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Cysteine Proteinase-1 and Cut Protein Isoform Control Dendritic Innervation of Two Distinct Sensory Fields by a Single Neuron

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SUMMARY

Dendrites often exhibit structural changes in response to local inputs. Although mechanisms that pattern and maintain dendritic arbors are becoming clearer, processes regulating regrowth, during context-dependent plasticity or after injury, remain poorly understood. We found that a class of *Drosophila* sensory neurons, through complete pruning and regeneration, can elaborate two distinct dendritic trees, innervating independent sensory fields. An expression screen identified *Cysteine proteinase-1* (*Cp1*) as a critical regulator of this process. Unlike known ecdysone effectors, *Cp1*-mutant ddaC neurons pruned larval dendrites normally but failed to regrow adult dendrites. Cp1 expression was upregulated/concentrated in the nucleus during metamorphosis, controlling production of a truncated Cut homeodomain transcription factor. This truncated Cut, but not the full-length protein, allowed *Cp1*-mutant ddaC neurons to regenerate higher-order adult dendrites. These results identify a molecular pathway needed for dendrite regrowth after pruning, which allows the same neuron to innervate distinct sensory fields.

INTRODUCTION

Dendrites are the primary sites of information input for neurons. Their initiation, arborization, targeting, and function are regulated by a series of finely tuned cellular events (Jan and Jan, 2010; Stuart et al., 2008). Critical for the proper wiring of neural circuits, defects in dendrite development and function have been linked to human neurodevelopmental and psychiatric diseases, including autism, fragile X syndrome, and schizophrenia (Kulkarni and Firestein, 2012; Penzes et al., 2011). Dendrites can also remodel after their initial arborization. This process is often coupled to neuronal activity inputs from external stimuli (Chen and Nedivi, 2010; Tavosanis, 2012) and presents a potential cellular basis for sensory map remodeling (Feldman and Brecht, 2005; Hickmott and Steen, 2005). Although the molecular mechanisms that pattern and maintain the proper dendritic tree/field are becoming clearer, the processes regulating dendritic rewiring remain poorly understood.

Drosophila peripheral nervous system dendritic arborization (da) neurons, classified into four classes (I–IV) based on their location and dendritic arbor complexity, have served as a powerful model system for studying conserved pathways controlling dendrite morphogenesis (Parrish et al., 2007). We showed previously that class IV da (C4 da) neurons undergo ecdysone hormone-induced pruning and subsequent regrowth of dendritic arbors during metamorphosis (Kuo et al., 2005). This remodeling is initiated by intracellular events downstream of nuclear hormone receptor signaling (Kanamori et al., 2013; Kirilly et al., 2009; Kuo et al., 2005, 2006; Lee et al., 2009; Williams et al., 2006) and extracellular events controlled by phagocytes (Williams and Truman, 2005) and epidermis (Han et al., 2014). After pruning, C4 da neurons regrow dendrites that innervate the adult sensory fields (Kuo et al., 2005), but the mechanisms controlling this dendrite regrowth remain largely unknown.

Here, we show that ddaC C4 da neurons regenerate adult dendritic arbors in a different manner after pruning than initially during development. Starting with an expression screen, we identified *Cysteine proteinase-1* (*Cp1*) and its critical role in regulating ddaC neuron dendrite regeneration to innervate the adult sensory fields.

RESULTS AND DISCUSSION

Regrowth of ddaC Sensory Neuron Dendrites

It is likely that many of the developmental pathways used to elaborate larval sensory neuron dendrites will be reused during regrowth. We reasoned that if mirrored programs were used,

then the regrown dendritic trees should morphologically resemble earlier larval shapes. The ddaC C4 da neurons maintain a stereotyped 2D dendritic morphology prior to metamorphosis (Han et al., 2012; Kim et al., 2012). This is established first by inserting early dendrites into the body wall during development, followed by dendritic growth that is scaled to concurrent expansion of the larval body wall and receptive fields (Parrish et al., 2009). Using live imaging of *pickpocket* (*ppk*)-*EGFP* reporter line to follow the abdominal segment ddaC neurons through metamorphosis, we found that their dendritic arbors changed into a different architecture after regrowth (Figure 1A; Movie S1). In addition to covering a smaller field, the soma and primary dendrites reside in a separate, deeper plane than higher-order dendritic branches that project to the body wall above (Figures 1A and S1A; Movie S1). To quantify the changes (Figure 1B), we developed a software script to track the depth of dendrites from the body wall and represented this distance colorimetrically (deeper arbors in red, shallower in blue, Figures 1C and S1B).

To understand the steps necessary to elaborate this dendritic tree after pruning, we performed time-lapse imaging of ddaC neurons during metamorphosis. Shortly after complete dendrite pruning at 24 hr after puparium formation (APF), ddaC neurons initiated dendrite regrowth, projecting primary dendrites along the wall from a lateral-to-medial direction (Figure S2A). This initial phase of dendritic growth was highly dynamic, with numerous neurite extensions/retractions (Figure S2A; Movie S2). Most of these neurites are transient structures because the primary dendrites continued to elongate without much elaboration of higher-order branches (Figures 1D and S2B). At later stages, between 60 and 72 hr APF, we observed the first stabilization of secondary dendrites branching from the primary dendrites toward the body wall above (Figure 1D). These secondary dendrites did not branch further until they reached the body wall, at which time there was a rapid expansion of higher-order dendritic branches close to the body wall (Figure 1D; Movie S3). This late expansion accounted for the majority of mature ddaC neuron dendritic field coverage at 95 hr APF just before eclosion (Figure S2C). Although initiation of the primary dendrite after pruning is rather stereotyped, the subsequent targeting/expansion of higher-order dendrites at the body wall differed between neighboring ddaC neurons. Quantification showed the temporal relationships of this process (Figure 1E), representing a different approach from receptive field scaling used by these same ddaC neurons during larval dendrite growth (Parrish et al., 2009).

Identification of *Cp1* Regulating ddaC Neuron Dendrite Regrowth

We hypothesized that if variations in molecular programs are needed to grow two different sets of dendrites in the same neuron, then the genes involved will likely change their expression levels in a context-dependent manner. We set out to identify such genes in ddaC neurons during dendrite regrowth. An expression screen of the EGFP-FlyTrap collection identified stock ZCL2854, corresponding to EGFP insertion into the *Cp1* gene, that showed increased EGFP expression during ddaC neuron dendrite remodeling (Figure 2A). We quantified *Cp1-EGFP* fluorescence levels in ddaC neurons by normalizing EGFP intensity to internal *UAS-mCD8::RFP* fluorescence driven by *ppk-Gal4*, which remained relatively constant throughout (Figure 2C; data not shown). We showed previously that dendrite remodeling in these neurons is initiated by nuclear hormone receptor signaling (Kuo et al., 2005). To confirm that the increase in *Cp1-EGFP* expression during metamorphosis is controlled by the *Drosophila* hormone ecdysone, we blocked ecdysone signaling by expressing a dominant-negative ecdysone receptor (*UAS-EcR-DN*) in ddaC neurons (Kirilly et al., 2009; Kuo et al., 2005). This effectively attenuated *Cp1-EGFP* upregulation during metamorphosis (Figures 2B and 2C).

Cp1 contains an evolutionarily conserved cysteine proteinase domain (Tryselius and Hultmark, 1997), but its function in *Drosophila* is poorly understood, with no previous link to neuronal development/function. Because in our hands *Cp1*-mutants were lethal (both $Cp1^{llcnbw38}$ and $Cp1^{c03987}$ alleles), we generated *Cp1*-mutant ddaC neuron clones. Unlike other signals downstream of ecdysone identified thus far during ddaC neuron dendrite remodeling (Kanamori et al., 2013; Kirilly et al., 2009), *Cp1*-mutant ddaC neurons pruned their larval dendrites normally during metamorphosis, followed by extension of their primary dendrites similar to controls (Figures S3A and S3B). But they failed to properly elaborate higher-order dendrites during the expansion phase when these dendrites target the body wall (Figures 2D–2F and S3B). These defects can be partially recovered by reexpressing Cp1 in mutant clones using a *UAS-Cp1* transgene (Figure S3D). In contrast to ecdysone control of endogenous Cp1 expression (Figure 2B), this Cp1 re-expression is under Gal4/UAS control; thus, it may not fully recover wild-type dendritic morphologies. We did not observe obvious dendritic morphology defects in larval ddaC neurons expressing Cp1 via *UAS-Cp1* transgene (Figure S3C; data not shown).

Cut Transcription Factor Isoform Regulates ddaC Neuron Dendrite Regrowth

Because *Cp1* function in *Drosophila* is unclear, we took a candidate approach to understand its regulation of ddaC neuron dendrite regrowth. One of the reported protein targets for cathepsin L (Ctsl), the mammalian homolog to Cp1, is homeodomain transcription factor Cut-like 1 (Cux1) (Goulet et al., 2004). During cell-cycle progression, Ctsl cleaves Cux1 between the first and second Cut repeats, generating a truncated protein containing the second and third Cut repeats and homeodomain, with different transcriptional properties to the full-length protein (Goulet et al., 2004; Moon et al., 2001). Cux1 is related to Cut, a key determinant of *Drosophila* peripheral sensory neuron dendrite arborization during development (Grueber et al., 2003). We asked whether Cut is part of the Cp1 pathway regulating ddaC neuron dendrite regrowth by first generating *cut*-mutant ddaC neuron clones. Consistent with earlier reported defects in larvae (Grueber et al., 2003), these neurons exhibited altered dendrites at the white pupae stage, but the dendrites pruned normally during metamorphosis, followed by regrowth of primary dendrites from the soma (data not shown). Thereafter, *cut*-mutant ddaC neurons showed a severe defect in arborization of higher-order dendritic branches targeting the body wall (Figure 3A). Quantification of multiple clones, followed continuously from identification at the start