Review Article • DOI: 10.2478/s13380-013-0125-2 • Translational Neuroscience • 4(3) • 2013 • 273-281

Translational Neuroscience

VERSITA

THE BRAKE WITHIN: MECHANISMS OF INTRINSIC REGULATION OF AXON GROWTH FEATURING THE CDH1-APC PATHWAY

Judith Stegmüller*

Max Planck Institute of Experimental Medicine, Hermann Rein Strasse 3, 37075 Göttingen, Germany

Center for Nanoscale Microscopy and Molecular Physiology of the Brain (CNMPB), Göttingen, Germany

Abstract

Neurons of the central nervous system (CNS) form a magnificent network destined to control bodily functions and human behavior for a lifetime. During development of the CNS, neurons extend axons that establish connections to other neurons. Axon growth is guided by extrinsic cues and guidance molecules. In addition to environmental signals, intrinsic programs including transcription and the ubiquitin proteasome system (UPS) have been implicated in axon growth regulation. Over the past few years it has become evident that the E3 ubiquitin ligase Cdh1-APC together with its associated pathway plays a central role in axon growth suppression. By elucidating the intricate interplay of extrinsic and intrinsic mechanisms, we can enhance our understanding of why axonal regeneration in the CNS fails and obtain further insight into how to stimulate successful regeneration after injury.

Keywords

• Axon growth • Development • Guidance molecules • Regeneration • Ubiquitin proteasome system

Received 16 July 2013
accepted 03 August 2013

1. Introduction

Neurodevelopment is a fundamental process involving axonal and dendritic growth to establish a functional neuronal network. Axon growth is crucial for making the appropriate connections with the target tissue. A large body of research in the neurodevelopment field led to advanced knowledge of various mechanisms underlying axon growth and guidance. During development of the nervous system, growth cones of neurons act as sensors to sample the environment for cues that promote axon growth and lead the way to the target area [1-3]. The growth cone harbors conserved receptors, which distinguish between attractive and repellent axon growth stimuli. Guidance cues including Netrins, Semaphorins and Slits, which bind to the respective Unc-5 or DCC, Plexin and Robo receptors direct outgrowing axons [4-8]. Further essential regulators of axon growth and guidance in the brain are Neurotrophins such as NGF and BDNF, which bind to Trk receptors and Ephrins together with Eph receptors [9-12]. Adhesion molecules are also crucial for axonal contact with its environment to ultimately establish a functional network [13,14].

Once fully developed, the CNS in contrast to the peripheral nervous system (PNS) has very little capacity for axonal repair and regeneration following brain or spinal cord injury [15,16]. Apart from damage to axonal tracts, CNS injury inevitably destroys surrounding structures like myelin and induces unfavorable responses by glial cells [15]. To date, we have a good understanding of the mechanisms that inhibit axonal regeneration as the major culprits have been extensively studied: Myelin components including Nogo, MAG (myelin associated protein) or OMGp (oligodendrocyte myelin glycoprotein) represent strong inhibitors of successful regeneration [17-20]. In addition, inflammation, formation of a glial scar, and chondroitin sulfate proteoglycans (CSPGs) create a hostile environment, which inhibits regeneration and CNS recovery [21-23]. Notably, the small GTPase RhoA does not only play a major role in axon growth during development but also during axonal regeneration [24-26]. Several of the axon growth-inhibiting mechanisms, e.g. those triggered by axonal contact of the Nogo-Receptor complex with myelin proteins and CSPGs, converge on RhoA, which negatively regulates the microtubule cytoskeleton [24-28]. Hence, besides overcoming myelin inhibition and recreating a permissive environment to stimulate reinnervation, Rho signaling has been the focus of regeneration models such as spinal cord injury and optic nerve crush, and in the treatment of CNS injuries [29-34]. Taken together, brain damage exposes axons to a milieu that is no longer supporting axonal health and growth but consists of a plethora of inhibitory components, which thwart any attempt of recovery.

2. Overview over intrinsic regulation of axon growth and regeneration

In addition to the unfavorable environment, it has become increasingly clear that the intrinsic potential of neurons to grow axons declines with neuronal maturation. Studies of retinal

* *E-mail: stegmueller@em.mpg.de*

neurons proved that while young neurons have the potential to extend long axons, older neurons fail to efficiently grow axons [35,36]. These findings posed another significant obstacle to axonal regeneration and thus ignited the search for intrinsic regulators of axon growth, which stimulate axon growth and possibly regeneration.

Owing to its pivotal role in axon growth, the growth cone is equipped with a sophisticated and tailored set of receptors. Over the past decade, a large body of evidence has emerged in support of transcriptional control of axon growth and guidance [37,38]. Several transcription factors have been identified to control the expression of receptors at the growth cone, which results in the intrinsic programming of the neuron's response to its environment [39]. Eph/ephrin and Trk are examples of crucial guidance receptors whose developmental expression is precisely regulated by homeobox transcription factors and Runx1, respectively [40-42]. Consistent with this concept of transcriptional control of axon growth, gene expression patterns associated with axon growth are very different in neurons when they are young as compared to when they are mature [43]. Similar to neuronal maturation, CNS injury also triggers a change in gene expression that affects axon growth and regeneration [44]. For example the transcription factor Krüppel-like factor 4 (KLF4), a suppressor of axon growth, is upregulated in mature neurons [43]. Consequently, deletion of KLF4 stimulates axon growth of cultured retinal neurons and axonal regeneration after optic nerve injury [43]. Injury-induced upregulation of *Smad1* gene expression turned out to be beneficial to axon growth as the activation of Smad1 by BMP4 was found to efficiently promote axon growth in spinal cord injury [44,45]. Further examples of successful induction of regeneration after optic nerve injury include the control of the JAK/STAT pathway and mTOR (mammalian target of rapamycin) pathway-regulated gene translation by manipulating the upstream factors SOCS3 and Phosphatase and tensin homolog (PTEN), respectively [46,47]. Simultaneous deletion of SOC3 and PTEN proved to be an even more potent promoter of regeneration in an optic nerve injury model [48,49], which supports the notion that a combinatorial approach is required to stimulate efficient axonal regeneration.

Although the growth cones display a highly regulated responsiveness owing to transcriptional control of receptor expression, recent findings revealed that growth cones of peripheral and central nervous system neurons display a different morphology of the microtubules. While PNS neurons have noticeably bundled microtubules (MT) [50], MTs in CNS neurons are disheveled and as a consequence are incapable to forcefully drive axon growth [50]. By stabilizing microtubules with low doses of the microtubule-stabilizing agent Taxol, the growth cone cytoarchitecture can be reshaped and axons could even overcome myelin inhibition [50]. Taxol has also proven to stimulate axonal regeneration in models of spinal and optic nerve injury [51,52]. Collectively, in addition to transcriptional regulation of receptors at the growth cone, MT stability contributes to the growth cone's intrinsic capability to stimulate axon growth.

Beyond transcription, growing evidence indicates that post-transcriptional regulation of gene expression by micro RNAs (miR) is also instrumental in controlling axon growth. Micro RNAs have gained tremendous interest in brain development and disease as they provide a means to specifically regulate gene expression [53-55]. The first clues that miRs are regulators of axon growth and thus relevant to axonal regeneration were established in non-mammalian organisms. miR-124 for example was found to affect the responsiveness of the growth cone to Sema3A in *Xenopus* retinal ganglion cells [56]. By targeting CoREST, a master regulator of neural cell fate, miR-124 delays the expression of the Sema3A receptor Neuropilin 1 [56]. The miR lin-4 affects the responsiveness of a specific neuronal cell type in *C. elegans* to the Netrin homologue Unc-6 by targeting the transcription factor lin-14 [57]. lin-14 is part of a regulatory loop that facilitates the regeneration of young neurons by downregulation of the miR let-7 [58]. In older neurons, lin-14 fails to support axonal regeneration owing to its downregulation by miR let-7 [58]. In mammals, miR-9 has been implicated in axon growth by regulating the microtubule binding protein MAP1b [59]. Mammalian mir-124 targets the mRNA of the small GTPase RhoG and thus promotes axonal branching [60]. These studies indicate that temporal regulation and fine-tuning of axon guidance is controlled by micro RNAs. Not surprisingly, miRs also take part in axonal regeneration. Here, miR-133b targets RhoA and promotes functional recovery after spinal cord injury in adult zebrafish [61]. A recent study identified miR-138 as an intrinsic suppressor of axon growth in rodents and demonstrated loss of axonal regeneration upon overexpression of miR-138 or by downregulating its target SIRT1 in a sciatic nerve lesion model [62].

Similar to changes in gene expression, injury triggers a differential expression of miRs. Axotomy-induced expression of miR-21 promotes axon growth in adult dorsal root ganglion neurons [63]. Among the microRNAs, that are upregulated in dorsal root ganglia after nerve injury, miR-222 was identified and found to target PTEN [64], suggesting that the injury-response disengages inhibitory mechanisms to stimulate regeneration in the PNS, which could be useful in designing therapies for CNS injuries.

Further epigenetic mechanisms, which include the modifications of histones, are rather unexplored but emerging evidence indicates a role in axon growth and regeneration. The histone acetyl transferases CBP/p300 and P/CAF appear to activate axon growth-stimulating transcriptional programs [65]. In an optic nerve crush model, expression of p300, which is downregulated in mature retinal ganglion neurons, has beneficial effects on axonal regeneration [66].

Collectively, this overview demonstrates a multi-layered regulation of axon growth and guidance by transcriptional and epigenetic mechanisms (Figure 1). The control of axon growth however does not stop at the DNA or RNA level. Protein degradation by the ubiquitin proteasome system (UPS) has emerged as an important regulator of axonal growth and guidance. Before I present in greater detail the regulation of axon growth by the E3 ubiquitin ligase Cdh1-Anaphase Promoting Complex (APC), I will briefly introduce the UPS and then discuss what is known about UPS components in axon growth regulation.

3. UPS-controlled axon growth

The UPS is unequivocally one of the most complex machineries of the cell and is certainly an uncharted area in neurodevelopmental biology. E1 ubiquitin-activating and E2-ubiquitin-conjugating enzymes together with E3 ligases constitute an enzymatic cascade that facilitates the ubiquitination of target proteins [67]. UPS component are numerous as there are 2 E1, approximately 35 E2 enzymes, and more than 600 E3 ubiquitin ligases encoded by the human genome [68], the ubiquitome thus outnumbers any other system in the cell. While the E1 enzyme activates ubiquitin in an ATP-dependent manner and mediates the transfer to the E2 enzyme, substrate recognition is conferred by E3 ubiquitin ligases. In addition to the substrate, E3 ligases also recruit the E2-ubiquitin conjugate to mediate the transfer of ubiquitin to the substrate. The two largest families are the RING (really interesting new gene)- and the HECT (homologous to E6AP C-terminus)-type E3 ligases. While RING ligases act as both mono- and multimeric scaffold proteins to facilitate the transfer of ubiquitin from the E2 to the substrate, HECT ligases harbor enzymatic activity and form a covalent E3-ubiquitin intermediate before passing ubiquitin on to the substrate. Ubiquitination of proteins is extremely versatile and can result in monoubiquitination, multi-monoubiquitination or conjugation of various types of polyubiquitin chains linked through the usage of distinct lysines of ubiquitin. Such modifications encode specific responses including proteasomal degradation, DNA repair, cell signaling, receptor internalization, and sorting of membrane proteins [69-72] (Figure 2). In contrast to enzymes that promote the assembly of ubiquitin chains, deubiquitinases (DUB) facilitate the cleavage and release of ubiquitin from substrates [73], adding another layer of complexity to an already intricate system.

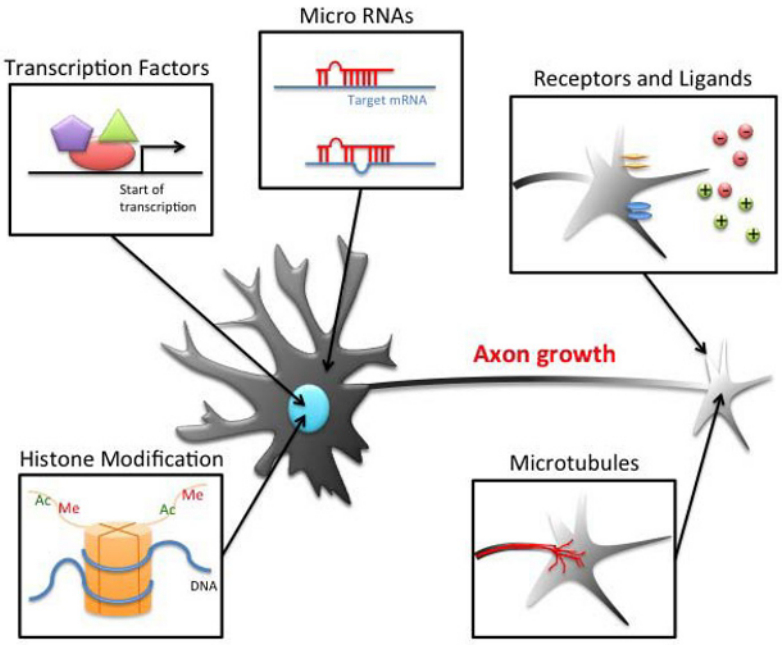

Figure 1. Extrinsic and intrinsic mechanisms of axon growth control. Aside from permissive and repulsive extrinsic factors that bind to specific receptors at the growth cone, nuclear events such as transcription and histone modification regulate axon growth. Furthermore, micro RNAs and microtubule stability affect axon growth. Ac= acetylation, Me= methylation.

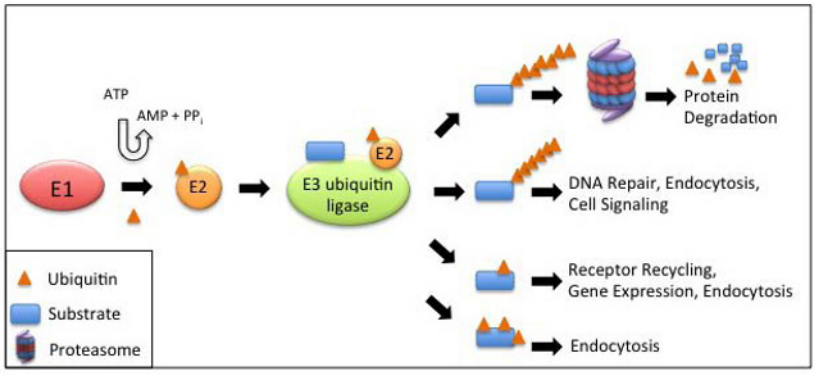

Figure 2. Schematic of E3 ubiquitin ligase-mediated ubiquitination. The E1 ubiquitin-activating enzyme activates ubiquitin in an ATP-dependent step and triggers the transfer to the E2 ubiquitin-conjugating enzyme. The E3 ubiquitin ligase recruits both the substrate protein and the E2-ubiquitin conjugate to mediate poly-, mono-, and multi-ubiquitination. Ubiquitination encodes the fate of a protein as the cellular response ranges from protein degradation to functional modification and change of localization.

Emerging evidence revealed pivotal roles of the UPS in various aspects of neurodevelopment and in neurological disorders [74-77]. A pioneering study by Campbell and colleagues underscored the importance of protein degradation in axon growth and guidance, by demonstrating that local inhibition of the proteasome at the growth cone altered its response to extrinsic cues such as Netrin-1 and Sema3A [78]. Another study that implicated an important role of the UPS in axon guidance reported that Netrin-1 induces the UPS-dependent degradation of DCC [79]. A role for the large RING E3 ligase Phr1 in axon guidance was first discovered in *C. elegans* (aka *rpm-1*), where it affects trafficking of Robo and unc receptors [80]. Also, after axotomy in *C. elegans*, Phr1-deficient motor

neurons regenerate more efficiently [81]. Rodent Phr1 was also found to be important for accurate pathfinding of motor and sensory neurons by associating with and stabilizing microtubules in axons [82]. Phr1 together with the F-box protein FBXO45 forms an alternative E3 complex [83]. FBXO45 knockout mice display severe axon tract defects similar to those observed in the Phr1 mutant mouse [82,83]. A closer look at Phr1 in the context of axonal regeneration in mammals might thus be worthwhile. Among the several hundred E3 ubiquitin ligases, RNF6 is another RING ligase, which has been identified as a regulator of axon growth [84]. By ubiquitinating LIM kinase 1 at the growth cone, RNF6 instructs the local removal of this target by the proteasome [84]. A member of the HECT-type ligases Smurf1 was also found to be important in different aspects in axon development. Phosphorylation in the axon switches Smurf1's substrate preference from Par6 to RhoA for proteasomal degradation, indicating that localized ubiquitination of substrates is crucial for acceleration of axon growth [85]. Conversely, disassembly of ubiquitination chains by DUBs appears to be as crucial as ubiquitination. The DUB USP33 interacts with and deubiquitinates Robo1 receptor thereby affecting the response of commissural neurons to Slits in midline crossing [86]. Given the large number of UPS components, future research is likely to establish a great role of the UPS in axon growth regulation and regeneration.

4. The Cdh1-APC pathway of intrinsic inhibition of axon growth

The large multisubunit E3 ligase Anaphase Promoting complex (APC) is of crucial importance for rapidly removing key proteins during mitosis by proteasomal degradation to facilitate smooth cell cycle transitions [87,88]. While the RING subunit APC11 binds the E2-ubiquitin conjugate, the subunits Cdh1 and Cdc20 are interchangeable activators of the APC. By binding to signature recognition motifs including destruction (D) box or KEN box [89,90], Cdh1 and Cdc20 recruit their substrate proteins in a very specific manner for ubiquitination.

Several years after discovering the puzzling presence of cell cycle regulator Cdh1-APC in postmitotic neurons [91], Konishi and colleagues established the E3 ubiquitin ligase Cdh1-APC as an intrinsic suppressor of axon growth regulation in a landmark publication [92]. Cdc20, which is also present in neurons, mediates the regulation of dendritic morphogenesis and synapse formation and thus exhibits complementary functions in neurons [93-96]. Several follow-up studies shed light onto an entire Cdh1-APC pathway of axon growth regulation and uncovered its relevance in axonal regeneration.

Neuronal Cdh1-APC harbors E3 ligase activity required for axon growth inhibition [91,92], indicating that controlled substrate turnover is essential in this process. In addition to its axon growth-inhibiting function, Cdh1-APC appears to control axonal patterning in the cerebellum reflected by defasciculation of the parallel fibers upon Cdh1 knockdown [92]. Strikingly, downregulation of Cdh1 in neurons by RNA interference overrides myelin inhibition of axon growth [92]. Beyond axonal development, these findings already forebode a crucial role for Cdh1-APC in axon regeneration.

4.1 Nuclear targets of Cdh1-APC

The search for substrates of Cdh1-APC in the control of axon growth led to the identification of the transcriptional regulator SnoN, as Cdh1 is enriched and crucially active in the nucleus [97]. SnoN has previously been identified as a substrate of Cdh1-APC to regulate Smad-dependent transcription in the TGFβ signaling pathway, but SnoN's role in the brain remained to be explored [98,99]. SnoN is expressed in postmitotic neurons and promotes axon growth. The degradation-resistant mutant SnoN D-Box mutant (DBM) is particularly efficient in stimulating axon growth [97]. Also, epistasis experiments revealed that SnoN acts downstream of Cdh1 and established the Cdh1-APC/SnoN pathway of axon growth. The role of SnoN in axonal development was also bolstered by *in vivo* electroporation analyses of the cerebellum, where knockdown of SnoN leads to underdeveloped parallel fibers in the cerebellar cortex [97]. In contrast to its proposed role as transcriptional repressor in the TGFβ signaling pathway in non-neural cells [100], neuronal SnoN acts as a co-activator together with the HAT p300 to promote axon growth by regulating Ccd1, a signaling scaffold protein enriched in axon terminals [101]. Consistent with this finding, p300 stimulates axonal regeneration in an optic nerve crush model [66,101]. In summary, the neuronal Cdh1-APC/SnoN pathway serves as a crucial regulator of axon growth.

SnoN is best known for its role in the TGFβ signaling pathway in tumor cells [100]. Here, the stability and thus activity of SnoN is regulated in a Smad2/3 dependent manner. TGFβ triggers the phosphorylation of Smad2/3, which results in the efficient recruitment of SnoN to Cdh1-APC followed by polyubiquitination and proteasomal degradation [98,99,102]. This finding thus raised the question if TGFβ signaling also influences the Cdh1-APC pathway of axon growth. Using an RNAi approach, Smad2/3 were shown to repress axon growth downstream of SnoN [103]. Furthermore, phosphorylated Smads and low levels of SnoN in neurons are indicative of active TGFβ signaling [103]. As a consequence of pharmacological inhibition of TGFβ signaling, SnoN becomes stabilized and axon growth is significantly enhanced [103]. Interestingly, Smad2 RNAi overcomes myelin inhibition of axon growth, which suggests a decreased sensitivity of axons to repulsive myelin components [103]. Collectively, these findings established an interaction of the Cdh1-APC and TGFβ signaling pathways and indicate a crosstalk of extrinsic cues with intrinsic pathways of axon growth (Figure 3A).

In addition to SnoN, Lasorella and colleagues identified the transcription factor Id2 as another nuclear substrate of Cdh1-APC, [104]. Id2 inhibits the E protein E47, which in turn controls the expression of axon growth-inhibiting genes such as *Nogo Receptor*, *Sema3F* and *Unc5A* [104]. Consequently, expression of E47 suppresses the axon growth-stimulating effect of both Cdh1 RNAi and the stabilized Id2 D-box mutant [104]. Collectively, these studies identified Id2 as a crucial target of Cdh1-APC, which critically regulates the expression of axon guidance receptors that alter the sensitivity of the growth cone (Figure 3A).

Apart from extrinsic factors affecting the Cdh1-APC pathway, phosphorylation of Cdh1 seems to play a significant role in axon growth regulation. Cdh1 has several highly conserved Cdk phosphorylation sites, which not only regulate binding of Cdh1 to the APC core [105] and Cdh1's localization but interestingly also the stability of Cdh1 [106]. The latter can be recapitulated by treatment of neurons with the Cdk inhibitor Roscovitine, which leads to destabilization of Cdh1 [106]. A hyperphosphorylated, stabilized mutant of Cdh1 is unable to restrict axon growth as it localizes predominantly in the cytoplasm and it fails to interact with the core complex [106]. A role for Cdk5 e.g. in axonal growth and axon formation has been previously shown [107], but Cdk5 appears to have little or no benefit in axon regeneration [108]. Phosphorylation of Cdh1 by Cdks might thus plays a greater role in development as compared to regeneration.

4.2 Cytoplasmic substrates of Cdh1-APC

Cdh1-APC is predominantly localized to the nucleus where it performs its function, however, it is also found to be active in the cytoplasm. The cytoplasmic localization together with the finding that Cdh1 RNAi overrules myelin inhibition suggested a crosstalk between the Cdh1-APC pathway and Nogo-R signaling. A key experiment revealed that the small GTPase RhoA, which acts downstream of Nogo-R, acts in the Cdh1-APC pathway [28,109]. Another important finding was that RhoA levels are reduced in growth cones upon Cdh1 knockdown [109]. Since both Cdh1 and RhoA suppress axon growth, it was unlikely that RhoA is a direct substrate of Cdh1-APC. This led to the identification of Smurf1, a downstream interactor of Cdh1 in the control of axon growth [109]. Smurf1 was previously found to target RhoA for proteasomal degradation [110]. Consequently, a degradation-resistant mutant of RhoA counteracts both Smurf1 DBM overexpression and Cdh1 RNAi-stimulated axon growth, integrating RhoA in the Cdh1-APC/Smurf1 pathway [109]. *In vivo* knockdown of Smurf1 in the developing cerebellum bolstered its role in axon growth regulation and revealed additional defects in neuronal migration [109].

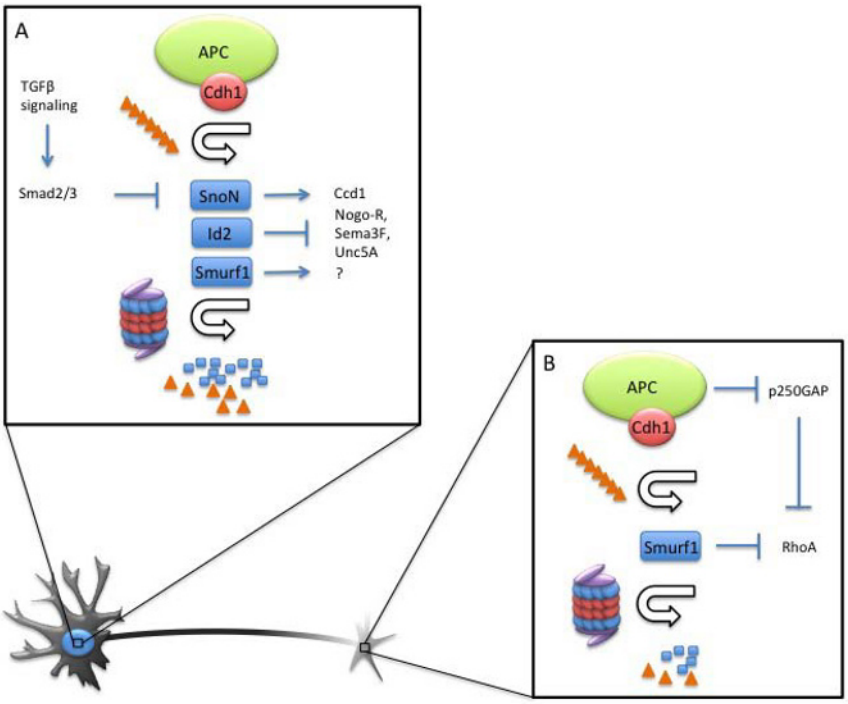

Figure 3. Cdh1-APC-regulated axon growth. (A) Cdh1-APC acts in the nucleus to ubiquitinate the transcriptional regulators SnoN and Id2 and the E3 ligase Smurf1. SnoN acts upstream of the Ccd1, which promotes axon growth. Also, SnoN is controlled by TGFβ signaling, which negatively regulates axon growth. TGFβ triggers the phosphorylation and subsequent translocation of Smad2/3 into the nucleus to regulate SnoN's stability together with Cdh1-APC. Id2 stimulates axon growth by blocking the expression of genes that encode inhibitory axon guidance factors including Nogo Receptor, Sema3F and Unc5A. (B) In the cytoplasm, Cdh1-APC acts upstream of the RhoGAP p250GAP and in addition ubiquitinates Smurf1 for proteasomal degradation. Both p250GAP and Smurf1 are negative regulators of RhoA, which has been identified as a component of the Cdh1-APC pathway.

Just like SnoN and Id2, Cdh1 binds to Smurf1 in a D-box motif-dependent manner to polyubiquitinate Smurf1, which leads to its proteasomal degradation [109]. Consequently, postnatal mouse brain with only one copy of the *Cdh1* gene revealed an increase in Smurf1 protein levels [109]. Further experiments also demonstrated a significant stimulation of axon growth by Smurf1-DBM expression in the presence of myelin, indicating that that low levels of Smurf1 contribute to myelin inhibition.

Since the activity of small GTPases such as RhoA is tightly regulated by GAPs (GTPase activating proteins) and GEFs (guanine exchange factors), a candidate screen revealed the RhoGAP p250GAP as a novel interactor of Cdh1 [111]. RhoGAP has previously been shown to act as a GAP for RhoA [112]. In addition to Smurf1, p250GAP was also identified as an axon growth-promoting RhoA regulator in the Cdh1-APC pathway [111]. These studies show that Cdh1-APC in association with p250GAP and Smurf1 controls RhoA, a key downstream component of extrinsic inhibition (Figure 3B).

4.3 The Cdh1-APC pathway as therapeutic target in spinal cord injury

As a suppressor of axon growth, the Cdh1-APC pathway and its targets were obvious candidates to test in axonal regeneration. Two recent studies using spinal cord injury models tested the potential of ID2 and SnoN, which yielded exciting results. Yu and colleagues showed that expression of the stabilized mutant Id2 (Id2 DBM) significantly prevents sensory axon dieback at the injury site [113]. Using the same injury model, Do and colleagues demonstrated that expression

of SnoN DBM led to regeneration of axonal processes into the injury site [114]. Collectively, these studies have validated that substrates of Cdh1-APC hold regenerative potential in spinal cord injuries.

5. Concluding remarks

By elucidating the Cdh1-APC pathway, it became evident that axon growth inhibition is an active, intrinsic process, highlighting the difficulties that must be overcome in order to stimulate axonal regeneration in the central nervous system. The complexity of the Cdh1-APC pathway also demonstrates that the control of axon growth inevitably includes the crosstalk between intrinsic and extrinsic pathways, leading to a highly intertwined mechanism with key inhibitors being engaged by multiples pathways. As a consequence, the release of either extrinsic or intrinsic brakes is not sufficient to promote regeneration. Future strategies to stimulate regeneration require both a permissive environment and an intrinsic boost for axons to grow and eventually make appropriate contact with the target tissue.

The Cdh1-APC pathway and its components represent potential therapeutic targets that have proven potencies to stimulate regeneration. Namely, it will be important to investigate the difference in effects of Smurf1 DBM expression and Cdh1 silencing on axonal regeneration. It will also be crucial to get further insight into mechanisms that regulate the activity of neuronal Cdh1-APC, particularly in young as compared to old neurons. A screen for kinases that phosphorylate Cdh1 and trigger its dissociation from the APC core might also be informative. In addition, small molecules that specifically inhibit Cdh1-APC activity could be useful for future therapeutic approaches. Alternatively, peptides that mimic Cdh1 substrates and block Cdh1-APC activity might be effective as well. Investigation of further crosstalk between the Cdh1-APC pathway and other axon growth and guidance factors might also help to understand if inhibition of the Cdh1-APC affects the response to extrinsic factors other than myelin. Collectively, it will be important to identify and understand the intrinsic programs underlying axon growth and guidance in neurons to eventually be able to successfully stimulate axonal regeneration in the CNS.

Acknowledgements

I thank Dr. Hiroshi Kawabe (MPI of Experimental Medicine, Göttingen) and Chaitali Mukherjee (MPI of Experimental Medicine, Göttingen) for critically reading this manuscript. This work is supported by the Max Planck Society, the Deutsche Forschungsgemeinschaft (DFG) and the CNMPB.

References

[1] Huber A.B., Kolodkin A.L., Ginty D.D., Cloutier J.F., Signaling at the growth cone: ligand-receptor complexes and the control of axon growth and guidance, Annu. Rev. Neurosci., 2003, 26, 509-563
[2] Dickson B.J., Molecular mechanisms of axon guidance, Science, 2002, 298, 1959-1964
[3] Tessier-Lavigne M., Goodman C.S., The molecular biology of axon guidance, Science, 1996, 274, 1123-1133
[4] Derijck A.A., Van Erp S., Pasterkamp R.J., Semaphorin signaling: molecular switches at the midline, Trends Cell Biol., 2010, 20, 568-576
[5] Rajasekharan S., Kennedy T.E., The netrin protein family, Genome Biol., 2009, 10, 239
[6] He Z., Wang K.C., Koprivica V., Ming G., Song H.J., Knowing how to navigate: mechanisms of semaphorin signaling in the nervous system, Sci. STKE, 2002, 2002, re1
[7] Nguyen-Ba-Charvet K.T., Chedotal A., Role of Slit proteins in the vertebrate brain, J. Physiol., Paris, 2002, 96, 91-98
[8] Dickson B.J., Gilestro G.F., Regulation of commissural axon pathfinding by slit and its Robo receptors, Annu. Rev. Cell Dev. Biol., 2006, 22, 651-675
[9] Markus A., Patel T.D., Snider W.D., Neurotrophic factors and axonal growth, Curr. Opin. Neurobiol., 2002, 12, 523-531
[10] Huang E.J., Reichardt L.F., Neurotrophins: roles in neuronal development and function, Annu. Rev. Neurosci., 2001, 24, 677-736
[11] Arévalo J.C., Chao M.V., Axonal growth: where neurotrophins meet Wnts, Curr. Opin. Cell Biol., 2005, 17, 112-115
[12] Klein R., Eph/ephrin signalling during development, Development, 2012, 139, 4105-4109
[13] Zhou F.Q., Zhong J., Snider W.D., Extracellular crosstalk: when GDNF meets N-CAM, Cell, 2003, 113, 814-815
[14] Schmid R.S., Maness P.F., L1 and NCAM adhesion molecules as signaling coreceptors in neuronal migration and process outgrowth, Curr. Opin. Neurobiol., 2008, 18, 245-250
[15] Bray G.M., Villegas-Perez M.P., Vidal-Sanz M., Carter D.A., Aguayo A.J., Neuronal and nonneuronal influences on retinal ganglion cell survival, axonal regrowth, and connectivity after axotomy, Ann. NY Acad. Sci., 1991, 633, 214-228
[16] Goldberg J.L., Barres B.A., The relationship between neuronal survival and regeneration, Annu. Rev. Neurosci., 2000, 23, 579-612
[17] Schwab M.E., Nogo and axon regeneration, Curr. Opin. Neurobiol., 2004, 14, 118-124
[18] Strittmatter S.M., Modulation of axonal regeneration in neurodegenerative disease: focus on Nogo, J. Mol. Neurosci., 2002, 19, 117-121
[19] Yiu G., He Z., Glial inhibition of CNS axon regeneration, Nat. Rev. Neurosci., 2006, 7, 617-627
[20] Fournier A.E., Strittmatter S.M., Repulsive factors and axon regeneration in the CNS, Curr. Opin. Neurobiol., 2001, 11, 89-94
[21] Busch S.A., Silver J., The role of extracellular matrix in CNS regeneration, Curr. Opin. Neurobiol., 2007, 17, 120-127
[22] David S., Zarruk J.G., Ghasemlou N., Inflammatory pathways in spinal cord injury, Int. Rev. Neurobiol., 2012, 106, 127-152
[23] Carulli D., Laabs T., Geller H.M., Fawcett J.W., Chondroitin sulfate proteoglycans in neural development and regeneration, Curr. Opin. Neurobiol., 2005, 15, 116-120

[24] Dickson B.J., Rho GTPases in growth cone guidance, Curr. Opin. Neurobiol., 2001, 11, 103-110
[25] Govek E.E., Newey S.E., Van Aelst L., The role of the Rho GTPases in neuronal development, Genes Dev., 2005, 19, 1-49
[26] Luo L., Rho GTPases in neuronal morphogenesis, Nat. Rev. Neurosci., 2000, 1, 173-180
[27] Dickendesher T.L., Baldwin K.T., Mironova Y.A., Koriyama Y., Raiker S.J., Askew K.L., et al., NgR1 and NgR3 are receptors for chondroitin sulfate proteoglycans, Nat. Neurosci., 2012, 15, 703-712
[28] Niederost B., Oertle T., Fritsche J., McKinney R.A.,Bandtlow C.E., Nogo-A and myelin-associated glycoprotein mediate neurite growth inhibition by antagonistic regulation of RhoA and Rac1, J. Neurosci., 2002, 22, 10368-10376
[29] Kopp M.A., Liebscher T., Niedeggen A., Laufer S., Brommer B., Jungehulsing G.J., et al., Small-molecule-induced Rho-inhibition: NSAIDs after spinal cord injury, Cell Tissue Res., 2012, 349, 119-132
[30] Kubo T., Yamashita T., Rho-ROCK inhibitors for the treatment of CNS injury, Recent Pat. CNS Drug Discov., 2007, 2, 173-179
[31] McKerracher L., Ferraro G.B., Fournier A.E., Rho signaling and axon regeneration, Int. Rev. Neurobiol., 2012, 105, 117-140
[32] Filbin M.T., Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS, Nat. Rev. Neurosci., 2003, 4, 703-713
[33] Benowitz L., Yin Y., Rewiring the injured CNS: lessons from the optic nerve, Exp. Neurol., 2008, 209, 389-398
[34] Giger R.J., Hollis E.R. 2nd, Tuszynski M.H., Guidance molecules in axon regeneration, Cold Spring Harb. Perspect. Biol., 2010, 2, a001867
[35] Chen D.F., Jhaveri S., Schneider G.E., Intrinsic changes in developing retinal neurons result in regenerative failure of their axons, Proc. Natl. Acad. Sci. USA, 1995, 92, 7287-7291
[36] Goldberg J.L., Klassen M.P., Hua Y.,Barres B.A., Amacrine-signaled loss of intrinsic axon growth ability by retinal ganglion cells, Science, 2002, 296, 1860-1864
[37] de la Torre-Ubieta L., Bonni A., Transcriptional regulation of neuronal polarity and morphogenesis in the mammalian brain, Neuron, 2011, 72, 22-40
[38] Butler S.J., Tear G., Getting axons onto the right path: the role of transcription factors in axon guidance, Development, 2007, 134, 439-448
[39] Polleux F., Ince-Dunn G., Ghosh A., Transcriptional regulation of vertebrate axon guidance and synapse formation, Nat. Rev. Neurosci., 2007, 8, 331-340
[40] Theil T., Frain M., Gilardi-Hebenstreit P., Flenniken A., Charnay P., Wilkinson D.G., Segmental expression of the EphA4 (Sek-1) receptor tyrosine kinase in the hindbrain is under direct transcriptional control of Krox-20, Development, 1998, 125, 443-452
[41] Kania A., Jessell T.M., Topographic motor projections in the limb imposed by LIM homeodomain protein regulation of ephrin-A: EphA interactions, Neuron, 2003, 38, 581-596
[42] Marmigere F., Montelius A., Wegner M., Groner Y., Reichardt L.F., Ernfors P., The Runx1/AML1 transcription factor selectively regulates development and survival of TrkA nociceptive sensory neurons, Nat. Neurosci., 2006, 9, 180-187
[43] Moore D.L., Blackmore M.G., Hu Y., Kaestner K.H., Bixby J.L., Lemmon V.P., et al., KLF family members regulate intrinsic axon regeneration ability, Science, 2009, 326, 298-301
[44] Zou H., Ho C., Wong K., Tessier-Lavigne M., Axotomy-induced Smad1 activation promotes axonal growth in adult sensory neurons, J. Neurosci., 2009, 29, 7116-7123
[45] Parikh P., Hao Y., Hosseinkhani M., Patil S.B., Huntley G.W., Tessier-Lavigne M., et al., Regeneration of axons in injured spinal cord by activation of bone morphogenetic protein/Smad1 signaling pathway in adult neurons, Proc. Natl. Acad. Sci. USA, 2011, 108, E99-107
[46] Park K.K., Liu K., Hu Y., Smith P.D., Wang C., Cai B., et al., Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway, Science, 2008, 322, 963-966
[47] Smith P.D., Sun F., Park K.K., Cai B., Wang C., Kuwako K., et al., SOCS3 deletion promotes optic nerve regeneration in vivo, Neuron, 2009, 64, 617-623
[48] Sun F., Park K.K., Belin S., Wang D., Lu T., Chen G., et al., Sustained axon regeneration induced by co-deletion of PTEN and SOCS3, Nature, 2011, 480, 372-375
[49] Luo X., Park K.K., Neuron-intrinsic inhibitors of axon regeneration: PTEN and SOCS3, Int. Rev. Neurobiol., 2012, 105, 141-173
[50] Erturk A., Hellal F., Enes J., Bradke F., Disorganized microtubules underlie the formation of retraction bulbs and the failure of axonal regeneration, J. Neurosci., 2007, 27, 9169-9180
[51] Hellal F., Hurtado A., Ruschel J., Flynn K.C., Laskowski C.J., Umlauf M., et al., Microtubule stabilization reduces scarring and causes axon regeneration after spinal cord injury, Science, 2011, 331, 928-931
[52] Sengottuvel V., Leibinger M., Pfreimer M., Andreadaki A., Fischer D., Taxol facilitates axon regeneration in the mature CNS, J. Neurosci., 2011, 31, 2688-2699
[53] Bhalala O.G., Srikanth M., Kessler J.A., The emerging roles of microRNAs in CNS injuries, Nat. Rev. Neurol., 2013, 9, 328-339
[54] Motti D., Bixby J.L., Lemmon V.P., MicroRNAs and neuronal development, Semin. Fetal Neonatal Med., 2012, 17, 347-352
[55] Saba R., Schratt G.M., MicroRNAs in neuronal development, function and dysfunction, Brain Res., 2010, 1338, 3-13
[56] Baudet M.L., Zivraj K.H., Abreu-Goodger C., Muldal A., Armisen J., Blenkiron C., et al., miR-124 acts through CoREST to control onset of Sema3A sensitivity in navigating retinal growth cones, Nat. Neurosci., 2012, 15, 29-38
[57] Zou Y., Chiu H., Domenger D., Chuang C.F., Chang C., The lin-4 microRNA targets the LIN-14 transcription factor to inhibit netrin-mediated axon attraction, Sci. Signal., 2012, 5, ra43
[58] Zou Y., Chiu H., Zinovyeva A., Ambros V., Chuang C.F., Chang C., Developmental decline in neuronal regeneration by the progressive change of two intrinsic timers, Science, 2013, 340, 372-376
[59] Dajas-Bailador F., Bonev B., Garcez P., Stanley P., Guillemot F., Papalopulu N., microRNA-9 regulates axon extension and branching by targeting Map1b in mouse cortical neurons, Nat. Neurosci., 2012, Epub ahead of print, doi: 10.1038/nn.3082
[60] Franke K., Otto W., Johannes S., Baumgart J., Nitsch R.,Schumacher S., miR-124-regulated RhoG reduces neuronal process complexity via

ELMO/Dock180/Rac1 and Cdc42 signalling, EMBO J., 2012, 31, 2908-2921

[61] Yu Y.M., Gibbs K.M., Davila J., Campbell N., Sung S., Todorova T.I., et al., MicroRNA miR-133b is essential for functional recovery after spinal cord injury in adult zebrafish, Eur. J. Neurosci., 2011, 33, 1587-1597

[62] Liu C.M., Wang R.Y., Saijilafu, Jiao Z.X., Zhang B.Y., Zhou F.Q., MicroRNA-138 and SIRT1 form a mutual negative feedback loop to regulate mammalian axon regeneration, Genes Dev., 2013, 27, 1473-1483

[63] Strickland I.T., Richards L., Holmes F.E., Wynick D., Uney J.B., Wong L.F., Axotomy-induced miR-21 promotes axon growth in adult dorsal root ganglion neurons, PLoS One, 2011, 6, e23423

[64] Zhou S., Shen D., Wang Y., Gong L., Tang X., Yu B., et al., microRNA-222 targeting PTEN promotes neurite outgrowth from adult dorsal root ganglion neurons following sciatic nerve transection, PLoS One, 2012, 7, e44768

[65] Gaub P., Tedeschi A., Puttagunta R., Nguyen T., Schmandke A., Di Giovanni S., HDAC inhibition promotes neuronal outgrowth and counteracts growth cone collapse through CBP/p300 and P/CAF-dependent p53 acetylation, Cell Death Differ., 2010, 17, 1392-1408

[66] Gaub P., Joshi Y., Wuttke A., Naumann U., Schnichels S., Heiduschka P., et al., The histone acetyltransferase p300 promotes intrinsic axonal regeneration, Brain, 2011, 134, 2134-2148

[67] Hershko A., Ciechanover A., The ubiquitin system, Annu. Rev. Biochem., 1998, 67, 425-479

[68] Deshaies R.J., Joazeiro C.A., RING domain E3 ubiquitin ligases, Annu. Rev. Biochem., 2009, 78, 399-434

[69] Peng J., Schwartz D., Elias J.E., Thoreen C.C., Cheng D., Marsischky G., et al., A proteomics approach to understanding protein ubiquitination, Nat. Biotechnol., 2003, 21, 921-926

[70] Lim K.L., Lim G.G., K63-linked ubiquitination and neurodegeneration, Neurobiol. Dis., 2011, 43, 9-16

[71] Ikeda F., Dikic I., Atypical ubiquitin chains: new molecular signals. 'Protein Modifications: Beyond the Usual Suspects' review series, EMBO Rep., 2008, 9, 536-542

[72] Welchman R.L., Gordon C., Mayer R.J., Ubiquitin and ubiquitin-like proteins as multifunctional signals, Nat. Rev. Mol. Cell Biol., 2005, 6, 599-609

[73] Komander D., Clague M.J., Urbe S., Breaking the chains: structure and function of the deubiquitinases, Nat. Rev. Mol. Cell Biol., 2009, 10, 550-563

[74] Yi J.J., Ehlers M.D., Emerging roles for ubiquitin and protein degradation in neuronal function, Pharmacol. Rev., 2007, 59, 14-39

[75] Kawabe H., Brose N., The role of ubiquitylation in nerve cell development, Nat. Rev. Neurosci., 2011, 12, 251-268

[76] Stegmuller J., Bonni A., Destroy to create: E3 ubiquitin ligases in neurogenesis, F1000 Biol. Rep., 2010, 2, 38

[77] Ciechanover A., Brundin P., The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg, Neuron, 2003, 40, 427-446

[78] Campbell D.S., Holt C.E., Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation, Neuron, 2001, 32, 1013-1026

[79] Kim T.H., Lee H.K., Seo I.A., Bae H.R., Suh D.J., Wu J., et al., Netrin induces down-regulation of its receptor, Deleted in Colorectal Cancer, through the ubiquitin-proteasome pathway in the embryonic cortical neuron, J. Neurochem., 2005, 95, 1-8

[80] Li H., Kulkarni G., Wadsworth W.G., RPM-1, a Caenorhabditis elegans protein that functions in presynaptic differentiation, negatively regulates axon outgrowth by controlling SAX-3/robo and UNC-5/UNC5 activity, J. Neurosci., 2008, 28, 3595-3603

[81] Hammarlund M., Nix P., Hauth L., Jorgensen E.M., Bastiani M., Axon regeneration requires a conserved MAP kinase pathway, Science, 2009, 323, 802-806

[82] Lewcock J.W., Genoud N., Lettieri K., Pfaff S.L., The ubiquitin ligase Phr1 regulates axon outgrowth through modulation of microtubule dynamics, Neuron, 2007, 56, 604-620

[83] Saiga T., Fukuda T., Matsumoto M., Tada H., Okano H.J., Okano H., et al., Fbxo45 forms a novel ubiquitin ligase complex and is required for neuronal development, Mol. Cell. Biol., 2009, 29, 3529-3543

[84] Tursun B., Schluter A., Peters M.A., Viehweger B., Ostendorff H.P., Soosairajah J., et al., The ubiquitin ligase Rnf6 regulates local LIM kinase 1 levels in axonal growth cones, Genes Dev., 2005, 19, 2307-2319

[85] Cheng P.L., Lu H., Shelly M., Gao H., Poo M.M., Phosphorylation of E3 ligase Smurf1 switches its substrate preference in support of axon development, Neuron, 2011, 69, 231-243

[86] Yuasa-Kawada J., Kinoshita-Kawada M., Wu G., Rao Y.,Wu J.Y., Midline crossing and Slit responsiveness of commissural axons require USP33, Nat. Neurosci., 2009, 12, 1087-1089

[87] Peters J.M., The anaphase promoting complex/cyclosome: a machine designed to destroy, Nat. Rev. Mol. Cell Biol., 2006, 7, 644-656

[88] Harper J.W., Burton J.L., Solomon M.J., The anaphase-promoting complex: it's not just for mitosis any more, Genes Dev., 2002, 16, 2179-2206

[89] Burton J.L., Solomon M.J., D box and KEN box motifs in budding yeast Hsl1p are required for APC-mediated degradation and direct binding to Cdc20p and Cdh1p, Genes Dev., 2001, 15, 2381-2395

[90] Pfleger C.M., Kirschner M.W., The KEN box: an APC recognition signal distinct from the D box targeted by Cdh1, Genes Dev., 2000, 14, 655-665

[91] Gieffers C., Peters B.H., Kramer E.R., Dotti C.G., Peters J.M., Expression of the CDH1-associated form of the anaphase-promoting complex in postmitotic neurons, Proc. Natl. Acad. Sci. USA, 1999, 96, 11317-11322

[92] Konishi Y., Stegmuller J., Matsuda T., Bonni S., Bonni A., Cdh1-APC controls axonal growth and patterning in the mammalian brain, Science, 2004, 303, 1026-1030

[93] Kim A.H., Puram S.V., Bilimoria P.M., Ikeuchi Y., Keough S., Wong M., et al., A centrosomal Cdc20-APC pathway controls dendrite morphogenesis in postmitotic neurons, Cell, 2009, 136, 322-336

[94] Yang Y., Kim A.H., Yamada T., Wu B., Bilimoria P.M., Ikeuchi Y., et al., A Cdc20-APC ubiquitin signaling pathway regulates presynaptic differentiation, Science, 2009, 326, 575-578

[95] Puram S.V., Kim A.H., Ikeuchi Y., Wilson-Grady J.T., Merdes A., Gygi S.P., et al., A CaMKIIbeta signaling pathway at the centrosome regulates dendrite patterning in the brain, Nat. Neurosci., 2011, 14, 973-983

[96] Yang Y., Kim A.H., Bonni A., The dynamic ubiquitin ligase duo: Cdh1-APC and Cdc20-APC regulate neuronal morphogenesis and connectivity, Curr. Opin. Neurobiol., 2010, 20, 92-99

[97] Stegmuller J., Konishi Y., Huynh M.A., Yuan Z., Dibacco S., Bonni A., Cell-intrinsic regulation of axonal morphogenesis by the Cdh1-APC target SnoN, Neuron, 2006, 50, 389-400

[98] Stroschein S.L., Bonni S., Wrana J.L., Luo K., Smad3 recruits the anaphase-promoting complex for ubiquitination and degradation of SnoN, Genes Dev., 2001, 15, 2822-2836

[99] Wan Y., Liu X., Kirschner M.W., The anaphase-promoting complex mediates TGF-beta signaling by targeting SnoN for destruction, Mol. Cell, 2001, 8, 1027-1039

[100] Liu X., Sun Y., Weinberg R.A., Lodish H.F., Ski/Sno and TGF-beta signaling, Cytokine Growth Factor Rev., 2001, 12, 1-8

[101] Ikeuchi Y., Stegmuller J., Netherton S., Huynh M.A., Masu M., Frank D., et al., A SnoN-Ccd1 pathway promotes axonal morphogenesis in the mammalian brain, J. Neurosci., 2009, 29, 4312-4321

[102] Bonni S., Wang H.R., Causing C.G., Kavsak P., Stroschein S.L., Luo K., et al., TGF-beta induces assembly of a Smad2-Smurf2 ubiquitin ligase complex that targets SnoN for degradation, Nat. Cell Biol., 2001, 3, 587-595

[103] Stegmuller J., Huynh M.A., Yuan Z., Konishi Y., Bonni A., TGFbeta-Smad2 signaling regulates the Cdh1-APC/SnoN pathway of axonal morphogenesis, J. Neurosci., 2008, 28, 1961-1969

[104] Lasorella A., Stegmuller J., Guardavaccaro D., Liu G., Carro M.S., Rothschild G., et al., Degradation of Id2 by the anaphase-promoting complex couples cell cycle exit and axonal growth, Nature, 2006, 442, 471-474

[105] Zachariae W., Schwab M., Nasmyth K., Seufert W., Control of cyclin ubiquitination by CDK-regulated binding of Hct1 to the anaphase promoting complex, Science, 1998, 282, 1721-1724

[106] Huynh M.A., Stegmuller J., Litterman N.,Bonni A., Regulation of Cdh1-APC function in axon growth by Cdh1 phosphorylation, J. Neurosci., 2009, 29, 4322-4327

[107] Su S.C., Tsai L.H., Cyclin-dependent kinases in brain development and disease, Annu. Rev. Cell Dev. Biology, 2011, 27, 465-491

[108] Bermel C., Tonges L., Planchamp V., Gillardon F., Weishaupt J.H., Dietz G.P., et al., Combined inhibition of Cdk5 and ROCK additively increase cell survival, but not the regenerative response in regenerating retinal ganglion cells, Mol. Cell. Neurosci., 2009, 42, 427-437

[109] Kannan M., Lee S.J., Schwedhelm-Domeyer N., Stegmuller J., The E3 ligase Cdh1-anaphase promoting complex operates upstream of the E3 ligase Smurf1 in the control of axon growth, Development, 2012, 139, 3600-3612

[110] Wang H.R., Zhang Y., Ozdamar B., Ogunjimi A.A., Alexandrova E., Thomsen G.H., et al., Regulation of cell polarity and protrusion formation by targeting RhoA for degradation, Science, 2003, 302, 1775-1779

[111] Kannan M., Lee S.J., Schwedhelm-Domeyer N., Nakazawa T., Stegmuller J., p250GAP is a novel player in the Cdh1-APC/Smurf1 pathway of axon growth regulation, PLoS One, 2012, 7, e50735

[112] Nakazawa T., Watabe A.M., Tezuka T., Yoshida Y., Yokoyama K., Umemori H., et al., p250GAP, a novel brain-enriched GTPase-activating protein for Rho family GTPases, is involved in the N-methyl-d-aspartate receptor signaling, Mol. Biol. Cell, 2003, 14, 2921-2934

[113] Yu P., Zhang Y.P., Shields L.B., Zheng Y., Hu X., Hill R., et al., Inhibitor of DNA binding 2 promotes sensory axonal growth after SCI, Exp. Neurol., 2011, 231, 38-44

[114] Do J.L., Bonni A., Tuszynski M.H., SnoN facilitates axonal regeneration after spinal cord injury, PLoS ONE, 2013, 8, e71906