The salt-inducible kinase KIN-29 regulates lifespan via the class II histone-deacetylase HDA-4

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Figure 1: Mutations in hda-4 suppress the extended lifespan of kin-29 mutants. Lifespan was determined by assaying the percent survival of animals as a function of time. Scoring was performed every other day on NGM plates containing 5 μM FUDR and concentrated E. coli OP50 bacteria (see methods). Mean lifespans were with n>100 animals for each genotype (days ± SEM): wild-type, kin-29(oy38) and hda-4(oy57) single mutants, and kin-29(oy38)hda-4(oy57) double mutants. Data is shown from a single biological repeat (#4) with all strains examined in parallel. The mean lifespan of hda-4 single and kin-
29hda-4 double mutants is significantly different from that of wild-type and kin-29 single mutants, whereas the mean lifespan of hda-4 single mutants is not statistically different from kin-29hda-4 double mutants (log-rank test, p<0.01). * indicates animals fed at a higher food density. See table for data of each biological repeat and a summary of lifespan statistics.

**Description**

kin-29 encodes the C. elegans homolog of mammalian Salt-Inducible Kinases (SIKs). kin-29 mutants are small, have increased propensity to develop into non-reproductive dauer larvae, have reduced chemoreceptor gene expression (Lanjuin and Sengupta, 2002; van der Linden et al., 2007), have reduced cellular ATP despite increased fat stores, and show reduced sleep (Grubbs et al., 2020). KIN-29 phosphorylates and inhibits the class II histone deacetylase 4 homolog HDA-4 to regulate gene expression in sensory neurons (van der Linden et al., 2007). The longevity phenotype of kin-29 mutants is suppressed by mutations in daf-16 (Lanjuin and Sengupta, 2002), which encodes a forkhead box protein O (FOXO) transcription factor (Kenyon et al., 1993). These results indicated that the increased lifespan of kin-29 mutants may be due to reduced insulin signaling.

To determine whether HDA-4 is required for the KIN-29 regulation of lifespan, we performed a survival analysis. As previously reported (Lanjuin and Sengupta, 2002), kin-29(oy38) mutants are long-lived. In contrast, hda-4(oy57) mutants are short lived (Fig. 1). The kin-29hda-4 double mutant longevity phenotype was similar to the phenotype of hda-4 single mutants (Fig. 1), suggesting that hda-4 acts downstream of kin-29 to regulate lifespan. Together with our previous findings (van der Linden et al., 2007), these genetic results suggest that KIN-29 may regulate lifespan via the action of HDA-4. Since SIKs can inhibit FOXO activity via HDAC4 to regulate gene expression (Mihaylova et al., 2011; Wang et al., 2011), it would be interesting in future studies to test the model that KIN-29/HDA-4 signaling converges on DAF-16/FOXO to modulate gene expression associated with longevity.

**Methods**

**Request a detailed protocol**

All strains were maintained at 20°C for at least two generations before the lifespan assay. Lifespan assays were conducted at 20°C by placing age-synchronized L4 larvae of wild-type and the indicated genotypes on 6-cm diameter plates containing nematode growth media (NGM) plates and 5 μM 5’-fluoro-2’-deoxyuridine (FUDR) (Sigma) seeded with ~10^{11} E. coli OP50 bacterial cells per ml and 10-15 worms per plate. The number of dead hermaphrodites was scored every two days until all worms were dead, and the percentage of survival was calculated. Death was scored as a lack of movement after a nose touch. Animals that experienced ventral rupture, bagging or walling were censored from the analysis. ~100-300 animals from each strain were used for each biological replicate. Statistical analyses and survival curves were performed with the Online Application for Survival Analysis software (OASIS 2, https://sbi.postech.ac.kr/oasis2/ (Han et al., 2016). The significance of differences in mean lifespan was calculated using the Log-Rank test.

**Reagents**

Strains used in this study are the wild-type strain N2 variety Bristol (Brenner, 1974), PY1476 kin-29(oy38), PY4111 hda-4(oy57), and PY4112 kin-29(oy38)hda-4(oy57) (Lanjuin and Sengupta, 2002; van der Linden et al., 2007). All strains are available at the CGC. The deletion/rearrangement in the oy38 allele was confirmed by the polymerase chain reaction (PCR) and the single nucleotide change in oy57 allele by sequencing of a PCR product.

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**References**


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