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PDF receptor signaling in *Caenorhabditis elegans* modulates locomotion and egg-laying

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ABSTRACT

In *Caenorhabditis elegans*, *pdfr-1* encodes three receptors of the secretin receptor family. These G protein-coupled receptors are activated by three neuropeptides, pigment dispersing factors 1a, 1b and 2, which are encoded by *pdf-1* and *pdf-2*. We isolated a PDF receptor loss-of-function allele (*lst34*) by means of a mutagenesis screen and show that the PDF signaling system is involved in locomotion and egg-laying. We demonstrate that the *pdfr-1* mutant phenocopies the defective locomotor behavior of the *pdf-1* mutant and that *pdf-1* and *pdf-2* behave antagonistically. All three PDF receptor splice variants are involved in the regulation of locomotor behavior. Cell specific rescue experiments show that this *pdf* mediated behavior is regulated by neurons rather than body wall muscles. We also show that egg-laying patterns of *pdf-1* and *pdf-2* mutants are affected, but not those of *pdfr-1* mutants, pointing to a novel role for the PDF-system in the regulation of egg-laying.

1. Introduction

The free-living nematode *Caenorhabditis elegans* is widely used to study the neuronal origins of behavior thanks to the existence of a detailed wiring diagram of the nervous system (White et al., 1986) combined with a fully sequenced genome (The *C. elegans* Sequencing Consortium, 1998). Although the physical connections between neurons are known, many functional relationships still have to be unraveled. While the nervous system of *C. elegans* appears simple and compact with only 302 neurons in adult hermaphrodites, these cells make up about one-third of all 959 somatic cells. It is now clear that behind this superficial simplicity, a complex network of neuronal circuits exists.

Communication between neurons and target cells is primarily established by the release of classical neurotransmitters like octopamine, tyramine, dopamine, serotonin, acetylcholine (ACh), gamma-aminobutyric acid (GABA), and glutamate. Additionally, the *C. elegans* genome contains a wide variety of predicted neuropeptide precursor genes (113) which can be processed to over 250 different neuropeptides (Nathoo et al., 2001; Li, 2005; Husson et al., 2005a,b, 2007b; Li and Kim, 2008). Mature, fully processed neuropeptides act as neurotransmitters and/or neuromodulators, mainly through G protein-coupled receptors (GPCRs). GPCRs transduce signals from the cell surface to the cytoplasm by means of heterotrimeric G proteins consisting of α, β and γ subunits and various second messenger pathways (Vanden Broeck, 2001; Schiöth and Fredriksson, 2005). The *C. elegans* genome is predicted to encompass approximately 1300 GPCR genes with the majority (~1000) encoding putative chemoreceptors (Bargmann, 1998; Fredriksson and Schiöth, 2005; Thomas and Robertson, 2008). Both vertebrate and invertebrate GPCRs can be grouped into five main families according to the GRAFS system: Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2 and Secretin type receptors are distinguished (Fredriksson and Schiöth, 2005). A database search based on putative transmembrane domains of the human secretin receptor family (also known as family B or II) genes predicted only six secretin receptor genes in *C. elegans*, one of which is the *pdfr-1* (C13B9.4) gene (Harmar, 2001; Cardoso et al., 2006). Through alternative splicing, *pdfr-1* produces three pigment dispersing factor (PDF) neuropeptide receptors (PDFR-1a, b and c) and is expressed in all body wall muscles, in several mechanosensory neurons, chemosensory neurons, ring motor neurons and pharyngeal

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interneurons (Janssen et al., 2008). These three receptors are the only GPCRs of the secretin type family in *C. elegans* that are deorphaned and they were shown to be activated by *C. elegans* PDF neuropeptides (Janssen et al., 2008, 2009). The *C. elegans* PDF receptors (PDFRs) are closely related to the fruit fly *Drosophila melanogaster* PDFR and more distantly to the mammalian VIP ($VPAC_2$) and calcitonin receptors (Janssen et al., 2008).

PDF neuropeptides belong to a conserved group of neuropeptides among insects, nematodes and crustaceans wherein they were originally discovered and named pigment dispersing hormones (PDHs) (also reviewed by Rao and Riehm, 1993; Meelkop et al., 2011). PDH is involved in the dispersion of pigment granules in the integument and compound eyes of crustaceans. Insect PDF contributes to the synchronization and transmission of signals from the circadian clock as was shown in the fruit fly *D. melanogaster* (Renn et al., 1999; Helfrich-Forster et al., 2000). Flies lacking the PDF receptor exhibit increased circadian arrhythmicity under constant darkness and display altered geotactic behavior, similar to flies lacking the PDF neuropeptide (Renn et al., 1999; Helfrich-Forster et al., 2000; Toma et al., 2002; Mertens et al., 2005). In addition, based on immunocytochemical experiments in the blow fly, PDF is suggested to be involved in reproduction (Hamanaka et al., 2005).

C. elegans expresses more than one PDF-like neuropeptide gene: *pdf-1* and *pdf-2*, encoding three different PDF-like neuropeptides named PDF-1a, b and PDF-2 (Janssen et al., 2009). According to their localization patterns, it is thought that PDF-1a, b and PDF-2 are mainly involved in the integration of environmental stimuli. The peptides already proved functional in locomotion as both the *pdf-1* loss-of-function allele *tm1996* and *pdf-2* over-expression induce decreased locomotion speed, increased reversal frequency and increased number of directional changes (Janssen et al., 2008). Each of the three PDF neuropeptides is able to bind to each of the three PDF receptors but with different affinities. While PDFR-1a and PDFR-1b signal through $G\alpha_s$ and adenylate cyclase and ultimately elevate cAMP levels, PDFR-1c inhibits cAMP levels through $G\alpha_{i/o}$ (Janssen et al., 2008).

This work aims to determine whether the absence of functional *C. elegans* PDF receptors results in similar locomotion defects as observed in *pdf-1* knockouts and to explore other possible *pdfr-1* knockout phenotypes. In this study we report the isolation of a *pdfr-1* loss-of-function allele (*lst34*) by means of a mutagenesis screen. *pdfr-1(lst34)* animals display aberrant locomotor behavior confirming the involvement of these receptors in the regulation of locomotion. Our results show a novel role for the PDF-system in the regulation of egg-laying.

2. Material and methods

2.1. Nematode strains and culturing

All *C. elegans* strains were cultivated at 20 °C in constant darkness on nematode growth medium (NGM) agar seeded with *Escherichia coli* OP50 bacteria. Strains used were Bristol N2, LSC39 *pdfr-1(lst34)* (6x) also see Section 2.2, LSC27 *pdf-1(tm1996)* (6x), LSC40 *pdf-2(tm4393)* (1x), *pdfr-1(tm4457)*, *pdf-2(tm4780)*, LSC10 = *pdf-1(tm1996)*; *lstIs1* [*pdf-1 +*] (Janssen et al., 2008), LSC84 = N2; *lstEx2*[*pdf-1 +*], LSC54 = N2; *lstEx3* [*pdf-2 +*], LSC85 = N2; *lstEx4* [*pdfr-1 +*] (Janssen et al., 2008) and BC11358 [$P_{pdfr\text{-}1}$*::gfp*]. JT191 *daf-28(sa191)* was cultured at 15 °C.

The vector containing $P_{myo\text{-}3}$*::mCherry* (a kind gift from E. Berezikov and M. Isik, Hubrecht Institute, Utrecht, The Netherlands) was used to microinject in wild type N2 and LSC39 *pdfr-1(lst34)* (6x) animals generating the following expression strains: LSC55 = N2; *lstEx13* [$P_{myo\text{-}3}$*::mCherry*], LSC56 = *pdfr-1(lst34); lstEx14* [$P_{myo\text{-}3}$*::mCherry*] and LSC57 = *pdfr-1(lst34)*; *lstEx15* [$P_{myo\text{-}3}$*::mCherry*].

Transgenic rescue strains generated in this work were accomplished by re-introducing the wild type gene including its promoter. The following primers were used to amplify these regions from genomic DNA: *pdf-2* forward primer 5′-TGCCCACAGATCGGCTTATG ATTGC-3′ and reverse primer 5′-ACTTTTACTTGCCAACGTCTCCAAGT CG-3′ and pdfr-1 forward primer 5′-TCTGATGGAGTTACTCGGAGA-3′ and reverse primer 5′-TGTCGTGTGATTTTCCCACT-3′. In order to rescue specific isoforms of the PDF receptor, we generated body wall muscle ($P_{hlh\text{-}1}$) and neuron ($P_{unc\text{-}119}$) specific fusion constructs (Hobert, 2002) whereby the *hlh-1* promoter region (3 kb prior to the ATG start codon) was amplified from the pKM1238 vector (a kind gift from M. Krause, NIDDK, National Institutes of Health, Bethesda MD, USA), the *unc-119* promoter (2210 bp prior to the ATG start codon) was isolated from genomic DNA and the PDF receptor splice variants from the pcDNA3.1D vector (Janssen et al., 2008) with the following primers: $P_{hlh\text{-}1}$ forward primer 5′-GCGTTTTTTGGGATTCTG AATGAT-3′ and reverse primer 5′-GAACGGTGACGTGGCATCCGCC ATTTCTGGAAAATTATTGGAAAATTTGG-3′, $P_{unc\text{-}119}$ forward primer 5′-GCAATTGTTTTGTGCCAAGCTTCA-3′ and reverse primer 5′-GAAC GGTGACGTGGCATCCGCCATATATGCTGTTGTAGCTGAAAATTTTGG-3′, *pdfr-1* forward primer (for *pdfr-1a*, *b* and *c*) 5′-GAACGGTGACGTG GCATCCGCCAT-3′, *pdfr-1a* and *pdfr-1b* reverse primer 5′-TTATGGA-GATTTTGTGAGCGATTGG-3′ and *pdfr-1c* reverse primer 5′-AATTTAT TCTTTGTTTTCTACTCTTCATAC-3′. Purified PCR products were microinjected at 10–50 ng/μl together with *elt-2::gfp* as a selection marker. At least three strains were analyzed for each rescue condition. The strains described in this paper are: LSC59 = *pdf-2(tm4393)*; *lstEx24* [*pdf-2 +*], LSC60 = *pdfr-1(lst34)*; *lstEx25* [*pdfr-1 +*], LSC64 = *pdfr-1(lst34)*; *lstEx112* [$P_{unc\text{-}119}$*::pdfr-1a*], LSC67 = *pdfr-1(lst34)*; *lstEx117* [$P_{unc\text{-}119}$*::pdfr-1b*] and LSC72 = *pdfr-1(lst34)*; *lstEx122* [$P_{unc\text{-}119}$*::pdfr-1c*].

2.2. Isolation of a pdfr-1 knockout allele

We generated an in-house mutant *C. elegans* library of 230,400 haploid genomes through random mutagenesis with 0.5 mg/ml trioxsalen (Sigma–Aldrich) followed by UV radiation (Colibri 360 nm at 3%, DAPI filter, A-Plan 5x/NA 0.12 objective, 10 s for 50 J/m^2 and 20 s for 100 J/m^2) (Zeiss Axio Imager, Carl Zeiss MicroImaging GmbH, Germany). Next, we distributed 50 mutagenized worms per plate over 2304 NGM plates and carried out a PCR screen as adapted from Lesa (2006). The PCRs were carried out according to the following PCR conditions: External PCR – 2 μl template DNA, 1.7 μl 10x rTaq PCR buffer (Invitrogen), 0.51 μl 50 mM $MgCl_2$, 0.34 μl 0.5 mM dNTPs, 0.34 μl 10 μM forward external primer, 0.34 μl 10 μM reverse external primer, 0.085 μl 5 U/μl rTaq (Invitrogen) and 11.685 MiliQ water. Step 1: 94 °C for 3 min; step 2: 94 °C for 10 s; step 3: 58 °C for 10 s; step 4: 72 °C for X seconds; repeat steps two to four 29 times; step 5: 72 °C for 2 min; end at 15 °C. X should be optimized so that there is no PCR product visible as to limit the amplification of wild type amplicons and prioritize the amplification of the shorter deletion alleles. Internal PCR – 0.7 μl external PCR product, 1.7 μl 10x rTaq PCR buffer (Invitrogen), 0.51 μl 50 mM $MgCl_2$, 0.34 μl 10 mM dNTPs, 0.34 μl 10 μM forward internal primer, 0.34 μl 10 μM reverse internal primer, 0.085 μl 5 U/μl rTaq (Invitrogen) and 11.685 MiliQ water. Step 1: 94 °C for 3 min; step 2: 94 °C for 10 s; step 3: 58 °C for 10 s; step 4: 72 °C for Y seconds; repeat steps two to four 29 times; step 5: 72 °C for 2 min; end at 15 °C. Y should be optimized so that there is a PCR product visible and is typically two times X. Oligonucleotide primers were designed with AcePrimer 1.3 (http://elegans.bcgsc.bc.ca/aceprimer/aceprimer.shtml). The following gene-specific primers (Sigma–Aldrich) were used to identify a deletion in the *pdfr-1* (C13B9.4) gene by two consecutive PCRs: forward external 5′-ATTCGGAACACCTAACAGCG-3′ and reverse external 5′-ACGTCATTATTGCCACGTCA-3′, 1784 bp apart; forward internal