Chapter 12
Molecular Control of Dendritic Remodeling

Kazuya Togashi, Hiroyuki Koizumi, Takahiro Kanamori, and Kazuo Emoto

Abstract Dendrites are the entry site of neural signals into neurons. Once formed, dendrites do not maintain their structure, rather are dynamically remodeled in vivo: some dendrites are pruned away, while others lengthen and branch out. Dendritic remodeling occurs not only during neural development but also in mature dendrites under both physiological and pathological conditions, suggesting its contribution to neural plasticity. The underlying cellular and molecular mechanisms remain poorly understood; however, they are just beginning to be elucidated from studies in vertebrate and invertebrate model systems. Here, we review recent advances in our understanding of dendritic remodeling by focusing particularly on insights obtained from layer IV spiny stellate neurons in the rodent somatosensory cortex and *Drosophila* sensory neurons.

Keywords Pruning • Drosophila sensory neurons • Barrel cortex

12.1 Introduction

Dendrites are the entry site of neural signals. In most types of neurons, neural signals, such as external stimuli for sensory neurons and synaptic inputs for postsynaptic neurons, are processed and converted to electrical signals in dendritic branches. Early anatomical studies pioneered by Ramón y Cajal revealed that dendritic structures could vary considerably between neurons (Cajal 1911). This led researchers to believe that dendritic divergence might explain, at least in part, why neurons show distinct functional properties in the brain. The fact that functional properties of neurons can often change in a plastic manner in vivo raises an intriguing question: do neurons remodel their dendrites structurally?

It has become increasingly clear that dendrites are remodeled in developmental, physiological, and pathological contexts. During neural development, neurons selectively eliminate exuberant dendritic branches to refine neural circuits. For

K. Togashi • H. Koizumi • T. Kanamori • K. Emoto, Ph.D. (✉)
Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
e-mail: emoto@bs.s.u-tokyo.ac.jp

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example, in the developing olfactory system of rats, mitral cells initially form multiple primary dendrites that contact adjacent glomeruli. However, they eventually prune all but one dendritic branch that remains in contact with a single glomerulus (Malun and Brunjes 1996). Developmental refinement of dendritic branches is also observed in other types of neurons, including retinal ganglion cells (Wang et al. 2001), spiny stellate cells in layer IV of the primary somatosensory cortex (Mizuno et al. 2014), and the cerebellar Purkinje cells (Kaneko et al. 2011). In a physiological condition, the superficial layer two-thirds interneurons in cerebral cortex dynamically add and eliminate dendritic branches, and the fraction of dynamic dendrites increases by three-fold after sensory deprivation, suggesting that neural activity influences the dynamics of dendritic remodeling (Chen et al. 2011; Lee et al. 2006). Pathologically, chronic stress and drug exposure can induce dendritic remodeling in a wide range of cerebral neurons in rodents (Ehlinger et al. 2012; Gourley et al. 2013; Li et al. 2012; Liston et al. 2006). Thus, increasing evidence indicates that both developing and mature neurons have the ability to remodel their dendritic branches. However, due to the lack of an appropriate model, the cellular and molecular mechanisms of dendritic remodeling have remained poorly understood.

Structural remodeling of dendrites reportedly occurs also in invertebrate nervous systems. In *Drosophila*, a variety of neurons in both the central nervous system (CNS) and peripheral nervous system (PNS) remodel their dendritic branches during metamorphosis from the larva to pupa and adult (Consoulas 2002; Kuo et al. 2005; Scott et al. 2011; Watts et al. 2003; Williams and Truman 2005). Taking advantage of the genetic tools available in *Drosophila*, a decade of extensive research has opened up new avenues for addressing the molecular and cellular basis of dendritic remodeling.

In this review, we summarize recent progress in understanding the cellular and molecular mechanisms of dendritic remodeling by focusing on layer IV neurons in the rodent somatosensory cortex and *Drosophila* PNS neurons, where major advances in knowledge have been made. The article begins with the cellular and molecular control of dendrite remodeling of developing neurons in the rodent somatosensory cortex. This is followed by a detailed account of genetic dissection of dendritic remodeling in the *Drosophila* PNS neurons. We also mention recent investigations using another invertebrate model system, *C. elegans* sensory neurons. We suggest that genetic studies using invertebrate model systems are better for gaining molecular insights into dendrite remodeling.

12.2 Dendritic Remodeling in the Mammalian Brain

12.2.1 Dendrite Remodeling of Developing Cortical Neurons in the Rodent Barrel Cortex

In the somatosensory cortex of rodents, the terminal arborization of thalamocortical afferents that represent each vibrissa or whisker is confined to separate barrels in layer IV. Layer IV spiny stellate cells initially develop symmetric trees, but they gradually orient their dendrites toward the center of their associated barrel, where the thalamic axons terminate. Interestingly, removal of one row of whiskers results in the formation of an expanded barrel. In contrast to the highly organized arborizations of normal animals, layer IV neurons in the center of the expanded barrel develop symmetric arborizations (Harris and Woolsey 1979; Steffen and Van der Loos 1980). It is thus likely that layer IV neurons reorganize their dendrites to preferentially contact active afferent inputs. Consistent with these notions, in the cortex-specific NR1 knockout, individual layer IV neurons lose oriented arborization and grow exuberant dendrites and spines (Datwani et al. 2002), suggesting that NMDA (*N*-methyl-D-aspartic acid) receptors mediate sensory stimulation-mediated dendrite orientation. Furthermore, by using the elegant single-cell knockout system MADM (mosaic analysis with double markers), Espinosa et al. (2009) demonstrated that NMDA activity is cell-autonomously required in cortical neurons for dendrite patterning but not for growth and branching. Indeed, a recent in vivo imaging study of layer IV dendrite dynamics during neonatal stages suggests that NMDA activity likely provides directional biases to dendrite dynamics during these stages (Mizuno et al. 2014).

12.2.2 Transcriptional Control of Dendrite Remodeling in the Barrel Cortex

A recent study has shown that the transcription factor BTBD3 is required for layer IV neurons to orient dendrites toward active axons (Matsui et al. 2013). BTBD3 is specifically expressed in the mouse somatosensory cortex during barrel formation, and its expression becomes detectable around postnatal day 2, at the time when thalamocortical axons innervate layer IV cortex. In cultured neural cell lines, BTBD3 is translocated from the cytosol into the nucleus in response to neural activity, implying its functions in activity-dependent neural processes. Indeed, short hairpin interference RNA (shRNAi)-mediated knockdown of *btbd3* in mouse layer IV neurons results in loss of dendrite orientation. More interestingly, ectopic *btbd3* expression in the visual cortex induces directed dendrite to active axon terminals. These data support a model in which BTBD3 mediates activity-dependent dendrite orientation in mammalian neurons. How the transcriptional factor may control dendrite orientation remains unclear. One possible scenario is that BTBD3 might

promote trimming of dendritic branches that initially grow toward few active axon terminals. Alternatively, BTBD3 could promote biased extension of dendritic branches toward active axons. In either scenario, how the transcriptional factor locally regulates dendritic structures remains to be elucidated.

12.3 Dendritic Remodeling in Drosophila Neurons

12.3.1 Dendritic Changes During Transition from Larval to Adult Circuits in the Fly PNS

Peripheral neurons of *Drosophila* larvae are an excellent model to study dendrites (Emoto 2012; Parrish et al. 2007). In each hemisegment, 15 dendritic arborization (da) neurons elaborate stereotypic dendritic branches underneath the epidermal tissue. Da neurons are divided into four classes, I, II, III, and IV, according to the complexity of their dendritic morphology. Each class of da neurons, expressing different sets of genes including ion channels, can sense different external stimuli. For example, class III da (C3da) neurons express a transient receptor potential (TRP) channel, NompC, which confers the ability to sense gentle touch (Yan et al. 2013); class IV da (C4da) neurons sense noxious mechanical, heat, and light stimuli, which are converted into neural signals by the degenerin/epithelial sodium channel (DEG/ENaC) family Pickpocket (for mechanical stimuli), the newly discovered mechanosensory ion channel Piezo (also for mechanical stimuli), and the TRP channel TrpA1(for heat and light stimuli) (Kim et al. 2012; Zhong et al. 2010, 2012). Taking advantage of the class-specific gene expression, several transgenic reporter lines have been developed to label a specific class of da neurons with single-dendrite resolution in vivo.

Live imaging studies using the reporter lines have revealed that during metamorphosis from the larva to pupa and adult, dendrites of larval da neurons dynamically remodel to develop the adult PNS. The remodeling process of da neuron dendrites can be divided into three phases: destructive, latent, and regenerating (Fig. 12.1). The first destructive phase takes place within 16–20 h after puparium formation (APF). During this phase, ddaB class II da (C2da) and ddaA C3da neurons undergo programmed cell death within ~10 h APF. In contrast, ddaD/ddaE class I da (C1da) and ddaC C4da neurons remain alive and completely prune their dendritic arbors, while their axons and cell bodies remain intact (Williams and Truman 2005). Dendrite pruning of both classes of da neurons is mediated by local degeneration rather than retraction of branches (Williams and Truman 2004, 2005). After the completion of dendrite pruning, da neurons stay "dormant" without net regrowth of dendritic branches (the latent phase). The length of this phase differs between da neurons: up to ~42 and ~72 h APF for ddaE C1da and ddaC C4da neurons, respectively (Lyons et al. 2014; Williams and Truman 2004). Finally, during the regenerating phase, da neurons regenerate their dendritic branches

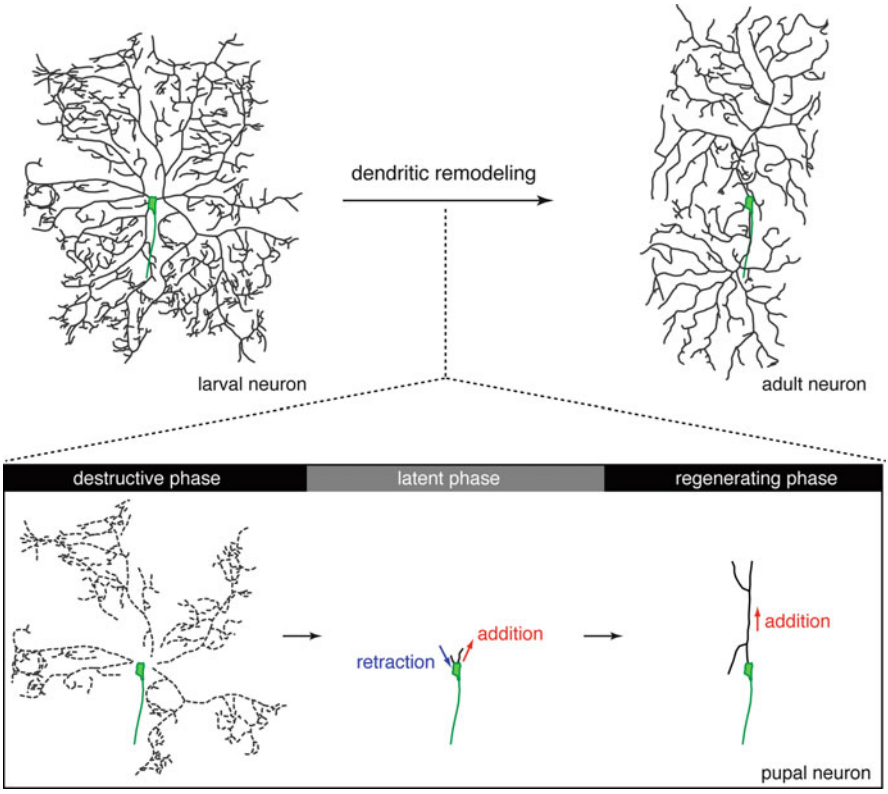

Fig. 12.1 Dendritic remodeling of *Drosophila* C4da sensory neurons. Dendritic branches of larval neurons (*upper left*) remodel during metamorphosis to form adult-specific dendrites (*upper right*). Dendrite remodeling is divided into three phases: destructive, latent, and regenerating phases (*bottom*). During the destructive phase, dendritic branches are pruned away by local degeneration, while axons and soma remain intact (*green*). After a latency of ~72 h, pupal neurons start to regenerate dendritic branches

through the rest of metamorphosis and are incorporated into the adult PNS circuits (Kuo et al. 2005; Lyons et al. 2014; Satoh et al. 2012; Shimono et al. 2009; Williams and Truman 2004). Hereafter, we will mainly focus on dendritic remodeling of ddaC C4da neurons, whose dendrites undergo pruning and regeneration during metamorphosis (Fig. 12.1), and review recently revealed molecular mechanisms underlying these processes.

12.3.2 Pruning of Larval Dendrites: The Molecular Mechanisms

12.3.2.1 Specification of Cell Responses

The molecular program of dendrite pruning is initiated by transcriptional control via the ecdysone-signaling cascade (Table 12.1). The steroid hormone ecdysone, which triggers a cascade of morphological changes during insect metamorphosis, acts through the ecdysone receptor (EcR), a member of the nuclear receptor superfamily, and its heterodimer partner ultraspiracle (Usp) (Fig. 12.2). On the prepupal pulse of ecdysone, the ecdysone receptor complex (EcR/Usp) is activated and induces the expression of a small set of genes, which triggers morphological changes of the larval tissue (Buszczak and Segraves 2000; Thummel 1996). Genetic inactivation of EcR/Usp functions inhibits dendrite pruning of C4da neurons in a cell-autonomous manner (Kuo et al. 2005; Williams and Truman 2005). It is thus plausible that dendrite pruning of C4da neurons is regulated by transcriptional induction of specific gene(s), which are vital for initiating and executing the pruning processes, through the ecdysone-signaling cascade.

Which are the genes induced by the ecdysone-signaling cascade to promote dendrite pruning of C4da neurons? A genetic study has identified a high-mobility group (HMG)-box transcription factor, Sox14, as a key gene that mediates dendrite pruning downstream of EcR/Usp (Kirilly et al. 2009). Sox14 expression is upregulated in the prepupal stage in an EcR/Usp-dependent manner. Interestingly, EcR forms a complex with a histone acetyltransferase CREB-binding protein (CBP) in response to ecdysone and is recruited to the *sox14* locus to facilitate H3K27 acetylation, the transcriptionally active chromatin mark (Kirilly et al. 2011). Since the upregulation of *sox14* also requires the function of CBP, it is likely that the EcR/Usp/CBP complex directly induces *sox14* expression. After being induced by ecdysone signaling, Sox14 upregulates the expression of at least two genes: molecule interacting with CasL (*mical*) and *cullin 1* (Kirilly et al. 2009; Wong et al. 2013). The expression level of Mical is increased in prepupal C4da neurons, similar to that of Sox14, and this increase is dependent not only on EcR/Usp but also on Sox14 (Kirilly et al. 2009). The mechanism by which Mical promotes dendrite pruning remains to be elucidated. However, given previous reports suggesting that Mical has a role in the regulation of actin cytoskeletons (Beuchle et al. 2007; Hung et al. 2010), this cytosolic protein might regulate the dynamics of actin filaments in pruning dendrites. The E3 ubiquitin ligase Cullin 1 (see below for details) is another possible downstream target of Sox14. Before initiation of axon pruning of the mushroom body (MB) γ neurons, an extensively studied model of developmental axon pruning, expression of Cullin 1 is upregulated in MB γ neurons in an EcR- and Sox14-dependent manner, although this upregulation in C4da neurons has not been directly examined (Wong et al. 2013). Given that this upregulation does not require Mical and that pruning defects of *mical* mutant C4da neurons are significantly enhanced by an additional mutation in

Table 12.1 Genes required for dendrite pruning of C4da neurons

Function in pruning	Fly gene name	Molecular function	References
Cell specification	*EcR*	Nuclear hormone receptor	Williams and Truman (2005); Kuo et al. (2005)
	ultraspiracle	Co-receptor of EcR	Williams and Truman (2005); Kuo et al. (2005)
	sox14	Transcriptional factor	Kirilly et al. (2009)
	CBP	Histone acetyltransferase	Kirilly et al. (2011)
	brahma	SWI/SNF chromatin remodeler	Kirilly et al. (2011)
	osa	SWI/SNF chromatin remodeler	Kirilly et al. (2011)
Nrg degradation	*rab5*	Rab family GTPase	Zhang et al. (2014)
	vps4	Disassembly of ESCRT-III complex	Zhang et al. (2014)
	vps28	ESCRT-I complex	Zhang et al. (2014)
	vps32	ESCRT-III complex	Zhang et al. (2014)
Thinning/severing of proximal regions	*mical*	Actin filament disassembly	Kirilly et al. (2009)
	headcase	Unknown	Loncle and Williams (2012)
	kat-60L1	Microtubule destabilization	Lee et al. (2009)
	ik2	Protein kinase	Lee et al. (2009)
	uba1	Ubiquitin activation enzyme	Kuo et al. (2005); Wong et al. (2013)
	mov34	19S proteasome particle	Kuo et al. (2005)
	ubcD1	E2 ubiquitin-conjugating enzyme	Kuo et al. (2006)
	vcp	Ubiquitin-selective AAA chaperone	Rumpf et al. (2011, 2014)
	pros26	20S proteasome core particle subunit	Rumpf et al. (2014)
	pros α7	20S proteasome core particle subunit	Rumpf et al. (2014)
	pros β5	20S proteasome core particle subunit	Rumpf et al. (2014)
	cullin 1	SCF complex, E3 ubiquitin ligase	Wong et al. (2013); Rumpf et al. (2014)
	roc1a	SCF complex, E3 ubiquitin ligase	Wong et al. (2013)
	skpA	SCF complex, E3 ubiquitin ligase	Wong et al. (2013)
	slimb	SCF complex, E3 ubiquitin ligase	Wong et al. (2013)
	nedd8	Neddylation of Cullin1	Wong et al. (2013)

(continued)

Table 12.1 (continued)

Function in pruning	Fly gene name	Molecular function	References
Compartmentalized Ca^{2+} transients	*ca-α1D*	Voltage-gated Ca^{2+} channel	Kanamori et al. (2013)
	cacophony	Voltage-gated Ca^{2+} channel	Kanamori et al. (2013)
	ca-beta	Voltage-gated Ca^{2+} channel	Kanamori et al. (2013)
	rab5	Rab family GTPase	Kanamori et al. (2015)
	shibire	Dynamin GTPase	Kanamori et al. (2015)
Distal degeneration	*dronc*	Caspase	Williams et al. (2006)
	drice	Caspase	Schoenmann et al. (2010)
	dcp-1	Caspase	Schoenmann et al. (2010)
	calpA	Calpain, Ca^{2+} dependent protease	Kanamori et al. (2013)
	calpB	Calpain, Ca^{2+} dependent protease	Kanamori et al. (2013)
Debris clearance	*draper*	Cell corpse engulfment receptor	Williams et al. (2006); Han et al. (2014)

a component of the Cullin 1 E3 ligase complex, *slimb*, it is likely that Mical and Cullin 1 act independently to promote dendrite pruning under the transcriptional control of Sox14 (Wong et al. 2013).

Another gene downstream of EcR/Usp is *headcase*. Screening genes that show genetic interactions with EcR mutants, Loncle and Williams identified *headcase* as a regulator of dendrite pruning (Loncle and Williams 2012). Similar to *sox14*, *headcase* expression follows the prepupal pulse of ecdysone in an EcR-dependent manner, although it remains to be elucidated whether EcR/Usp is recruited to the *headcase* locus to induce its transcription directly. Interestingly, the upregulation of *headcase* does not require *sox14* expression, and vice versa, suggesting that these two genes are independently regulated downstream of EcR/Usp. Consistent with this, pruning defects in a null allele of *headcase* are additively enhanced by RNAi knockdown of *mical*, a downstream gene of *sox14*. The molecular function of headcase in dendrite pruning remains unknown.

Sox14 and *headcase* are unlikely to be the only genes that C4da neurons express in response to the ecdysone-signaling cascade to initiate dendrite pruning. Although overexpression of *sox14*, but not that of *headcase* (Loncle and Williams 2012), accelerates dendrite pruning to occur earlier than in wild-type neurons, this precocious pruning is observed only after the prepupal pulse of ecdysone, suggesting that EcR/Usp regulates the expression of other genes in addition to these two. Identification of additional regulators that promote dendrite pruning downstream of EcR/Usp awaits further research.

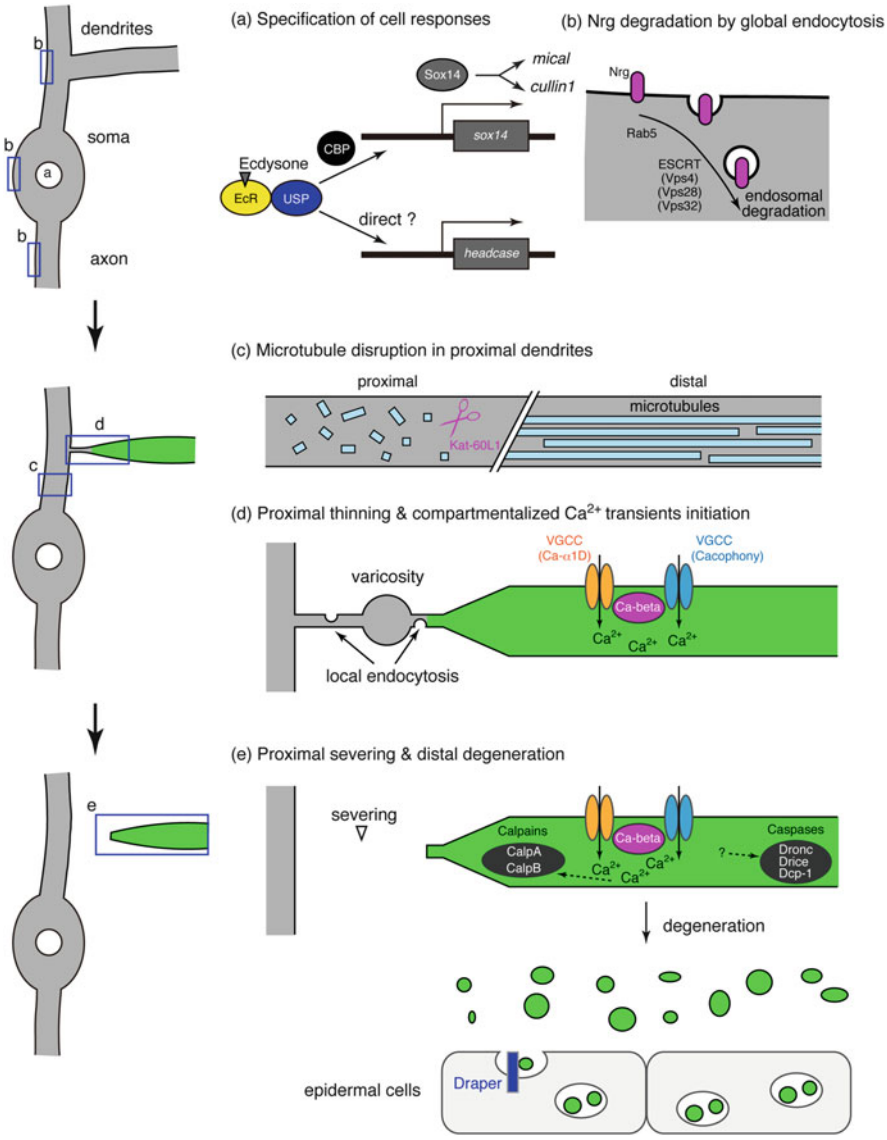

Fig. 12.2 Molecular mechanisms governing dendrite pruning of *Drosophila* C4da neurons. (**a**, **b**) Early events in the prepupal stage. Specification of cell responses is regulated by ecdysone signaling (**a**). The steroid hormone ecdysone binds to EcR/USP complex to induce expression of downstream genes, such as *sox14* and *headcase*. Induction of *sox14* expression also requires a histone acetyltransferase CBP. Sox14 induces expression of *mical* and *cullin1*. Downregulation of Nrg by endosomal degradation is a prerequisite for dendrite pruning (**b**). This involves Rab5 and the ESCRT complex components (Vps4, Vps28, and Vps32). (**c**, **d**) Local events in proximal dendrites. Microtubule disruption in proximal regions involves Kat-60L1 (**c**). Endocytic activity is locally elevated in proximal dendrites to induce branch thinning (**d**). Proximal thinning accompanies varicosity formation and tightly linked with the initiation of compartmentalized Ca^{2+} transients. Two types of VGCCs (L-type, Ca-α1D; and PQNR-type, cacophony), which require the beta subunit (Ca-beta) for their functions, are responsible for generating Ca^{2+} transients.

12.3.2.2 Thinning and Severing of Proximal Branches

After the initial specification driven by ecdysone signaling, the first morphological alterations are observable in the proximal regions of dendrites (Fig. 12.2): proximal dendrites actively form varicosities, around which dendritic branches progressively become thinner (Kanamori et al. 2015; Kirilly et al. 2009; Williams and Truman 2005). Some of the thinning proximal dendrites also form dynamic filopodia-like extensions (Williams and Truman 2005; our unpublished observations). The thinning dendritic branches are eventually severed (i.e., the distal regions become physically detached from the rest of the cell) (Kuo et al. 2005; Williams and Truman 2005). The initial severing of proximal dendrites occurs in a spatially correlated manner with the location of a distal edge of dendrites wrapped by the glial membrane, suggesting the existence of extrinsic mechanisms for dendrite severing (Han et al. 2011). Although it is not known whether each of these events is regulated by distinct molecular mechanisms, recent loss-of-function studies have begun to provide molecular insights into these early processes in dendrite pruning.

Regulators of the cytoskeleton are one group of genes involved in these early events. Cytoskeletal changes are prominent features of the thinning proximal dendrites: microtubules are fragmented locally in proximal dendrites in the early pupal stage (Lee et al. 2009; Williams and Truman 2005); and actin filaments are also disrupted in proximal regions of dendrites (Lee et al. 2009). Actin disruption is retarded compared with that of microtubules (Kanamori et al. 2015). *Katanin p60-like 1* (*kat-60L1*) is a key molecule for the microtubule destabilization in proximal dendrites. Given that Kat-60L1 is homologous to a p60 subunit of the microtubule-severing protein Katanin, this protein likely promotes dendrite pruning by directly severing microtubules in proximal dendrites. In addition, as mentioned above, Mical is likely to be involved in disrupting actin filaments to facilitate dendrite severing at the proximal regions. One important, but as yet unanswered, question is how these cytoskeletal regulators act locally in proximal dendrites during the pruning processes.

Another group of genes encodes regulators of the ubiquitin-proteasome system (UPS). Proper severing of proximal dendrites requires several UPS genes such as ubiquitin activation enzyme 1 (*Uba1*), a component of the 19S particle of the proteasome, *Mov34*, and the E2 ubiquitin-conjugating enzyme *UbcD1* (Kuo et al. 2005, 2006). At least three mechanisms are proposed for the function of the UPS in dendrite pruning. Firstly, it has been suggested that proximal severing requires UPS-mediated degradation of the E3-ubiquitin ligase *Drosophila* inhibitor of apoptosis protein 1 (DIAP1). This is mainly based on the following evidence:

Fig. 12.2 (continued) (**e**) Severing of proximal dendrites and degeneration of their distal regions. Proximal dendrites are severed during the repetition of Ca^{2+} transients, and the distal regions undergo degeneration via the local activation of calpains (CalpA and CalpB) and caspases (Dronc, Drice, and Dcp-1). Debris clearance is mediated by a cell surface protein Draper that is expressed in epidermal cells

(1) a gain-of-function mutation in *diap1*, which results in a DIAP1 protein insensitive to UPS-mediated degradation, compromises proximal severing (Kuo et al. 2006), and (2) inhibition of valosin-containing protein (VCP), a ubiquitin-selective AAA chaperone that is required for DIAP1 degradation in vitro, causes a similar severing defect, which is significantly suppressed by reducing DIAP1 expression (Rumpf et al. 2011). Supporting this idea, the noncanonical IκB kinase family member Ik2, which functions as a negative regulator of DIAP1 by promoting DIAP1 degradation (Kuranaga et al. 2006; Oshima et al. 2006), is also required for proper severing of proximal dendrites (Lee et al. 2009). Because severing of proximal dendrites apparently occurs in mutants of the cell death protease (caspase) Dronc, a well-known DIAP1 substrate, DIAP1 might degrade some substrates other than Dronc for proximal severing (Kanamori et al. 2013; Rumpf et al. 2014; Williams et al. 2006).

The second proposed mechanism by which the UPS controls dendrite severing involves downregulation of the insulin signaling pathway (Wong et al. 2013). Substrate specificity of the UPS-mediated protein degradation is achieved via E3 ligase substrate binding, which results in ubiquitination of the bound substrates. The Cullin1-based SCF (Skp1-Cullin-F-box) complex is an E3 ligase required for proximal severing. Genetic evidence suggests that a signaling pathway composed of insulin receptor (InR), phosphatidylinositol 3-kinase (PI3K), and target of rapamycin (TOR) (InR/PI3K/TOR pathway) is aberrantly activated in *cullin1* knockdown C4da neurons. Interestingly, the protein kinase Akt, a positive regulator of the InR/PI3K/TOR pathway, is more abundant in *cullin1* knockdown neurons compared with control neurons at the beginning of the pupal stage and physically interacts with the substrate-recognizing F-box Slimb. Based on these findings, it is proposed that the SCF E3 ligase complex targets Akt to downregulate the InR/PI3K/TOR pathway. Further investigation is needed to clarify how this downregulation is linked to the cell biological processes underlying proximal severing.

Regarding the third mechanism involving the UPS, Rumpf and colleagues recently reported that VCP also regulates proximal severing in a manner independent of protein degradation (Rumpf et al. 2014). Inhibition of VCP causes two kinds of defects in mRNA metabolism: missplicing of *mical* mRNA and aberrant localization and expression of RNA binding proteins, which could at least in part explain the pruning defects in *vcp* mutant neurons. Interestingly, these defects in mRNA metabolism are not observed in the mutants of the 20S proteasome component. Thus, the UPS likely promotes dendrite pruning both in a protein degradation-dependent and degradation-independent manner. Which cell biological process the UPS regulates to promote proximal severing remains to be elucidated.

12.3.2.3 Dendrite Degeneration and Its Clearance

After a proximal dendrite is severed, the dendrites distal to a site of severing degenerate rapidly after a latency of less than 2 h. Degeneration involves rapid

blebbing and fragmentation of an entire dendritic branch into short segments (Williams and Truman 2005). These changes in cellular morphology are similar to Wallerian degeneration in live preparations of mouse axons, where the distal portion of axotomized peripheral nerves degenerates after about 1.5 days of latency without affecting the main cell bodies (Coleman and Freeman 2010). This argues for a potential conservation of degenerative mechanisms between these two different contexts (i.e., developmental dendrite pruning and Wallerian degeneration of mouse axons). This possibility was tested and supported by recent studies utilizing the causal gene product of the slow Wallerian degeneration mouse (C57BL/*Wld*^{S}) (Lunn et al. 1989), in which Wallerian degeneration is delayed tenfold. In flies overexpressing Wld^S proteins in C4da neurons, degeneration of the pupal dendrites during pruning was slightly but significantly retarded, while proximal dendritic branches seemed to be severed normally (Schoenmann et al. 2010; Tao and Rolls 2011), suggesting that developmental dendrite pruning may share molecular mechanisms with Wallerian degeneration (although this was not the case in developmental axon pruning of MB γ neurons (Hoopfer et al. 2006)). Because pruning defects seen in Wld^S overexpressing C4da neurons were subtle, it is likely that other mechanisms also contribute to the degeneration process.

Recently, local activation of caspases was suggested as an additional mechanism of dendritic degeneration in C4da neurons. Using the genetically encoded detector of effector caspases (such as Drice and Dcp-1), caspase activity was first readily detected within degenerating C4da dendrites, but not in the cell bodies or axons, during pupal stages (Williams et al. 2006). The authors also demonstrated that overexpression of the viral effector caspases inhibitor p35 had an inhibitory effect on dendrite pruning in C4da neurons (Kuo et al. 2006). Consistent with this are recent observations that flies lacking Drice or Dcp-1 were slightly but significantly defective in dendrite degeneration, while dendrite severing at proximal regions was apparently normal (Schoenmann et al. 2010). Therefore, caspase activation in dendritic branches could act as a degeneration mechanism in dendrite pruning. In addition, given that in C4da neurons overexpressing both Wld^S and p35 proteins dendrite pruning was inhibited slightly more strongly than in those expressing either of them (Schoenmann et al. 2010), it is possible that these two mechanisms contribute cooperatively to the degeneration process.

After dendritic branches are degenerated into short fragments, the neuronal debris is completely removed from the surrounding tissue, paving the way for subsequent dendritic regrowth during late metamorphosis. Which are the cell types responsible for clearing the degenerating dendrites? Hemocytes, highly motile macrophage-like cells, have been previously suggested as a possible candidate for this process (Williams and Truman 2005). Live imaging showed that some hemocytes were closely associated with degenerating sensory dendrites labeled with green fluorescent protein (GFP) and that they progressively exhibited green fluorescent cytoplasmic particles that are also labeled with the lysosome marker LysoTracker (Williams and Truman 2005). These results raise the possibility that hemocytes may phagocytose and clear the degenerating dendritic branches. Subsequently, a recent study from Jan and colleagues reexamined this possibility and

showed that hemocytes play only a minor role (if any) in debris clearance although they appear to contribute partially to dendrite fragmentation during degeneration with an unknown mechanism (Han et al. 2014). Instead, the authors argue that epidermal cells adjacent to C4da dendrites are the major phagocytes clearing the neuronal debris (Fig. 12.2). They performed high-resolution 3D confocal imaging of degenerating dendrites and found that fragmented dendritic debris dispersed into the epidermal layer within several minutes after degeneration and eventually incorporated into the phagolysosomal compartments in the epidermal cells. This phagocytosis was inhibited in flies overexpressing mutant dynamin, which is known to regulate phagocytosis (Yu et al. 2006), specifically in epidermal cells; a significant amount of fragmented dendrites was not cleared and remained outside of the epidermal layer (Han et al. 2014). Taken together, these findings suggest that epidermal cells are likely the major phagocytes responsible for debris clearance.

The first clues to the molecular mechanisms of neuronal debris clearance came from studies of developmental pruning and injury-induced degeneration of *Drosophila* axons. These studies uncovered the role of the engulfment receptor *draper*, required for the clearance of apoptotic cells in *C. elegans* (Zhou et al. 2001) and phagocytosis in cultured *Drosophila* cells (Manaka et al. 2004), in the clearance of degenerating axons by glial cells (Awasaki et al. 2006; Hoopfer et al. 2006; MacDonald et al. 2006). Recently, astrocytes have been reported to be at least one of the glial cells that execute Draper-mediated phagocytosis during axon pruning of MB γ neurons (Hakim et al. 2014; Tasdemir-Yilmaz and Freeman 2014). Similarly, it was later shown that *draper* also has a role in the clearance of degenerating C4da dendrites during metamorphosis and its function is required, at least in part, in epidermal cells (Williams et al. 2006; Han et al. 2014). This Draper-mediated dendrite clearance by epidermal cells does not require the phagocytic receptor *six-microns-under* (*simu*), which functions upstream of *draper* in the context of apoptotic cell clearance in the developing CNS (Han et al. 2014; Kurant et al. 2008). This raises the intriguing possibility that different engulfment pathways are utilized depending on whether neuronal debris was generated through neurite pruning or apoptotic cell death. Evidence in support of this notion is also found in studies on developmental axon pruning of MB γ neurons (Kuraishi et al. 2009; Tasdemir-Yilmaz and Freeman 2014). The molecular and genetic dissection of the engulfment pathways in these different contexts should provide further insight into the molecular mechanisms underlying neuronal debris clearance.

Intriguingly, Draper-mediated phagocytic mechanisms might have an active role in the neural circuit remodeling. In other words, phagocytes (i.e., astrocytes and epidermal cells in the axon and dendrite pruning, respectively) are likely not just passive participants that scavenge the already degraded debris, but they could actively promote the degeneration of axonal and dendritic branches. This notion is based on the following observations: (1) before any visible signs of axon pruning, larval astrocytes transform into phagocytes in a cell-autonomous manner to infiltrate axon lobes destined for pruning and to increase the expression level of Draper and phagocytic activity (Hakim et al. 2014; Tasdemir-Yilmaz and Freeman 2014);

(2) genetic inactivation of glial infiltration into axon lobes significantly delays axon fragmentation (Awasaki and Ito 2004); (3) glial cells, presumably those other than astrocytes (Hakim et al. 2014), around MB γ axon branches destined to be pruned increase in number prior to branch fragmentation (Watts et al. 2004); (4) whole-body knockout or phagocyte-specific knockdown of *draper* causes defects not only in debris clearance but also in branch fragmentation (Han et al. 2014; Tasdemir-Yilmaz and Freeman 2014; but see also Hakim et al. 2014). In good agreement with this idea, Chung, Barres, and colleagues recently demonstrated that astrocytes utilize MEGF10 (mouse Draper homolog) and the engulfment receptor MERTK to eliminate excessive synapses formed between retinal ganglion cells (RGCs) and dorsal lateral geniculate nucleus (dLGN) neurons in the developing mouse retinogeniculate system. In addition, they showed that in mice lacking these two genes, the retinogeniculate connections were not refined normally and excessive weak but functional synapses did remain, suggesting an active role of Draper/MEGF10 in eliminating excessive connections (i.e., more than just scavenging neural debris) (Chung et al. 2013). It will be interesting to determine how phagocytes selectively eliminate unnecessary neurites and synapses among many others and whether or not there is a neuronal molecular machinery that could be activated by phagocytes to facilitate branch fragmentation.

12.3.2.4 An Emerging New Paradigm: Calcium Signaling and Endocytosis in Dendrite Pruning

The current view on dendrite pruning that proximal severing induces the distal degeneration of dendritic branches is based on the observation of dendritic shapes using membrane-targeted fluorescent markers. However, recent evidence suggests that this prevalent view is probably an oversimplification (Kanamori et al. 2013) (Fig. 12.2). Time-lapse imaging of pruning dendrites with simultaneous Ca^{2+} imaging has revealed that Ca^{2+} transients occur in a compartmentalized manner in dendritic branches destined to be pruned. Interestingly, compartmentalized Ca^{2+} transients are observed before dendrite severing and perfectly predict the location and timing of the dendrite pruning. Voltage-gated Ca^{2+} channels (VGCCs) are responsible for generating Ca^{2+} transients, and mutant C4da neurons lacking VGCC activity show significant defects in dendrite pruning. Subsequent calcium signaling activates the serine protease calpains, which promote the degeneration of dendrites cooperatively with the activity of caspases. Thus, compartmentalized Ca^{2+} transients act as spatial and temporal cues to trigger dendrite pruning before proximal severing occurs. How are dendrites compartmentalized into those with and without Ca^{2+} transients? A recent study has addressed this question and provided evidence suggesting that local endocytosis in proximal dendrites contributes to compartmentalization and initiation of Ca^{2+} transients by inducing branch thinning of proximal dendrites (Kanamori et al. 2015) (Fig. 12.2).

Involvement of endocytosis in dendrite pruning is also reported by Wong et al., showing that global endocytosis occurs in the entire neurons, including axons,

dendrites, and cell bodies, and degrades the L1-type cell adhesion molecule Neuroglian (Nrg) through the endo-/lysosomal degradation pathway (Zhang et al. 2014) (Fig. 12.2). Nrg degradation starts from the onset of metamorphosis (i.e., the white pupal stage), and loss-of-function *nrg* mutant neurons show precocious dendrite pruning. Thus, removal of Nrg from the cell surface is a prerequisite for dendrite pruning. Interestingly, genetic evidence suggests that this global endocytosis for Nrg degradation promotes dendrite pruning cooperatively with local endocytosis for compartmentalized Ca^{2+} transients (Kanamori et al. 2015). These recent studies reveal that two distinct endocytic mechanisms act together in developmental dendrite pruning.

12.4 Regrowth and Reshaping of Adult Dendrites

After all larval dendrites are eliminated, C4da neurons regrow dendritic branches for the adult PNS circuit during the rest of the pupal stage. Dendrite regrowth in the late pupal stage can be divided into the latent and regenerating phases (Fig. 12.1). After the completion of dendrite pruning at 16 h APF, C4da neurons stay "dormant" until ~72 h APF without extensive dendritic regrowth (the latent phase), whereas numerous neurites extend and retract from the cell bodies. There is little net increase of dendritic length or branch point numbers during this phase (Lyons et al. 2014). Strikingly, however, dendritic branches grow in an exponential fashion between 72 and 96 h APF (the regenerating phase), eventually resulting in asymmetric dendritic fields that are characteristic of the adult ddaC C4da neurons (Lyons et al. 2014; Shimono et al. 2009) (Fig. 12.1). These observations suggest the existence of mechanisms that inhibit or promote dendritic regrowth during the latent or regenerating phase, respectively. Which molecular mechanisms contribute to dendritic regrowth? A recent study provided the first molecular insight into the regulation of dendritic regrowth during the late metamorphosis (Lyons et al. 2014). Kuo and colleagues showed that this process requires *Cysteine proteinase-1* (*Cp1*), the functions of which are not well characterized in the nervous system. Expression of Cp1 in C4da neurons increases during the latent phase in an EcR-dependent manner. Cp1 cleaves the homeodomain transcription factor cut to change its subnuclear localization from heterochromatin to the entire nucleus, suggesting that temporal Cp1 activation contributes to initiating the transcriptional program underlying the temporal switch between the two phases in dendrite regrowth. Interestingly, Cp1 is dispensable for dendritic regrowth of ddaE C1da neurons, which have a shorter latent phase for regrowth compared with that of C4da neurons (Lyons et al. 2014; Williams and Truman 2004). Thus, each class of da neurons might use distinct strategies for regrowing dendritic branches during late metamorphosis.

Which are the mechanisms that shape the regrowing dendrites for the adult PNS circuit? Several recent studies on v'ada neurons, another subclass of C4da neurons, have provided insights into this question. Firstly, actively regrowing v'ada

dendrites extend in the same path with those of another cell type of da neurons (Satoh et al. 2012). This suggests a possible role of the physical interaction between dendrites of different neurons or between dendrites and extracellular substrates in the regulation of dendritic regrowth. Secondly, an HSP90 co-chaperone protein, CHORD, regulates the final size of dendritic arbors together with the insulin/IGF signaling pathway (Shimono et al. 2014). Finally, and most strikingly, v'ada C4da neurons reshape their newly regrown dendrites from radial shape to lattice shape in the early adult stage (Shimono et al. 2009; Yasunaga et al. 2010). This radial-to-lattice transformation involves remodeling of the extracellular matrix (ECM) surrounding dendrites, which is controlled by temporal upregulation of matrix metalloproteinase Mmp2 in the epidermal tissue (Yasunaga et al. 2010). How do dendritic branches sense the extracellular environment? Which molecular cues direct dendritic branches toward their final shapes? These are the questions to be addressed in future research.

12.5 Dendritic Remodeling in Other Invertebrate Model Systems

C. elegans sensory neurons are an emerging model system for studying dendrite development and remodeling. Recent genetic screens have identified several genes involved in dendrite branching, extension, and self-avoidance of the PVD multidendritic neurons (Liu and Shen 2011; Smith et al. 2012, 2013). For example, the type I membrane protein EFF-1, which was originally identified as a cell fusion-promoting factor, regulates the complexity of dendritic arbors by pruning excessive dendritic branches (Oren-Suissa et al. 2010). The pruning process involves not only dendrite severing and retraction but also dendrite-dendrite autofusion, which is never observed in C4da dendrite pruning, thus providing additional mechanistic insights into dendrite pruning.

With regard to dendrite remodeling, a recent study has reported that in response to adverse environmental conditions, the IL2 bipolar sensory neurons undergo dendrite arborization and shift from bipolar to multipolar neurons (Schroeder et al. 2013). This process is reversible: the arborized dendrites are pruned away after worms are returned back to the normal (non-stress) environment. The Furin proprotein convertase KPC-1, whose expression in IL2 neurons increases in stress conditions, is identified as a critical regulator of this reversible arborization of dendritic branches (Schroeder et al. 2013). Given that KPC-1/Furin also controls the dendritic development of PVD neurons in the same genetic pathway as the leucine-rich transmembrane receptor (LRR) DMA-1 (Liu and Shen 2011; Salzberg et al. 2014), KPC-1/Furin might regulate the IL2 dendrite remodeling by proteolytically processing DMA-1/LRR, which indeed contains a predicted Furin cleavage site. Further genetic and biochemical studies are required to elucidate this point.

Thus, studies of the worm sensory neurons have started to uncover a new path that will pave the way for a deeper understanding of dendrite remodeling.

12.6 Conclusions and Future Directions

What information have invertebrate model systems provided about dendrite remodeling? Cellular mechanisms underlying dendrite pruning are extensively described in *Drosophila* C4da neurons, which are amenable to live imaging during the whole pruning processes. Early events in dendrite pruning are observed in the proximal dendrites: cytoskeletal remodeling and dendritic thinning. Compartmentalized Ca^{2+} transients, spatiotemporal cues to trigger dendrite pruning, occur in a correlated manner with dendritic thinning (Kanamori et al. 2013, 2015). Proximal dendrites are eventually severed and their distal regions undergo rapid degeneration after a latency period. Genetic screens also uncovered key factors in dendrite pruning of C4da neurons. Interestingly, many of these factors are evolutionarily conserved from fruit flies to vertebrates. We end this review by outlining the key questions remaining to be addressed.

First, despite extensive research, our understanding of the molecular mechanisms downstream of the ecdysone-signaling cascade is rudimentary. Elucidating this point will require unbiased identification of downstream genes not only by performing conventional genetic screens, which are not yet saturated, but also by using microarray expression analysis. The latter approach was successfully taken to identify the key regulators of MB γ axon pruning (Hoopfer et al. 2008). Applying current state-of-the-art sequencing technologies, such as the single-cell RNA-seq technique, will facilitate identification of additional EcR-dependent regulators.

Second, although it is clear that in response to ecdysone-signaling C1da and C4da neurons prune their dendrites, while C2da and C3da neurons undergo programmed cell death, it is unclear how such distinct responses are induced in response to the same steroid hormone. Are there any genes expressed specifically in distinct classes of neurons to induce their cellular responses? Epigenetic mechanisms might be involved in the regulation of distinct cell responses. Although both *mical* and *headcase* are required for C4da dendrite pruning but not for apoptotic cell death of C3da neurons, the expression of these genes is induced in both classes of da neurons in an EcR-dependent manner (Kirilly et al. 2009; Loncle and Williams 2012). Therefore, expression analysis from single da neurons would be a more straightforward approach than loss-of-function screening for genes required specifically for dendrite pruning or apoptotic cell death.

Finally, it is important to consider whether molecular mechanisms elucidated from studies using invertebrate models are evolutionarily conserved from invertebrates to vertebrates. Establishing vertebrate model systems, which are amenable not only to live imaging but also to genetic manipulation, is the first step to answer this question. The recent availability of genetic tools in mice, genetically

engineered viruses, and multiphoton microscopy makes it easier to establish such systems.

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