Characterization of The Voltage-Gated K^+ Channel Gene, A Novel Cilia Gene $K_V3.2$, in Cilia Biogenesis

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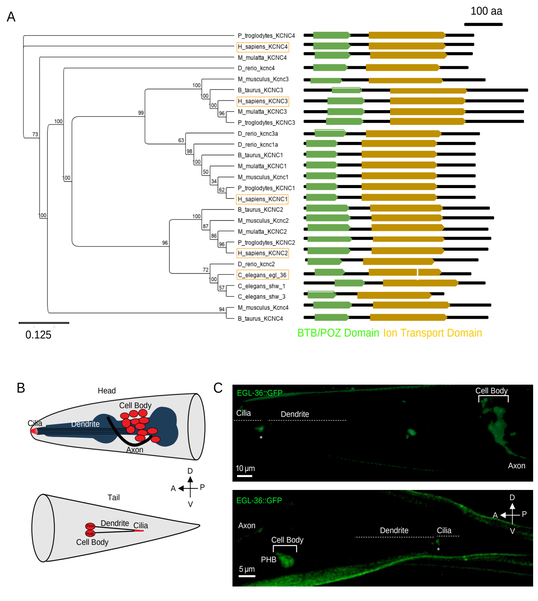
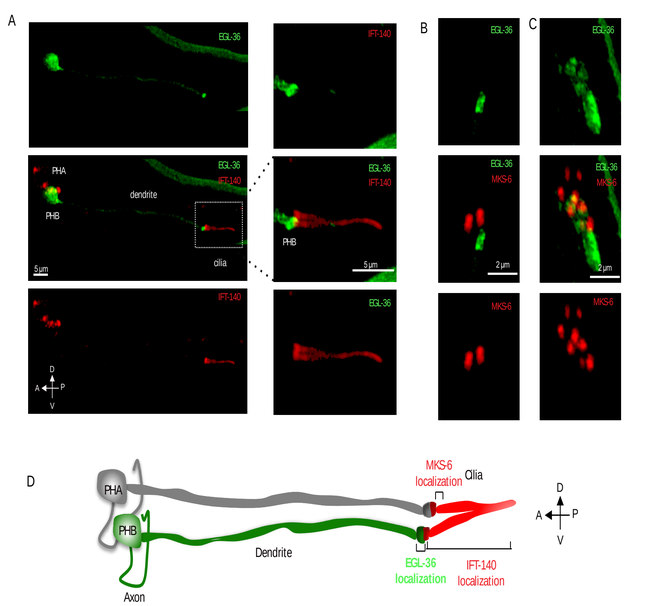
Abstract

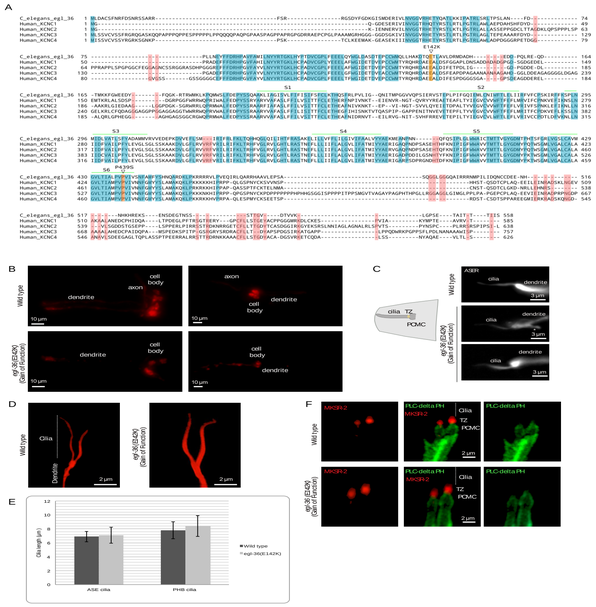
Though discovery of cilia coincided with the use of microscopes by Antonie van Leeuwenhoek over 300 years ago, cilia have received great attention in the last 25 years and have emerged as major cellular organelle that plays a key role in the growth of embryos, signaling pathways, and chemosensation. Because of the functional importance of cilia, cilia were implicated in a variety of human pathologies, such as Bardet-Biedl syndrome (BBS), Meckel-Gruber syndrome (MKS) Alström syndrome (ALMS), polycystic kidney disease, or retinitis pigmentosa, collectively called ciliopathy. Though tremendous efforts have been made to discover the molecular architecture of cilia, we are still far away from knowing the full molecular composition of cilia.

Here, we used the nematode *Caenorhabditis elegans* as a model organism to understand the ciliary involvement of the voltage-gated K+ channel EGL-36 , a member of the Shaw subfamily (Kv3). We first generated transgenic strains expressing the C-terminal tagged EGL-36 with GFP followed by co-expression with a ciliary and transition zone markers to reveal the subcellular localization of EGL-36 in the ciliate sensory neurons. Confocal microscopy analysis revealed that EGL-36 is concentrated at the base of cilia, with distribution of axon, dendrite and cell soma. Our mutant analysis with a gain of function variant (*egl-36*(n728) reveals that EGL-36 is not essentially needed for ciliogenesis though we are still analysing the individual cilia morphology in *egl-36*(n728) mutant.

Results







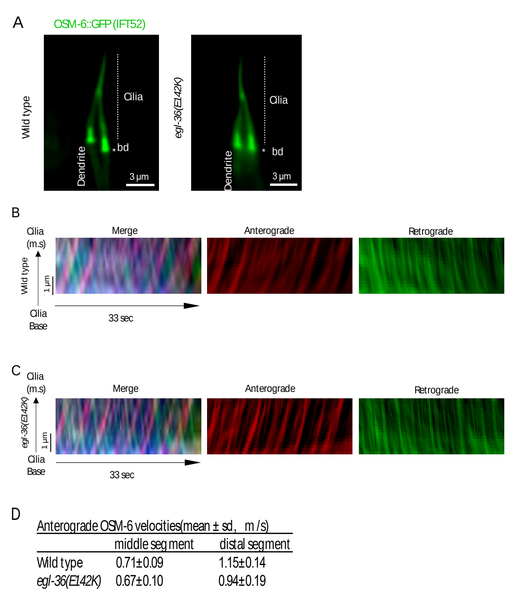


Figure 1: Distributions of EGL-36 in the ciliated sensory neurons
A) Phylogenetic tree of the KCNC-type potassium channel amino sequences from human, chimp, mice, Zebrafish, cow, Rhesus monkey and C. elegans is displayed. Bootstrap values are indicated at nodes of the phylogenetic tree. Nodes with 100% imply great support for them. KCNC family shares the protein domain (BTB/POZ Domain and Ion Transport Domain) B) Schematic representation of sensory neurons showing the anatomical positions of cell body (red), axon, dendrite and cilia (red) in the head (amphid) and tail (phasmid). C) EGL-36::GFP localizations are displayed in the head and tail sensory neurons. EGL-36 staining is observed in the cell body, dendrite, axon and cilia base. Asterisk indicates EGL-36 signal at the cilia base. A, anterior; P, posterior; D, dorsal; V, ventral. (L) Ventral view. Scale bar: 5 µm

Figure 2: EGL-36 is enriched at the base of cilia in C. elegans.
A, B and C) Confocal fluorescence microscopy analysis of worms expressing EGL-36::GFP (driven by a cilia specific promoter) together with MKS-6::mCherry, a transition zone marker, (a single copy insertion) or IFT-140::mCherry (a single copy insertion) revealed that EGL-36::GFP concentrates at the base of cilia in head (C, right column) and tail sensory neurons (PHB not PHB) (A and B, left and middle columns) in C. elegans. EGL-36::GFP is not observed inside of cilia. Scale bar: 5 µm (A), 2 µm (B) D) An illustration showing the localization of EGL-36 in relation to cilia in the tail sensory neurons (PHA/PHB). IFT-140 and MKS-6 stain cilia and transition zone, respectively. EGL-36 is exclusively observed at the base of cilia in the PHB sensory neurons but EGL- 36 signal is not seen in the PHA sensory neuron. A, anterior; P, posterior; D, dorsal; V, ventral. (L) Ventral view.

Figure 3: Investigation of cilia morphology in egl-36 mutants
A) A diagram displaying multiple sequence alignment of human KCNC1, KCNC2, KCNC3 and KCNC4 and C. elegans EGL-36 protein sequences. Identical amino acids are marked blue and pink. B) egl-36(n728) and egl-36(n728n398) mutants exhibit wild type-like dye uptake. Fluorescence images depicting the uptake of fluorescence in the head (left) and tail (right) are shown. Normal fluorescence dye uptake demonstrates correctly formed cilia. Scale bar: 5 µm. C) A schematic of ASE cilia is shown. Fluorescence images displaying ASE cilia and distal dendrite in wild type and egl-36(n728). Scale bar: 3 µm. Asterisk (*) indicates an abnormal projection from the distal dendrite in egl-36(n728) mutants. D) Confocal images display Y like AWB cilia in the head of wild type and egl-36(n728). Scale bar: 2 µm. F) Confocal images show colocalization of tdTomatto tagged MKSR-2, a transition zone marker, and GFP-tagged PLC-delta PH. GFP-tagged PLC delta PH decorates exclusively at the periciliary membrane compartment (PCMC) and dendrite but is excluded from cilia in wild type and egl-36(n728) mutants. E) ASE and PHB cilia length is played for wild type and egl-36(n728).

Figure 4: IFT localization and IFT speed in egl-36 mutants
A) GFP-labelled OSM-6/IFT52, an IFT complex B protein, decorates exclusively the tail cilia and basal body in wild-type, displaying a single projection from the basal body. Fluorescence images are displaying IFT localization in wild type and egl-36(n728). Scale bar: 3 µm. B and C) Representative kymographs of OSM-6/IFT52::GFP translocating in the PHB cilia of wild type and egl-36(n728) mutants were shown. Color kymographs for anterograde (Red), retrogrades (Green) and merged (Red and Green) were created with ImageJ equipped with KymographClear. The angle of the trajectory in each kymograph was measured to reveal the average speed of OSM-6/IFT52::GFP in wild type and egl-36(n728). Kymographs show travel time and travel distance. D) Average speeds of OSM-6 were presented in wild type and egl-36(n728) mutants. Sd: standard deviation