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Structure–function analyses of tyrosine phosphatase PTP69D in giant fiber synapse formation of *Drosophila*

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ABSTRACT

PTP69D is a receptor protein tyrosine phosphatase (RPTP) with two intracellular catalytic domains (Cat1 and Cat2) and has been shown to play a role in axon guidance of embryonic motoneurons as well as targeting of photoreceptor neurons in the visual system of *Drosophila melanogaster*. Here, we characterized the developmental role of PTP69D in the giant fiber (GF) neurons, two interneurons in the central nervous system (CNS) that control the escape response of the fly. Our studies revealed that PTP69D has a function in synaptic terminal growth in the CNS. We found that missense mutations in the first immunoglobulin (Ig) domain and in the Cat1 domain, present in $Ptp69D^{10}$ and $Ptp69D^{20}$ mutants, respectively, did not affect axon guidance or targeting but resulted in stunted terminal growth of the GFs. Cell autonomous rescue experiments demonstrated a function for the Cat1 and the first Ig domain of PTP69D in the GFs but not in its postsynaptic target neurons. In addition, complementation studies and structure–function analyses revealed that for GF terminal growth Cat1 function of PTP69D requires the immunoglobulin and the Cat2 domains, but not the fibronectin III or the membrane proximal region domains. In contrast, the fibronectin III but not the immunoglobulin domains were previously shown to be essential for axon targeting of photoreceptor neurons. Thus, our studies uncover a novel role for PTP69D in synaptic terminal growth in the CNS that is mechanistically distinct from its function in photoreceptor targeting.

1. Introduction

Receptor protein tyrosine phosphatases (RPTPs) are a family of transmembrane proteins that are strongly expressed in the nervous system and are crucial for the formation of functional neuronal circuits (Stoker, 2014; Van Vactor, 1998). RPTPs have modular ectodomains, which resemble cell adhesion molecules, and intracellular tyrosine phosphatase domains, which antagonize tyrosine kinase signaling. PTP69D is a *Drosophila* RPTP that has been shown to play an important role during neurodevelopment (Desai and Purdy, 2003; Desai et al., 1996; Garrity et al., 1999). The extracellular domain of PTP69D is comprised of two immunoglobulin (Ig) domains, three fibronectin type III (FNIII) domains and a membrane-proximal region (MPR), while the intracellular region has two protein tyrosine phosphatase domains (Cat1 and Cat2, Fig. 1A). PTP69D has a role during periods of axon outgrowth (Desai and Purdy, 2003) and along with other RPTPs, such as DLAR and PTP99A, is required for proper motor axon guidance (Desai et al., 1996, 1997; Song et al., 2008). PTP69D is also known to play a role in axon fasciculation and branching in the mushroom bodies (Kurusu and Zinn, 2008). In the *Drosophila* visual system, PTP69D is required for lamina specific targeting of the photoreceptor neurons R1–R6 (Garrity et al., 1999). These structure–function studies revealed that the FNIII domains, but not the Ig domains, are required for axon targeting. It was shown that mutations disrupting the function of both Cat1 and Cat2 domains prevented proper targeting of R1–R6 to the lamina, but catalytic activity of one of the two domains was sufficient for normal neuronal development. Furthermore, phosphatase activity of PTP69D was also shown to be involved in R7 targeting to the medulla (Hofmeyer and Treisman, 2009; Newsome et al., 2000).

In the present work, we characterize the function of PTP69D in the adult giant fiber circuit (GFC), which mediates the escape response of the fly (Allen et al., 2006). The two giant fiber (GF) neurons extend their axons in the brain during the L3 larval stage, and the axons project into the second thoracic neuromere during early pupal development. During mid-pupal development, they establish electrical and chemical synapses with the peripheral synapsing interneurons (PSIs) and the tergotrochanteral motorneurons (TTMns, Fig. 1B). Here, we show that PTP69D, in addition to its previously characterized roles in axon guidance and targeting, also has a function in terminal growth in the central nervous system (CNS). We found that the disruption of the catalytic function of the membrane proximal Cat1 domain in $Ptp69D^{20}$ mutants had no effect on axon guidance or targeting, but resulted in severely

Abbreviations: CNS, central nervous system; RPTP, protein tyrosine phosphatase; GF, giant fiber; Ig, immunoglobulin; FNIII, fibronectin type III; MPR, membrane-proximal region; Cat, catalytic domain; GFC, giant fiber circuit; TTM, tergotrochanteral muscle; TTMn, tergotrochanteral motorneuron; PSI, peripheral synapsing interneuron; DLM, dorsal longitudinal muscle; RL, response latency; FF, following frequency

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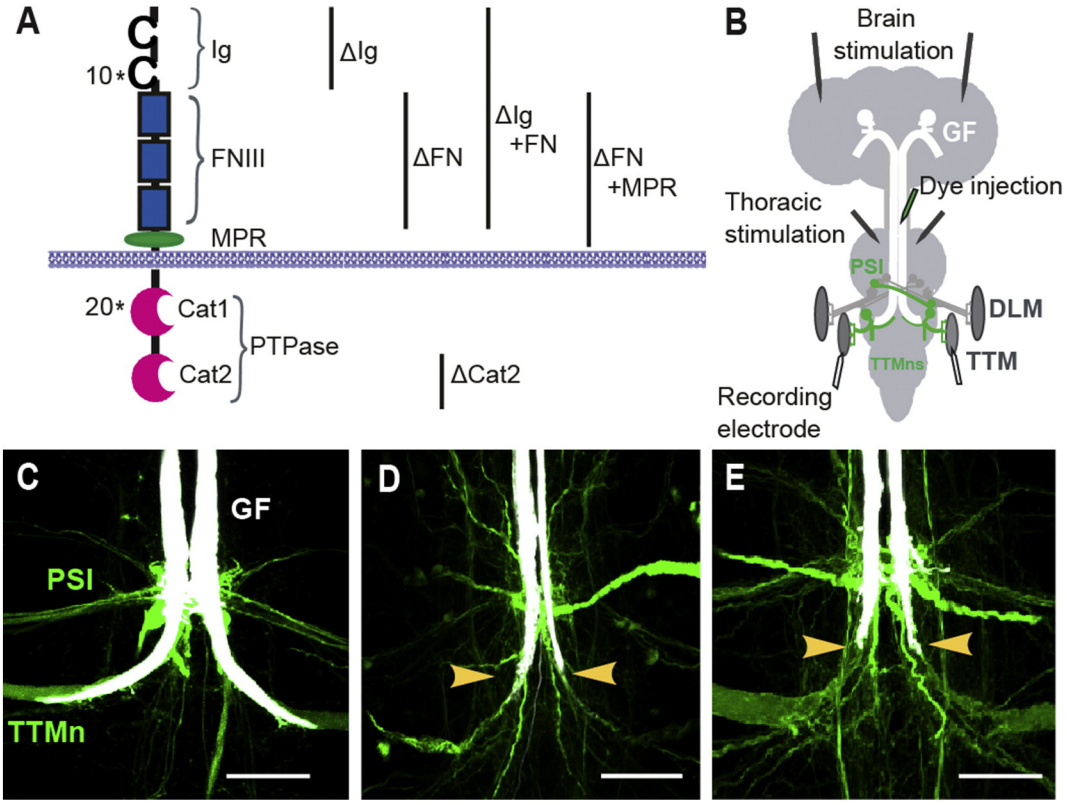

Fig. 1. Anatomical phenotypes of *Ptp69D* missense mutants in the GFC. A) Schematic of PTP69D protein structure, indicating the mutation sites (*) representative of the $Ptp69D^{10}$ and $Ptp69D^{20}$ alleles (Desai and Purdy, 2003). Deletions (Δ) in transgenic constructs used for structure–function analysis are depicted by lines adjacent to the molecule (Garrity et al., 1999). A construct with D–A conversions in both Cat1 and Cat2 domains is not depicted. B) Schematic of the CNS of *Drosophila* depicting the GFC within the brain and the ventral nerve cord (modified from Allen and Godenschwege, 2010). The two GFs (white), somas and dendrites are located in the brain. In the GF–TTM pathway, the GF axon forms a large synaptic terminal onto the TTMn (green) in the second thoracic neuromere, which innervates the TTM. In the GF–DLM pathway, the GF synapses with PSI (green). The PSI synapses with the DLMns (gray), which innervates the DLM. Placement of stimulation and recoding electrodes as well as site of dye injections are indicated. C–E) GF synaptic terminals and dye-coupling to the postsynaptic target neurons were visualized by the co-injection of rhodamine-dextran (white) and neurobiotin (green) into the GF in the cervical connective. Projection views of confocal stacks of the GF terminals are shown. Scale bars are 20 μm. In w^{1118} wild type control animals (C), the GFs exhibited large GF terminals and dye-coupled with the TTMns and the PSIs. In homozygous $Ptp69D^{10}$ (D) and $Ptp69D^{20}$ (E) mutants the GFs were severely stunted (arrowheads) but dye-coupled with the TTMns and the PSIs in all cases. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

stunted GF terminals that are dysfunctional, yet still connected to the TTMn. Structure–function analyses revealed that Cat1 function in GF terminal growth required the Ig domains, but not the FNIII domains. In summary, our studies suggest that during GF terminal growth outside-in signaling of PTP69D involves the Cat1 and the Ig domains and this is mechanistically distinct from how it functions in axon targeting.

2. Results

2.1. Characterization of Ptp69D missense mutants in the GFC

Ptp69D null mutants exhibit lethality and typically do not survive past the larval stage (Desai et al., 1996). Therefore, we characterized the phenotypes of the viable $Ptp69D^{10}$ and $Ptp69D^{20}$ alleles, which carry missense mutations in the first Ig domain and Cat1 domain, respectively, in adult GF neurons (Fig. 1A) (Desai and Purdy, 2003; Desai et al., 1996). The GF axons can be reliably identified in the cervical connective using differential interference contrast microscopy. We used dye injections of rhodamine-dextran and neurobiotin into the GF axons to reveal the morphology of the terminals and to determine if the GFs are coupled to their synaptic targets via the gap junctions of their electrical–chemical synapses. All GFs in both mutant alleles reached the synaptic target area in the second thoracic neuromere without any guidance defects and only disrupted the GF–TTMn connection anatomically and functionally, but not the GF to PSI (GF–PSI) connection (Figs. 1B–E, 2, data not shown). In homozygous $Ptp69D^{10}$ ($n = 22$) and $Ptp69D^{20}$ ($n = 20$) mutants, the GF terminals were dramatically shorter when compared to control animals (Fig. 1C–E). However, all GF terminals dye-coupled with the TTMns, although often weakly, suggesting that terminal growth and not synaptic targeting was disrupted in these mutants.

Consistent with the morphological defects of the GF terminal, we found that the function of the GF–TTM pathways was severely impaired. In control animals, the GF–TTM pathway is able to respond in a 1:1 ratio when the GFs are stimulated with 10 pulses at 100 Hz (Figs. 1A, 2A). In contrast, the average FF of the GF–TTM pathway was severely reduced in $Ptp69D^{10}$ (FF = 27%) and $Ptp69D^{20}$ (FF = 32%) animals (Fig. 2B, C). Correlating with the decrease to follow stimulations at 100 Hz, the average RL of the GF–TTM pathway to an individual stimulus significantly increased in *Ptp69D* mutants (≥1 ms), when compared to the control animals (0.8 ms) (Fig. 2D). In control animals the RL remained constant in individual flies, when 10 stimuli were given at 5 Hz (Fig. 2B). In contrast, in the *Ptp69D* mutants the RL varied and the duration usually increased further (Fig. 2B) or the GF–TTM pathways failed to respond, similar to repetitive stimulation seen at 100 Hz (Fig. 2A) revealing a severe weakening of the synaptic connection. When we used thoracic stimulation to bypass the GFs and activate the TTMns directly, 100% FF of the neuromuscular junctions and a RL below 0.8 ms were observed (data not shown). This confirmed that the site of the synaptic defect was at the GF–TTMn synapse. The functional defects were slightly enhanced when the $Ptp69D^{10}$ and $Ptp69D^{20}$ alleles were tested over a chromosome deficient ($Df(3L)^{8ex34}$) for the *PTP69D* gene demonstrating that the observed defects are PTP69D-specific (Fig. 2C, D). Finally, we