

Supplemental Data

C. elegans *daf-6* Encodes a Patched-Related Protein

Required for Lumen Formation

Elliot A. Perens and Shai Shaham

Supplemental Experimental Procedures

General Methods and Strains

C. elegans were cultured at 20°C as described by Brenner (1974), unless otherwise indicated. The wild-type strain used was *C. elegans* var. Bristol (N2). The following strains were used: LGI: *che-3(e1124)*, *che-13(e1805)*, *che-14(e1960, ok193)*; LGII: *daf-19(m86, m344)*; LGIV: *daf-10(e1387)*, *osm-3(p802)*; LGV: *che-11(e1810)*, *osm-6(p811)*, *ntl-1* (P_{gcy-5} -GFP); LGX: *daf-6(e1377, m176, m186, m197, m205, m506, n1543)*. *Is*[$P_{T08G3.3}$ DsRed ADF] is unmapped. *mcEx178* (P_{che-14} *che-14*::GFP), *nsEx42* (P_{daf-6} GFP), *nsEx71* (P_{vap-1} DsRed), *nsEx75* (P_{HS} *daf-6*), *nsEx85* (P_{daf-6} *daf-6*::GFP), *nsEx94* (P_{daf-6} *daf-6*::GFP), *nsEx105* (P_{vap-1} *vap-1*::GFP), *nsEx110* (P_{daf-6} *daf-6*::GFP), *nsEx131* (P_{HS} *daf-6*), *nsEx164* (P_{daf-6} *daf-6*::GFP), *qpEx*[P_{vha-1} GFP], and *utEx*[P_{che-2} *che-2*::GFP].

Plasmid Constructions

For P_{daf-6} GFP (pEP1), we amplified by PCR a 3 kb DNA fragment from cosmid F31F6 containing sequences upstream of the *daf-6* ATG as well as the coding sequence for the first four amino acids. The resulting amplicon was ligated to the GFP vector pPD95.69 (Miller et al., 1999) digested with XbaI and BamHI. For P_{daf-6} *daf-6*::GFP (pEP3C), we amplified by PCR DNA containing the *daf-6* genomic coding region and 3 kb of upstream sequences from cosmid F31F6. The resulting amplicon was ligated to the GFP vector pPD95.75 (Miller et al., 1999) digested with XbaI and BamHI. For P_{vap-1} DsRed (pEP6), we amplified by PCR a 2.8 kb genomic DNA fragment containing sequences upstream of the *vap-1* ATG. The resulting amplicon was ligated to the DsRed gene (pEP5) digested with XbaI and BamHI. For P_{HS} *daf-6* (pEP7), we amplified by PCR the *daf-6* cDNA in plasmid pBSKS (Stratagene) using the T3 and T7 primers. The resulting amplicon was digested with BamHI and KpnI and ligated into the BamHI and KpnI restriction sites of the heat shock construct pPD49.78 (Fire et al., 1990). For P_{HS} *che-14* (pEP36), we amplified by PCR the *che-14* cDNA from a mixed stage cDNA library. The resulting amplicon was digested with EcoRV and NheI and ligated into the EcoRV and NheI restriction sites of the heat shock construct pPD49.78 (Fire et al., 1990). Point mutations in pEP3C were generated using QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene). The “SSD alone” construct (pEP29) was generated by PCR and included 3 kb of genomic sequence upstream of the *daf-6* ATG, the coding region for the first 24 amino acids of DAF-6 (including the putative signal sequence), and the coding region for amino acids 264-439 (amplified from the *daf-6* cDNA) cloned into pPD95.75. The “ Δ loop2” construct (pEP33) was generated by amplifying pEP3C by PCR except for the region coding for amino acids 564-735, and inserting sequence encoding amino acids LDL in its place.

Supplemental References

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics* 77, 71–94.

Fire, A., Harrison, S.W., and Dixon, D. (1990). A modular set of *lacZ* fusion vectors for studying gene expression in *Caenorhabditis elegans*. *Gene* 93, 189–198.

Miller, D.M., Desai, N.S., Hardin, D.C., Piston, D.W., Patterson, G.H., Fleenor, J., Xu, S., and Fire, A. (1999). Two-color GFP expression system for *C. elegans*. *Biotechniques* 26, 914–918, 920–921.

A

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DAF-6 (264-435)  A F V P N F V S F F V L A M Y A . . . . . L V S S F T L K S S S A T K I D W T I S K .
Dm AAF57274 (270-432)  V V P Y F S S T L L M G L F S . . . . . I I T C M M G . . . . . D A V F S K .
Hs FLJ30296 (162-327)  V S E R Y L V T S C I L V V T M A . . . . . I L C C M G . . . . . D C V R S K .
Hs FLJ44037 (382-545)  T V P V E H A Y L L I L F A . . . . . V T S C F R F . . . . . D C L R N K .
PTC-1 (656-818)      L A G Y A L M L A Y A I V T Q A R . . . . . F D N C L P A . . . . . T E S S .
CHE-14 (300-477)    S K K N K F H L K G L L M D V K R D R L L A A A L R D S M L A L L A A G . . L V M I V V A V H S Q S
NPC-1 (632-807)     V T A L A F L I G V I F S L G R . . . . . Y F V C E N Q L W S . . . . . I L V H S R .
NPC-2 (595-772)     A A V L I W V I T M L G T I N H W P . . . . . E S S T L S A . . . . . L V H H K .

DAF-6 (264-435)  P W L A A A G M F S I V L S I T S A F G F L F I L G V R Y N V I N T . I P F L L I A G I D D M F
Dm AAF57274 (270-432)  P F L G L L G N V S R L M A T L L A A F G L A M Y C G E F I G I N I . A A P F L M I G I G I D D T F
Hs FLJ30296 (162-327)  P W L G L L G L V T I S T A T L L T A A G I I N L T G G K Y N S T F L . G V P F V M L G H G L Y G T F
Hs FLJ44037 (382-545)  M C V A A F G V I S A F L A V V S G F G L L L H I G V P F V I I V A . N S P F L L L G V G V D M F
PTC-1 (656-818)      M G L A L A G V L V V T F A S V A G L G L A T W F G I E F N A A T T Q . T V P F L L L G I G V D N M F
CHE-14 (300-477)    L L Y A F I V V I L L A L S V V G A L G V Y S L F T D E F P L L N L . V T F V L L I A I G S D A F
NPC-1 (632-807)     I C L G M L S V I N L L S S F C S W G I F S M F G T H P V K N A L V V Q F F V V T L L G V C R T F
NPC-2 (595-772)     L L I S T S A V M I S V I S V W C S I G M F S L F G V H A T D N A I I V L F E V I T C L G I N R I F

DAF-6 (264-435)  L M N A C W D Q T S K S L S V P . . . . . E R M S K T L S H A G . V A V I T I N V T D V M S F
Dm AAF57274 (270-432)  V M L A G W R R T K A K M P V A . . . . . E R M G L M M S E A A . V S I T I T S V T D F I S F
Hs FLJ30296 (162-327)  E M L S S W R K T R E D O H V K . . . . . E R T A A V Y A D S M . L S F S L T T A M Y L V T F
Hs FLJ44037 (382-545)  T M I S A W H K T N L A D D I R . . . . . E R M S N V Y S K A A . V S T T I T I T I N I L A L
PTC-1 (656-818)      M L L H N Y R D V V K L A G H . . . . . A E M A I L M R E T G . M S I C T S I N N I L S F
CHE-14 (300-477)    T L K S N F P K H L N E D T F H . . . . . T F S H I T S . F I M F I C F S T G I W P F
NPC-1 (632-807)     W V V K Y Y A Q Q R V S M P Y M S P D Q . C P E I V G M V M A G T I . P A M F S S S L G A T S F
NPC-2 (595-772)     V I I R T F Q A N G H C Y G L P N I S Y R E M N R H I S N V M R R S T P I V L N S L L C S T C L F

DAF-6 (264-435)  A I G C . . . . . I D L P G T R F F C T Y A C V S V A F S Y F Y Q L T F F S G A M A T M G E V I E
Dm AAF57274 (270-432)  L I G T . . . . . I S P F R S V R I F C T Y S V F A V C F T F L W H I T F F A A C M A I S G Y I R E
Hs FLJ30296 (162-327)  G I G A S P . . . . . F N T E A A R I F C C N S C T A I F N Y L Y V L S F Y G S S L V F T G Y T E
Hs FLJ44037 (382-545)  Y T G I . . . . . M S F R S V Q C F C T T T B U L L F C Y F Y N I I F G F A F M A L D G K E F
PTC-1 (656-818)      L I G T . . . . . L E P T P A L R S F C A Q S T L L T I F N F T A I L T I I P A I I S I D L R R K
CHE-14 (300-477)    F I N I . . . . . T S N V I V F R C F G L F A G V T V T E N Y F E M V V S F L P A F L I Q Y R H F
NPC-1 (632-807)     F I G G . . . . . F I D L P A T R T F C L Y A G L A V L T D V V L H C T I F L A L F V W D T Q R E
NPC-2 (595-772)     L A G G V L P Y V S V S M P A V E V F A R H A G L A I L M D T A F Y L L V M L P L F O Y D A R R E

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B

Relationship between DAF-6 and closely related SSD containing proteins

| | overall | | SSD | |
|-------------|-----------|-------------|-----------|-------------|
| | %identity | %similarity | %identity | %similarity |
| PTC-1 | 21.0% | 38.7% | 20.8% | 43.4% |
| NPC-1 | 20.4% | 37.2% | 19.7% | 39.3% |
| NPC-2 | 20.9% | 36.8% | 26.0% | 42.8% |
| CHE-14 | 16.5% | 32.2% | 21.4% | 40.5% |
| Dm AAF57274 | 29.0% | 46.2% | 36.4% | 54.9% |
| Hs FLJ44037 | 19.5% | 31.3% | 33.5% | 53.2% |
| Hs FLJ30296 | 18.9% | 33.7% | 23.7% | 47.7% |

Supplemental Figure S1. DAF-6 Is a Conserved Sterol-Sensing Domain-Containing Protein

(A) Sequence comparison of putative SSDs of DAF-6 (amino acids 264-435), *Drosophila* AAF57274 (270-432), human FLJ30296 (162-327), human FLJ44037 (382-545), *C. elegans* PTC-1 (656-818), *C. elegans* CHE-14 (300-477), *C. elegans* NPC-1 (632-807), and *C. elegans* NPC-2 (595-772). Boxes, similarities and identities. Bold letters and dark shading, identities. Light shading, similarities.

(B) Identities and similarities between DAF-6 and closely related SSD-containing proteins. Percentages are shown for the entire proteins (overall) and for the SSD alone (SSD).

Supplemental Table S1. *daf-6* Amphid Mutant Defects

| | Genotype | L1 | L2 | L3 | L4 | Adult |
|--|---------------------|----------|----------|----------|----------|----------|
| (A) % Dye-filling defective | N2 | 0 (50) | 0 (50) | 0 (50) | 0 (50) | 0 (50) |
| | <i>daf-6(e1377)</i> | 100 (50) | 100 (50) | 100 (50) | 100 (50) | 100 (50) |
| (B) % Cilia fail to extend through sheath/socket | N2 | ND | 0 (20) | 0 (21) | 0 (22) | 0 (20) |
| | <i>daf-6(e1377)</i> | ND | 100 (20) | 100 (23) | 100 (23) | 100 (22) |
| (C) % Curved cilia | N2 | 0 (25) | 0 (27) | 0 (37) | 0 (22) | 0 (28) |
| | <i>daf-6(e1377)</i> | 16 (44) | 69 (49) | 100 (39) | 100 (33) | 100 (20) |
| (D) % Vacuole accumulation | N2 | 0 (21) | 0 (30) | 0 (18) | 0 (34) | 0 (20) |
| | <i>daf-6(e1377)</i> | 38 (32) | 66 (32) | 89 (36) | 92 (36) | 100 (20) |

Numbers in parentheses are the total number of animals scored.
 ND, not determined.