Mutant alleles

\textit{ham-3(n1654)III}, \textit{ham-3(tm3309)III}, \textit{swsn-2.2(ak3161)I/hT2[bl1-4(e937) let-7(q782) qls48]I;III}, \textit{psa-1(os22)IV}, \textit{psa-4(os13)IV, swsn-7(gk1041)/min1[mls14 dpy-10(e128)] II, let-526(gk816) I / hT2 (I;III)}

Transgenes

\textit{zdIs13: Is[tph-1::gfp], otIs266: Is[cat-1::mCherry], otIs225: Is[cat-4::gfp], otIs226: Is[bas-1::gfp], inIs179: Is[ida-1::gfp], uts22: Is[mec-3::gfp], otIs33: Is[kal-1::gfp], otIs337: Is[unc-86 fosmid::yfp; ttx-3p::mCherry] (kind gift from Pat Gordon), kuls34: Is[sem-4p::sem-4::gfp] (kindly provided by Min Han), NG2656: Ex[ham-3::gfp; rol-6], otEx5092, otEx5142, otEx5143: Ex[ham-3 rescuing fosmid (WRM0626dF04); rol-6(d)], otEx5093, otEx5145, otEx5146: Ex[ham-3::gfp; elt-2::dsRed], otEx5094, otEx5148, otEx5149: Ex[swsn-2.2::mChOpti; ttx-3::gfp]}

Generation of transgenes

\textit{ham-3 and swsn-2.2} reporter constructs were generated by PCR fusion (HOBERT 2002). The \textit{ham-3} genomic locus was fused to \textit{gfp} and injected into N2 wildtype at 10 ng/µL with \textit{elt-2::dsRed} at 5 ng/µL as an injection marker. The \textit{swsn-2.2} genomic locus was fused to \textit{mChOpti} (a codon-optimized version of \textit{mCherry}) and injected into N2 wildtype at 5 ng/µL with \textit{ttx-3::gfp} at 5 ng/µL as an injection marker. For rescue experiments, the fosmid WRM0626dF04 was linearized and injected at 10 ng/µL with a linearized plasmid containing \textit{rol-6} at 5 ng/µL directly into OH9422, a strain containing the \textit{ham-3(n1654)} mutation as well as the transgene \textit{zdIs13}, an integrated \textit{tph-1::gfp} reporter. All arrays were generated as complex arrays with 100-125 ng/µL of sonicated bacterial genomic DNA.

Whole Genome Sequencing

Genomic DNA was prepared from \textit{ham-3(n1654)} mutant animals as previously described (SARIN et al. 2010). DNA was sequenced using an Illumina Genome Analyzer II platform and sequence analysis was done using MAQGene (BIGELOW et al. 2009).

RNA interference

RNAi was performed using a bacterial feeding protocol in an \textit{nre-1 lin-15b} mutant background (SCHMITZ et al. 2007).

Microscopy

A Zeiss Axioplan 2 equipped with Nomarski and fluorescence optics was used. DIC and fluorescent images were collected and processed using Micro-manager (EDELSTEIN et al. 2010).


