Targeting cyclic nucleotide phosphodiesterase as a potential nematicide by using chemical and biochemical agents

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Abstract

Nearly 4,100 species of plant parasitic nematodes cause a serious constraint in global food security, and about ~100B crops are damaged due to them. Nematode behavior and lifecycle depend on cyclic nucleotide signaling. PDEs are major determinants of cellular levels of cyclic nucleotides because the main process of lowering active intracellular cyclic nucleotide second messengers (cAMP and cGMP) in the cells is through PDEs. However, there is currently limited knowledge of the role of individual PDEs in mediating *C. elegans* signaling pathways. Mammals possess 11 PDE families (PDE1-PDE11), and nematodes have six PDE genes representing six families. Our studies show that exposing wildtype *C. elegans* N2 strain to a human PDE1-specific inhibitor reduced chemotactic response to the odorant. To mimic the phenotype observed by exposing human PDE1- specific inhibitor, exposed *C. elegans* strain in which PDE1 was ablated ("knocked out") can also disrupt chemosensation. In addition, exposing a PDE-1/PDE-4 double mutant *C. elegans* strain to the pde-3 RNAi construct showed a greater reduction in reproduction and developmental delays, and nematodes appeared to be lethargic. Altogether, these findings will help us to understand the role of individual PDEs in *C. elegans* and lay the foundation for discovering novel chemical or genetic controls for phyto parasitic nematodes that minimize adverse effects on the host or the environment. The outcome of this work may lead to increased crop productivity globally, thereby enhancing access to a safe and secure food supply.

Methodology

1. **Creation of transgenic *C. elegans* strains with ablated PDE genes-** Ablation of each of the six PDE genes in *C. elegans* was performed by In Vivo Biosystems by targeted genome editing using CRISPR/Cas9. For each PDE knockout (KO), guide RNAs were designed to (at minimum) remove the catalytic domain (Fig.1). Whole genome sequencing by the Hubbard Center for Genome Studies (HCGS) at UNH confirmed the targeted deletion of each of the PDE KOs, with no off-target effects of genome editing observed.

Fig. 1. Whole genome sequencing analysis of transgenic *C. elegans* strains with PDE gene ablation.

2. **Use of RNA interference (RNAi) to knock down expression of *C. elegans* PDE genes-**
Starved L1-stage *C. elegans* [either PDE KO strains on an N2 background were transferred to agar plates with *E. coli* HT115 lawns harboring one or two PDE RNAi constructs (or empty L4440 plasmid as a negative control) and nematode growth and behavior monitored at 20°C (Timmons and Fire, 1998).

3. **Growth and locomotion assays-**
Synchronized L1 nematodes were cultured at 20°C for various times on the indicated bacterial lawn, then washed and transferred to NGM agar plates and allowed to disperse for 10 min before recording videos with the WormLab Imaging System (MBF Bioscience). Nematode growth (length, µm) and locomotion (velocity, µm per sec) were quantified (~150 worms per treatment) with WormLab software (v 3.0.0) at a resolution of 1280 x 960 pixels at 15 frames per sec (Roussel et al., 2014).

4. **Fecundity assay-**
L1-stage nematodes were transferred to plates containing an *E. coli* HT115 lawn with or without PDE RNAi-containing plasmids and allowed to grow at 20°C to the L3 stage. Individual L3-stage nematodes were transferred daily to 6-well plates containing the same bacterial lawn to quantify the total number of progeny produced over the subsequent 3-day period (Brooks and Johnson, 1991).

References

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Results

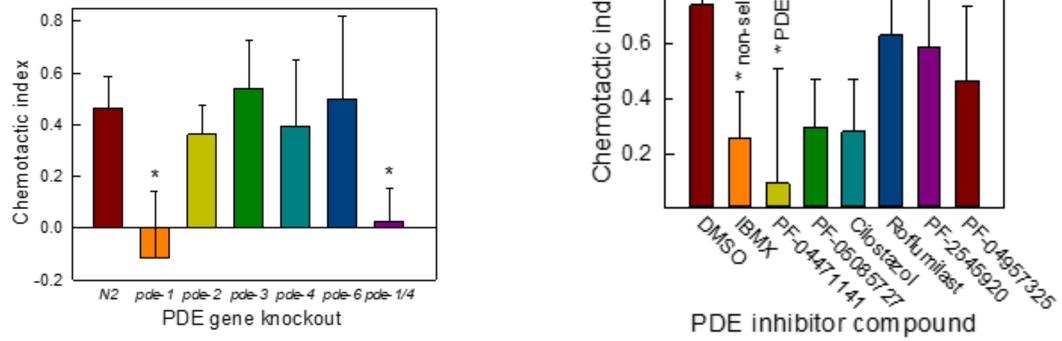

Fig. 2. Chemoattraction to 2-butanone is abolished in PDE1 KO strains and upon exposure to a human PDE1-selective inhibitor. *Left:* L3 stage nematodes of each PDE KO strain were assayed for their chemotactic response to the attractant 2-butanone. *Right:* L3 stage *C. elegans* (N2) were pre-incubated for 1 h with a pan-specific inhibitor (IBMX) or human PDE family-selective compounds at concentrations near their aqueous solubility limit (ranging from 500 µM for IBMX to 25 µM for PF-05085727, with most inhibitors at ~125 µM; final [DMSO] = 0.5%) before initiating the chemotaxis assay. In both panels, * denotes $p < 0.05$ (Holm-Siak pairwise test; n = 3).

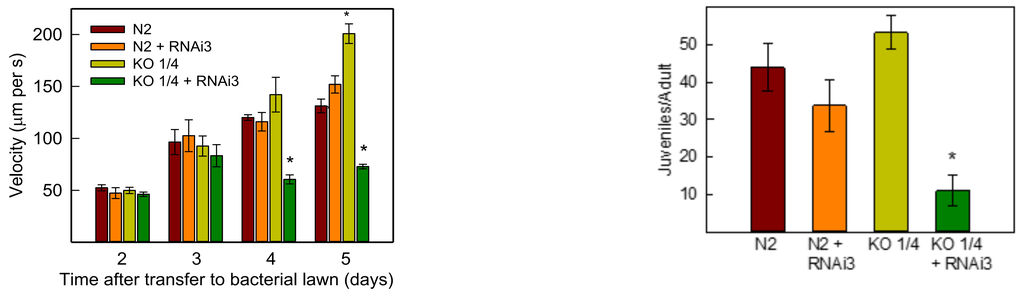

Fig. 3. Disruption of *pde-1, pde-3,* and *pde-4* markedly reduces growth rate and fecundity. L1 stage N2 or PDE 1/4 KO strains were grown in bacteria containing RNAi3 or the empty L4440 vector. Upon reaching L3 stage, one portion of each treatment was transferred to agar plates for locomotion assays (*left panel*). The following day, individual L3 animals were replica plated daily onto fresh bacterial lawns containing the same vector, and the total progeny produced over three days was determined (*right panel*). * Indicates statistically significant difference ($p < 0.001$; n = 3) using Dunnett's t-test.

Conclusions and future directions

- Disruption of an individual PDE by gene deletion or RNAi knockdown does not show any strong phenotype.
- Deletion of *pde-1* mimics exposure to a PDE1 inhibitor in abolishing chemoattraction.
- Disruption of *pde-1, pde-3,* and *pde-4* through a combination of knockouts and knockdowns resulted in developmental delays and lethargic movement.
- cAMP and cGMP metabolism appears to be coordinated by multiple PDEs in most cells; disruption of one PDE gene may be compensated for by upregulation of other PDEs.
- Generate double/triple mutant by genetic crosses and screen for phenotypes with combinations of PDE "knockout/knockdown"

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