A ccpp-6 deletion mutation does not impair gross cilia integrity in C. elegans

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Abstract

Tubulin glutamylation is a reversible modification that regulates microtubule function in cilia. The removal of glutamylation from microtubules is carried out by a family of cytosolic carboxypeptidase (CCP) enzymes. C. elegans has two deglutamylating enzymes, CCPP-1 and CCPP-6, homologs of mammalian CCP1 and CCP5 respectively. CCPP-1 is required for ciliary stability and function. To determine whether CCPP-6 is similarly required for cilia integrity in C. elegans we analyzed the ccpp-6(ok382) deletion mutant. We find that both dye-filling and male mating are normal, suggesting that CCPP-6 is not required for ciliary integrity in C. elegans.

Figure 1. Markers of cilia function are unaltered in ccpp-6(ok382) mutants.

A) Diagram of the ccpp-6 gene structure indicating the location of the ok382 deletion (red), coding exons in black; Scale bar: 1kbp. B) images of dye filling, and C) quantification of numbers of worms that filled with dye. Number of worms analyzed: WT n=43; ccpp-6 n=70. D) Percentage of males completing the response step of male mating. Response rate for three independent trials shown (·), and the mean of the replicates indicated. Error bars indicate s.d.

Description

The microtubules of the ciliary axoneme are subject to a variety of post-translational modifications, which regulate cilia function and structural integrity. One such modification, glutamylation, involves the reversible addition of glutamic acid to the C terminal tail of tubulin. Both hypo- and hyper-glutamylation have been associated with ciliary dysfunction in C. elegans.
(O’Hagan et al. 2011; Chawla et al. 2016). Loss of the deglutamylating enzyme, CCPP-1, causes a dye-filling phenotype indicative of a progressive degeneration of amphid and phasmid cilia. In male-specific neurons PKD-2 is mislocalized when CCPP-1 is lost, leading to male mating defects (O’Hagan et al. 2011, 2017). In addition to CCPP-1, C. elegans possesses a second cytosolic carboxypeptidase, CCPP-6. Similar to CCPP-1, CCPP-6 is expressed in the amphid neurons, moreover the ccpp-6(ok382) mutation leads to hyperglutamylation, indicating that CCPP-6 has deglutamylating activity (Kimura et al. 2010). We analyzed amphid and male-specific cilia in the ccpp-6(ok382) mutant worms to determine whether CCPP-6, like CCPP-1, is required for cilia integrity.

We first obtained and outcrossed the ccpp-6(ok382) deletion mutant. The ok382 deletion removes the start codon of the gene and both the first and second exons in their entirety (Fig 1A). The ccpp-6(ok382) worms did not show a defect in dye-filling with dye detected in 100% of adult amphid cilia (Fig1B&C), suggesting that the cilia are structurally intact. This contrasts with ccpp-1 mutants, where adult worms do not uptake dye due to structural defects in the cilia (O’Hagan et al. 2011). Loss of CCPP-1 also leads to defects in the male-specific CEM cilia and an inability to complete the response step of male mating (O’Hagan et al. 2011, 2017). We therefore analyzed male mating in the ccpp-6(ok382) mutants (Fig 1D). The lov-1; him-5 strain is known to have a defect in male mating and was used as a control. We found no significant difference between the him-5 control strain, which does not have a mating defect, and the ccpp-6; him-5 strains (student’s t-test P>0.05) indicating that CCPP-6 is not required for proper male mating response and thus the cilia are likely structurally intact.

In summary, we have found that the ccpp-6(ok382) mutants do not have overt defects in the amphid cilia nor impairment of the cilia required for male mating. Because the ok382 deletion allele removes the start codon (fig 1A) and leads to hyperglutamylation of the ciliary microtubules (Kimura et al. 2010) we infer that this allele severely diminishes CCPP-6 function. Thus the lack of ciliary impairment in our assays suggests that the CCPP-6 protein is dispensable for gross ciliary function, although we cannot rule out the presence of subtle defects. This contrasts with CCPP-1 which is absolutely required for the integrity of both amphid and male-specific cilia (O’Hagan et al. 2011, 2017).

### Methods

We carried out the response assay as detailed in (Chawla et al. 2016). Briefly, young males were isolated and kept at 15°C overnight, and warmed to room temperature before use. Plates were seeded with 10μl of concentrated OP50, and ~30 unc-119 hermaphrodites placed in the food. Two males were placed in the center of the plate, and individually monitored for the execution of the response step of male mating during a 5 min period. A male was scored as positive if it began scanning a hermaphrodite with his tail and maintained contact for 10s or more. The response rate for each of three independent trials is shown, and the average of the three trials is plotted. Each trial assayed at least 10 worms of each genotype.

### Reagents

| Strain name | Genotype | Available from |
|-------------|----------|----------------|
| NIN59       | ccpp-6(ok382) II | authors        |
| NIN81       | ccpp-6(ok382) II; him-5(e1490) V | authors        |
| PS3151      | lov-1(sy552) II; him-5(e1490) V | CGC            |
| DR466       | him-5(e1490) V | CGC            |
| HT1593      | unc-119(ed3) III | CGC            |

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