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Dendrite Reshaping of Adult *Drosophila* Sensory Neurons Requires Matrix Metalloproteinase-Mediated Modification of the Basement Membranes

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SUMMARY

In response to changes in the environment, dendrites from certain neurons change their shape, yet the mechanism remains largely unknown. Here we show that dendritic arbors of adult *Drosophila* sensory neurons are rapidly reshaped from a radial shape to a lattice-like shape within 24 hr after eclosion. This radial-to-lattice reshaping arises from rearrangement of the existing radial branches into the lattice-like pattern, rather than extensive dendrite pruning followed by regrowth of the lattice-shaped arbors over the period. We also find that the dendrite reshaping is completely blocked in mutants for the matrix metalloproteinase (Mmp) 2. Further genetic analysis indicates that Mmp2 promotes the dendrite reshaping through local degradation of the basement membrane upon which dendrites of the sensory neurons innervate. These findings suggest that regulated proteolytic alteration of the extracellular matrix microenvironment might be a fundamental mechanism to drive a large-scale change of dendritic structures during reorganization of neuronal circuits.

INTRODUCTION

Neuronal circuits in the brain are not static. In many systems, especially during critical periods of development, neurons exhibit a period of juvenile plasticity in which connectivity can be modified in response to sensory input or following specific experiences, thereby providing neurons with new response properties, tailored to the new environment. To achieve these changes in connectivity, certain neurons modify the shape of their dendritic arbors in response to various stimuli (Cline, 2001; Lohmann and Wong, 2005; Wong and Ghosh, 2002). For example, retina ganglion cells (RGCs) modify their dendritic arbors as they mature in a sensory-evoked, activity-dependent manner (Bodnarenko and Chalupa, 1993). Likewise, mitral and tufted cells in the olfactory bulb initially have multiple primary dendrites that contact adjacent glomeruli; however, they eventually lose all but one dendritic branch that remains in contact with a single glomerulus (Malun and Brunjes, 1996). Over time, many types of neurons exhibit a reduction in structural plasticity, with neurons progressively reducing branch dynamics and stabilizing their dendritic arbors. For example, in hippocampal pyramidal neurons, the proportion of stable dendritic spines increases over time (Holtmaat et al., 2005; Zuo et al., 2005). Even in the case of adult-born neurons that integrate into existing neural circuits, dendrites enter a maintenance phase after a short period of dynamic growth and dendrite arbor rearrangement (Mizrahi, 2007). However, dendritic arbors of mature neurons often undergo drastic reshaping under pathological conditions, such as epilepsy and after ischemia. (Spigelman et al., 1998; Ruan et al., 2006). Therefore, understanding the mechanisms that underlie the dendrite arbor reshaping has important implications for understanding normal development of dendrite arbors, regulation of structural plasticity of dendrites, and dendritic pathology.

Although the mechanisms responsible for regulating structural plasticity of mature dendrites remain elusive, reshaping of dendrite arbors appears to be controlled not only by intrinsic factors, but also by extrinsic mechanisms, including modification of the extracellular matrix (ECM) (Cline, 2001; Wong and Ghosh, 2002; Pavlov et al., 2004). The ECM exerts a strong influence on dendrite morphogenesis in cultured neurons, as ECM can affect dendrite patterning, in part through ECM-neurite adhesive contacts mediated by cell adhesion molecules, such as integrins (Reichardt and Tomaselli, 1991). ECM-neurite interactions have also been implicated in regulating structural plasticity of dendrites in vivo, since blockage of the integrin-ECM interaction in RGCs (Marrs et al., 2006) or genetic ablation of the integrin-mediated signaling in adult cortical neurons (Moresco et al., 2005) causes progressive retraction of dendritic branches. How broadly the ECM is involved in regulating remodeling of neuronal circuits is currently unknown, but recent studies demonstrate that the ECM undergoes dynamic changes in the developing brain, and that the ECM development is affected in pathological conditions (Pavlov et al., 2004; Yong, 2005), suggesting that the ECM modifications may accompany remodeling of neuronal circuits. Indeed, recent studies suggest that local

degradation of the ECM plays an important role in structural plasticity of dendritic spines in response to neuronal activity (Frischknecht et al., 2009; Magata et al., 2004; Oray et al., 2004). The ECM modifications in the nervous system are likely achieved by the concerted actions of several different proteases that are secreted by neurons and glial cells (Dzwonek et al., 2004; Page-McCaw et al., 2007; Yong, 2005). Among these many proteases, the members of the matrix metalloproteinase (MMP) family stand out as likely regulators of the dendrite development and pathology, because the MMPs are dramatically upregulated in particular neurons of the developing brain and in pathological conditions, and are often colocalized with dendrites. The MMPs stand out because they are dramatically upregulated in particular neurons of the developing brain and in pathological conditions, and are often colocalized with dendrites (Sekine-Aizawa et al., 2001; Szklarczyk et al., 2002). However, in large part due to issues of redundancy and compensation among over 20 vertebrate MMP family members, the in vivo role of MMPs in the nervous system remains to be established. In this regard, *Drosophila* affords an attractive genetic model system in which to study MMP functions, since there are only two MMP family members in the fly: Mmp1 and Mmp2 (Llano et al., 2000, 2002; Page-McCaw et al., 2003).

The *Drosophila* dendrite arborization (da) sensory neurons provide a suitable system for systematic analysis of dendritic morphogenesis (Gao et al., 1999; Jan and Jan, 2003; Parrish et al., 2007). Recent studies have demonstrated that the subtype-specific dendritic patterns of class IV da (C4 da) neurons are determined by intrinsic factors, such as transcription factors (Gao, 2007; Parrish et al., 2007), as well as extrinsic cues, such as repulsive interactions between neighboring dendrites (Emoto et al., 2004, 2006; Grueber et al., 2002; Koike-Kumagai et al., 2009). The C4 da neurons are born by mid-embryogenesis, and extend their two-dimensional dendrites between the epidermis and the underlying musculature during the late-embryonic and larval stages (Bodmer and Jan, 1987; Grueber et al., 2002). Following a period of growth and development in larval stages, the larval dendritic arbors are completely replaced with adult-specific processes as a result of extensive pruning and subsequent regeneration of dendrite arbors during metamorphosis (Kuo et al., 2005; Shimono et al., 2009; Williams and Truman, 2005). In contrast to the significant progress being made to understand embryonic/larval dendrite morphogenesis in C4 da neurons, much less is known about how C4 da neurons remodel and regenerate their dendritic arbors in the pupal/adult stages.

In this study, we investigated the arbor dynamics of C4 da neurons following elaboration of the adult-specific dendrite arbors, and found a rapid, highly stereotyped reshaping of these dendrite arbors following eclosion. Similar to their larval counterparts, dendrites of adult C4 da neurons initially elaborated dendritic trees in a radial fashion, and covered the whole body wall prior to eclosion. However, in contrast to what is observed during larval development, this radial arrangement of the dendritic arbor was rapidly rearranged to a lattice-like shape within 24 hr after eclosion. Time-lapse imaging revealed that this radial-to-lattice reshaping was largely due to rearrangement of the existing radial processes into a lattice-like pattern, rather than extensive pruning of the radially arranged dendrites followed by regrowth of new arbors into a lattice pattern. Mutations in *Mmp2*, which encodes a GPI-anchored MMP, blocked this radial-to-lattice reshaping of C4 da dendrites without affecting other aspects of dendrite growth or development, and Mmp2 expression in epithelial cells adjacent to C4 da dendrites was transiently increased at exactly the time when C4 da dendrites undergo the radial-to-lattice reshaping. Therefore, Mmp2 is a critical regulator of the dendrite arbor reshaping. Furthermore, we have found that epithelial Mmp2 promotes the dendrite reshaping through local modification of the basement membrane (BM) upon which C4 da dendrites grow, suggesting that alteration of the ECM microenvironment might be a general mechanism for driving the structural plasticity of dendritic arbors in vivo.

RESULTS

Dendrite Reshaping in Adult Sensory Neurons

During *Drosophila* metamorphosis, all three C4 da neurons degrade their larval dendrites by 15 hr after puparium formation (APF), and two of them, the dorsal ddaC neuron and the ventral-lateral v'ada neuron (Figure 1A), subsequently regenerate adult dendrites starting at ~50 hr APF while the ventral vdaB neuron undergoes apoptosis (Kuo et al., 2005; Williams and Truman, 2005). We focused on the v'ada neurons for our studies of the arbor dynamics during dendrite regrowth because the dorsal ddaC dendrites are difficult to image due to tanning of the cuticle as adults age. We monitored the morphological changes of the v'ada neurons throughout the regeneration process by using the C4 da neuron-specific *pickpocket* (*ppk*)-Gal4 driving mCD8GFP (Kuo et al., 2005). Consistent with prior reports (Kuo et al., 2005; Williams and Truman, 2005), dendritic processes of the larval C4 da neurons were completely removed by 15 hr APF, while the soma and axonal projections remained intact. Beginning at ~50 hr APF, these neurons began to extend fine protrusions, and by the time of pupal eclosion, these neurons elaborated dendritic arbors that cover the ventral-lateral body wall in a complete but nonredundant manner (Figures 1B and 1C). This tiling arrangement of C4 da dendrites, as well as the radial shape of individual dendrite arbors, is reminiscent of what has been observed in the larval C4 da neurons (Emoto et al., 2004, 2006; Grueber et al., 2002). To our surprise, however, the dendrite arbors of these adult C4 da neurons were dramatically rearranged within 24 hr after eclosion. Although no directional preference was detectable in C4 da dendrites at the time of eclosion (Figure 1C), many major dendrite branches, as well as the majority of terminal dendrite branches, became oriented along the dorsal-ventral (DV) axis by 24 hr after eclosion (Figure 1D). This lattice-like structure became more pronounced by 96 hr after eclosion (Figure 1E) and persisted throughout the adult stages (data not shown).

The radial-to-lattice reshaping of dendritic trees could result from pruning of the radially arranged dendrite branches followed by growth of the new lattice-shaped branches over the time interval, or from rearrangement of the existing radially oriented branches along the DV axis. To distinguish between these possibilities, we conducted time-lapse analysis of single adult neurons over a 72 hr time interval beginning at the time of eclosion (Figures 1F and 1G). All of the major branches and most of the