavelength and amplitude of sinusoidal tracks in millimeters (mm) of strains N2 (WT), XZ2314 flp-1(ok2811), RB2126 flp-1(ok2811); daf-11(yak155); yak156, and XZ2394 flp-1(ok2811); yak156 strains. Photos of worm tracks from wild type (WT), XZ2314 flp-1(ok2811), RB2126 flp-1(ok2811); daf-11(yak155); yak156, and XZ2394 flp-1(ok2811); yak156 strains. The updated genotype of strain RB2126 is flp-1(ok2811); daf-11(yak155); yak156.

Description

The FLP family of neuropeptides contains at least 30 genes in C. elegans (Li and Kim, 2008). Some of the flp genes were found to play roles in locomotion, egg laying, and sleep, but most flp genes have unknown function (Li and Kim, 2008; Chang et al. 2015). flp-1 is one of the few flp genes whose mutations are reported to cause overt behavioral defects (Nelson et al. 1998; Hums et al. 2016; Buntschuh et al. 2018; Oranth et al. 2018). The initial flp-1 deletion alleles that were analyzed behaviorally (yn2 and yn4; Nelson et al. 1998) included a deletion of part of daf-10 (Allion and Thomas, 2003; Buntschuh et al. 2018). This resulted in some of the initial behaviors that were attributed to FLP-1 peptides being caused by loss of daf-10 activity (Buntschuh et al. 2018). Subsequent analysis of newer flp-1 deletion alleles suggests that FLP-1 peptides are involved in nose touch sensitivity, locomotion, and reproduction (Buntschuh et al. 2018).

flp-1(ok2811) is a prospective null allele (Hums et al., 2016; Buntschuh et al., 2018). We obtained strain RB2126 (Caenorhabditis Genetics Center, CGC) in order to analyze flp-1(ok2811) null mutant locomotion behavior. When the strain was grown at room temperature (23°C), some animals formed noticeable dauers similar to strains containing mutations in constitutive dauer (Daf-c) genes (Hu, 2007) (Fig. 1A). To examine whether the Daf-c phenotype was because of a background mutation, strain RB2126 was outcrossed three times. This outcross resulted in the segregation of two different mutants with readily apparent phenotypes: one resulting in deep body bends and a second in the Daf-c phenotype. PCR genotyping showed that the deep body bends co-segregated with the flp-1(ok2811) deletion. The mutation causing the Daf-c phenotype was mapped to chromosome V close to the daf-11 gene and a complementation test using daf-11(sa195) (a presumed null allele of daf-11) showed that it is in daf-11 (Birnby et al. 2000). These results suggest that flp-1(ok2811) mutants exhibit elevated body curvature, and that strain RB2126 has a background mutation in daf-11. This new daf-11 allele in strain RB2126 was named yak155.

Methods

Request a detailed protocol
Strain maintenance

Strains were maintained at 15˚C or room temperature (23˚C) on NGM plates with *E. coli* strain OP50 as a food source (Brenner, 1974).

Waveform quantification

First-day adult animals were placed on an OP50 plate and allowed to move forward until they had completed at least five tracks. Each animal’s tracks were imaged at 50X magnification using a Nikon SMZ745 stereoscope and an iLabCam. Worm track period and 2X amplitude were measured using the line tool in Image J. For each worm, three to five periods and amplitudes were measured. Each experiment was performed on 5-10 worms.

Statistics

P values were determined using GraphPad Prism 9.0 (GraphPad Software). Normally distributed data sets requiring multiple comparisons were analyzed by a one-way ANOVA followed by a Bonferroni test. Normally distributed pairwise data comparisons were analyzed by two-tailed unpaired t tests.

Strains

Bristol N2: wild type
EG7765: oxT76[Peft-3::GFP::H2B::tbb-2 3’UTR, unc-18(+)] IV; unc-18(md299) X
JT195: daf-11(sa195) V
JT6709: unc-42(e270) V daf-11(sa195) V
RB2126: flp-1(ok2811) IV; daf-11(yak155) V; yak156
XZ2314: flp-1(ok2811) IV [3X outcrossed from RB2126]
XZ2370: oxT76[Peft-3::GFP::H2B::tbb-2 3’UTR, unc-18(+)] IV; daf-11(yak155) V
XZ2380: flp-1(ok2811) IV; daf-11(sa195) V
XZ2394: flp-1(ok2811) IV; yak156
XZ2406: flp-1(ok2811) IV; daf-11(yak155) V

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