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HDAC signaling in neuronal development and axon regeneration

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Summary

The development and repair of the nervous system requires the coordinated expression of a large number of specific genes. Epigenetic modifications of histones represent an essential principle by which neurons regulate transcriptional responses and adapt to environmental cues. The post-translational modification of histones by chromatin-modifying enzymes histone acetyltransferases (HATs) and histone deacetylases (HDACs) shapes chromatin to adjust transcriptional profiles during neuronal development. Recent observations also point to a critical role for histone acetylation and deacetylation in the response of neurons to injury. While HDACs are mostly known to attenuate transcription through their deacetylase activity and their interaction with co-repressors, these enzymes are also found in the cytoplasm where they display transcription-independent activities by regulating the function of diverse proteins. Here we discuss recent studies that go beyond the traditional use of HDAC inhibitors and have begun to dissect the roles of individual HDAC isoforms in neuronal development and repair after injury.

Introduction

Epigenetic changes refer to the modifications of chromatin, including histone and DNA that contribute to regulate the transcriptional response. One of the best-characterized epigenetic modifications is lysine acetylation of histones, which is mediated by two groups of enzymes, HATs and HDACs. The C-terminal tails of histones are normally positively charged to condense the DNA structure, thereby repressing gene expression. Whereas acetylation mediated by HATs opens the chromatin and allows transcription, HDACs have a general repressive effect on gene expression, restricting transcription factor access to regulatory regions [1,2]. HDACs can also have direct roles in transcription by deacetylating and regulating transcription factors or interacting with co-repressors [1,2]. In addition to these HDACs' roles in regulating gene transcription, the acetylation and deacetylation of lysine residues is emerging as a mechanism analogous to phosphorylation to control the function,

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activity and stability of various proteins beyond histones. HDACs thus have the potential to be involved in multiple aspects of neuronal development and repair.

Mammalian HDACs are organized into a super-family of four classes based on domain organization (Figure 1) [1,3]. Class I members (HDAC1, -2, -3 and -8) are ubiquitously expressed with predominant nuclear localization. Class II and class IV members are more selectively expressed and can undergo stimulus-dependent shuttling between nucleus and cytoplasm. Class II members can be further subdivided into two classes. Class IIa members (HDAC4, -5, -7, and -9) contain, in addition to the HDAC domain, an extended N-terminal regulatory domain [4]. Class IIb is represented by HDAC6 and HDAC10, which possess distinct C-terminal domains. HDAC6 expression is mostly cytoplasmic and was the first identified tubulin deacetylase [5]. HDAC11, the only Class IV HDAC, has characteristics of both class I and class II HDACs, although little is known of its function. The sirtuins family of deacetylases represents class III but these are functionally unrelated to HDACs: their deacetylase activity depends on the co-factor NAD⁺, whereas other HDACs are known as zinc-dependent histone deacetylases. All HDACs classes display high structural conservation of their active sites. A catalytic tyrosine is conserved in all HDACs except for vertebrate class IIa enzymes where it is replaced by histidine [4], strongly reducing their catalytic activity. It has been suggested that vertebrate class IIa HDACs may have evolved to efficiently process restricted sets of specific substrates [4].

Although much has been learned through the use of HDAC inhibitors, recent studies are beginning to reveal the biological function of each of these individual enzymes. In this review we discuss recent findings on the role of HDACs in regulating gene expression during neuronal development and repair following injury, as well as studies that depart from this traditional focus and reveal new cytoplasmic functions for HDACs in neurons. The often divergent roles of specific HDAC family members are discussed. The role of HDACs in synaptic plasticity, brain function and neurodegeneration has been reviewed elsewhere [6–10] and will not be discussed here.

HDAC signaling in neuronal development

Neurogenesis in the developing brain

Neurogenesis is the process of generating new neurons from progenitor cells during development and throughout adulthood, which includes cell proliferation, migration, and differentiation. The differentiation of neuronal progenitor cells to neurons requires the transduction of signals to the genome to de-repress neuron-specific genes. It has been known for several years that HDAC inhibitors induce differentiation of both embryonic and adult cortical neuronal progenitor cells to neurons specifically [11–15]. The regional and cell-type specific expression of individual HDACs in the rat central nervous system (CNS) [16] suggests that each enzymes may play specific roles in neuronal development and emphasizes the need for more specific approaches to study the function of each HDAC isoform.

To study the role of HDAC1 in the progression of neural precursors to neurons during brain development, Montgomery et al. used a conditional deletion approach to avoid early

lethality associated with global deletion. Using mice expressing Cre recombinase under the control of the human glial fibrillary acidic protein (GFAP) promoter, which is widely expressed in the central nervous system, including neural stem cells, conditional deletion of both HDAC1 and HDAC2 revealed a redundant role for these enzymes in the progression of neuronal precursors to neurons during brain development [17] (Figure 2). Neuronal progenitors lacking HDAC1 and HDAC2 are unable to differentiate into mature neurons and undergo cell death, resulting in defects in superficial cortical layering and death by postnatal day seven [17]. In zebrafish, HDAC1 represses Notch target genes expression during neurogenesis and favors the generation of motor neurons in response to hedgehog signaling [18], as well as to promote retinal neurogenesis [19] (Figure 2). Although HDACs are generally considered to repress gene expression, HDAC1 can also act as a positive regulator of gene transcription during development of the zebrafish CNS [20] (Figure 2). The HDAC1-regulated genes encode DNA-binding transcription factors that are implicated in promoting neuronal specification and CNS patterning, including the proneural bHLH proteins *Ascl1*, as well as *Neurod* and *Neurod4* [20]. Chromatin immunoprecipitation experiments reveal that HDAC1 is specifically and stably associated with the promoter region of a subset of actively transcribed genes, including *Ascl1*, thereby promoting the expression of a neurogenic program in zebrafish embryos [20].

HDAC3 is also a candidate in the control of gene expression during development and maintenance of the neural stem cell state. In rat cortical neurons HDAC3 maintains the nuclear localization of the transcriptional repressor Silencing Mediator of Retinoic acid and Thyroid hormone receptors (SMRT), which controls neuronal responsiveness of several transcription factors [21]. Knockdown of HDAC3 or inhibition of HDAC3 with valproate or apicidin triggers SMRT nuclear export [21] (Figure 2). Given that SMRT can regulate neurogenic pathways [21] future studies may reveal whether HDAC3 also controls neurogenesis.

These studies clearly point to a role for class I members HDACs in developmental neurogenesis through the control of gene expression. Other HDAC classes are likely involved in regulating this important developmental stage. Indeed, HDAC10 has been shown to interact with and maintain Pax3, a key transcription factor in stem cell maintenance and neurogenesis, in a deacetylated state [22] (Figure 2). Pax3 acetylation regulates its function by favoring *Neurog2* transcription over *Hes1* and thereby promoting neuron differentiation [23,24]. Interestingly, Sirt1 was also proposed to be a key component in regulating Pax3 acetylation levels [24].

Adult neurogenesis

In adult rodents, neurogenesis occurs mostly in the subventricular zone of the lateral ventricle and the subgranular zone of the hippocampal dentate gyrus. The distinct expression pattern of HDACs in the adult rat brain [16] suggests that HDAC isoforms expression may also be developmentally regulated. Hence, does adult neurogenesis employ the same battery of epigenetic mechanisms as developmental neurogenesis? Studies on the role of HDAC2 would argue that they do not. Indeed, the catalytic function of HDAC2 is required in adult brain, but not during embryonic neurogenesis [25] when HDAC1 can compensate for the

lack of HDAC2 [17]. In mice lacking the catalytic activity of HDAC2 or in mice with conditional deletion of HDAC2, neurons derived from adult neurogenesis die at a specific maturation stage, whereas brain development and adult stem cell fate are normal [25]. This study suggests that HDAC2 is critically required to silence progenitor transcripts during neuronal differentiation of adult generated neurons [25]. In agreement with specific role for HDACs in adult neurogenesis, different subsets of neural stem cells and progenitors switch expression from HDAC1 to HDAC2 as they commit to a neurogenic lineage in the subventricular zone and the dentate gyrus [26], with HDAC1 enriched in neural stem cells and HDAC2 in neuroblasts. This study further demonstrates that HDAC inhibitors trichostatin (TSA) and valproate perturb postnatal neurogenesis *in vivo* and profoundly inhibit the production and expansion of neurospheres *in vitro* [26]. Together, these studies highlight the pleiotropic activities of HDACs in brain development.

The class II HDAC5 has also been implicated in neuronal differentiation. In a screen for small molecules that induce a cardiogenic phenotype, Schneider et al. found a molecule, isoxazole, that triggers robust neuronal differentiation in adult neural stem cells via a mechanism involving calmodulin kinase-dependent nuclear export of HDAC5 and MEF2-dependent gene expression [27] (Figure 2). Given that nuclear export of class II HDACs can be triggered in response to extracellular stimuli, other class II HDACs may be involved in activity-dependent adult neurogenesis.

In summary, both embryonic and adult neurogenesis engage HDAC family members to control gene expression and cell fate. The role of HDACs in directly controlling the activity of transcription factors is becoming apparent. Importantly, these studies emphasize that the clinical use of HDAC inhibitors to treat diseases ranging from schizophrenia to neurodegeneration could impair ongoing brain development and should be carefully considered.

Neurite formation, dendrite and axon growth

Breaking the symmetry of neurons to form axon and dendrites depends on cytoskeletal rearrangements [28]. Several HDAC family members deacetylate components of the microtubule and actin cytoskeleton, tubulin [5] and cortactin [29], respectively, and can impact neurite formation. Indeed, HDAC6 control microtubule dynamics to optimize neurite formation through septins, which provide a physical scaffold for HDAC6 to achieve efficient microtubule deacetylation [30]. Acute knockdown or knockout of septin7 in cerebrocortical neurons results in excessive stabilization of microtubules which inhibits their growth *in vitro* and impairs axon growth *in vivo* [30].

Compared to the mechanisms regulating axon development, relatively little is known about the mechanisms regulating the patterning of dendrites, which is essential for the establishment of neuronal connectivity. Several studies revealed that HDACs play an important role in dendrite development. Knock down of HDAC6 in cerebellar and hippocampal neurons decreases dendrite length and dendritic branching, suggesting that HDAC6 has a generalized dendritogenic function [31]. Interestingly, this HDAC6 function is independent of its deacetylase activity and rather requires a direct interaction between HDAC6 ZnF domain (Figure 1) and Cdc20-APC, which stimulates Cdc20-APC activity via

stabilization of Cdc20 polyubiquitination [31]. The observations that Aurora A kinase activates HDAC6 at the basal body to disassemble ciliary microtubules [32] and that Aurora A pathway regulates microtubule remodeling during neurite extension [33] suggest that HDAC6 could also regulate dendrite development via the Aurora kinase.

Using the *Drosophila* olfactory system, Tea et al. found a role for HDAC1 and HDAC2 in dendrite wiring. Rpd3, which is homologous to mammalian HDAC1 and HDAC2, but not, HDAC3, the only other class I HDAC in the fly, regulates dendrite targeting largely through the action of the transcription factor Pros [34]. In rat cortical neurons, HDAC2 regulates dendrite development in response to neurotrophic factors. Brain derived neurotrophic factor (BDNF) triggers nitric oxide synthesis and S-nitrosylation of HDAC2, resulting in dendritic growth and branching [35] (Figure 2). S-nitrosylation of HDAC2 does not affect its deacetylase activity, but induces its release from chromatin, which in turn increases acetylation of histones surrounding neurotrophin-dependent gene promoters [35]. Recent studies also indicate that the herbal derivative genipin increases S-nitrosylation of HDAC2 and has both neuroprotective and neurite outgrowth activities in retinal ganglion cells [36]. S-nitrosylation of HDAC2 occurs on cysteine residues (Cys1262 and Cys1274) that are conserved in HDAC1 and HDAC3, suggesting that nitrosylation might also control their function. HDAC9 activity has also been implicated in the regulation of dendrite development. In cultured mouse cortical neurons, increase of spontaneous firing activity causes HDAC9 translocation from nucleus to cytoplasm during postnatal development [37]. Introduction of a mutant HDAC9 that is trapped in the nucleus decreases the length of dendritic branches, whereas knockdown of HDAC9 promoted dendritic growth [37]. Although these studies point to HDAC9 functioning as a transcriptional repressor, whether HDAC9 also plays a role outside the nucleus to regulate dendrite growth remains to be determined.

Axon Regeneration: Axonal roles of HDACs

Injured axons need to transform their damaged axon tip into a new growth cone to initiate regeneration, a process that results in part from the reorganization and dynamic properties of the local microtubule cytoskeleton [38,39]. Studies in our laboratory revealed that HDAC5 controls microtubule dynamics in injured dorsal root ganglia (DRG) neurons [40]. Injury-induced calcium influx activates HDAC5 to decrease the level of acetylated tubulin, known to correlate with stable microtubules, in a gradient-like fashion from the site of injury. This pathway, which is not shared by axons in the CNS, locally decreases microtubule stability needed for axon regeneration [40] (Figure 3). It is worth noting that heightening microtubule stabilization with taxol treatment increases axon regeneration in the CNS [38,41,42], and induces axon formation in cultured neurons [43]. One explanation could be that in the injured CNS, stabilizing microtubules fortifies the microtubule array at the tip of the axon to generate more powerful driving force through the inhibitory environment, whereas in the PNS, local and controlled destabilization of the microtubule network optimizes growth cone dynamics.

Whereas HDAC5 is involved in injury-induced tubulin deacetylation and axon regeneration in DRG neurons, HDAC6 does not play a major role in this process [40]. In contrast, when

DRG neurons are cultured in the presence of myelin-associated glycoprotein to mimic the inhibitory environment of the CNS, inhibiting HDAC6 enhances tubulin acetylation levels and promotes axon regeneration [44] (Figure 3). This is intriguing because inhibition of HDAC6 in hippocampal neurons increases tubulin acetylation, but does not affect axon growth properties [43]. Similarly, mice lacking HDAC6 display hyperacetylated microtubules but are otherwise viable with no apparent developmental defects [45]. Interestingly, HDAC6, but no other class II HDACs, is robustly up-regulated in cultured cortical neurons undergoing oxidative stress or neurite growth inhibition [44]. These studies suggest that HDAC6 may play a specific role following axon injury or in other pathological conditions in the CNS, but not during normal neuronal development or in the peripheral nervous system (PNS).

The apparent opposite roles of HDAC5 and HDAC6 may also result from different substrate specificity beyond tubulin. Indeed, unlike HDAC5, HDAC6 has two deacetylase domains (Figure 1), which are required for its interaction with the F-actin-binding protein cortactin [29]. Cortactin deacetylation increases its affinity with F-actin and enhances actin-dependent cell motility [29]. The role of cortactin in growth cone morphology and spreading in hippocampal neurons [46] suggests that HDAC6 regulation of axon regeneration may involve actin-based mechanisms. HDAC6 also possesses a ZnF-UBP C-terminal domain that mediates its interaction with the Cdc20-APC complex to regulate dendrite development [31] and possibly axon growth [47].

The upstream signals regulating HDAC5 and HDAC6 activities are also likely to differ. Whereas HDAC5 activity in injured DRG neurons depends on calcium activated PKC μ [40], whether and how HDAC6 activity towards tubulin or other substrates is regulated to control growth on inhibitory substrates remains elusive. The extracellular signal-regulated kinase (ERK) may represent a candidate regulator of HDAC6. ERK-mediated phosphorylation of HDAC6 promotes migration of embryonic fibroblasts via deacetylation of α -tubulin [48] and axon injury was shown to activate ERK [49]. Another candidate regulator of HDAC6 involves the scaffolding protein sequestosome 1/p62, which was shown to negatively regulate HDAC6 deacetylase activity [50]. In the absence of p62, HDAC6 is hyper-activated and deacetylation of α -tubulin and cortactin occurs [50]. Finally, the NIMA-related kinases Nek3 was also shown to control HDAC6-dependent deacetylation of microtubules in neurons [51]. Future studies will elucidate the signaling pathways controlling HDAC6 activity, and define the protein ensembles regulated by HDAC5 and HDAC6 in axons. These findings may shed light on the apparent opposite functions these enzymes play in axon regeneration.

Axon Regeneration: Nuclear roles of HDACs

Studies based on HDAC inhibitors have suggested a role for histone acetylation, or the lack thereof, in the poor regenerative ability of CNS neurons. HDAC inhibition was shown to improve locomotion *in vivo* after spinal cord injury [52], improve regeneration in injured retinal ganglion cells [53], partially overcome neurite outgrowth of cerebellar granule neurons cultured on inhibitory substrates [54] and increase regeneration of ascending sensory axons following spinal cord injury [55]. These studies suggest that if provided with

the appropriate epigenetic landscape, CNS neurons can express the genes required to stimulate axon regeneration. In contrast, peripheral neurons possess the intrinsic ability to increase histone acetylation in response to injury. Our laboratory revealed a calcium-initiated signaling mechanism in DRG neurons that elicits HDAC5 nuclear export via PKC α activation, thereby increasing the level of acetylated histone H3 and accelerating axon regeneration via expression of pro-regenerative genes [56] (Figure 3). In addition to histone H3 acetylation, Finelli et al. showed that injury to the peripherally projecting DRG axons induces histone H4 acetylation, which also correlates with expression of regeneration-associated genes [55] (Figure 3). Whether acetylation of histone H4 in response to injury also depends on the calcium-PKC-HDAC5 pathway remains to be determined.

CNS neurons appear unable to respond to incoming injury signals and elicit the appropriate epigenetic response. Indeed, key elements of the pathway leading to HDAC5 nuclear export in peripheral neurons fail to be activated in retinal ganglion cells (RGCs) [56], suggesting that failure to export HDAC5 in these cells limits their regenerative ability. Similarly, injury to the sensory axon ascending into the spinal cord fails to increase histone H4 acetylation in DRG neurons [55]. Furthermore, whereas HDAC3 is exported to the cytoplasm in injured DRG [56], HDAC3 accumulates in the nucleus of injured RGCs [57], which may contribute to decrease histone acetylation and repress gene transcription. Interestingly, in an HDAC5 knockout mouse, HDAC3 localizes mostly to the nucleus of injured DRG neurons, rather than to the cytoplasm in the wild type [56]. It appears that in response to injury, HDAC5 knockout DRG neurons behave similarly to RGCs, with respect to HDAC3 localization. Could HDAC5 be required for injury-induced HDAC3 nuclear export? The known interaction between HDAC5 and HDAC3 [58] suggests that injury-induced HDAC5 nuclear export may be coupled to HDAC3 localization.

Which genes are associated with increased histone acetylation to control axon regeneration? Using chromatin immunoprecipitation approaches, inhibition of HDACs with TSA in cerebellar neurons was found to induce expression of the well-known axonal growth associated marker GAP-43, as well as the HATs, CBP, p300 and P/CAF [54]. With a similar approach, Finelli et al. found that in DRG neurons, injury-induced histone H4 acetylation is associated with promoter regions of regeneration-associated genes [55], including *Smad1*, *Atf3*, *Sprr1a*. Interestingly, inhibition of HDAC1/3 with MS-275 treatment did not increase the acetylated levels of histone H4 at the promoters of *Smad1* or *Atf3* [55]. By creating a mutant HDAC5 that is trapped in the nucleus (HDAC5nuc), our laboratory compared the effects of injury in wild type and HDAC5nuc-expressing DRG neurons and identified a number of genes that are activated by injury in manner that depends on HDAC5 nuclear export, including several transcription factors implicated in the regenerative response, such as jun, fos, KLFs, Gadd45a [56]. A large proportion of the HDAC5-dependent genes have prominent roles in the regulation of transcription [56], suggesting that HDAC5 controls an early response phase to injury. These studies emphasize that multiple regulatory mechanisms control the epigenetic landscape of injured neurons. The activity of multiple HDACs likely converges to regulate a favorable, pro-regenerative epigenetic landscape around regeneration-associated gene promoters. Furthermore, HDACs can deacetylate transcription factors in addition to histones and inhibit transcription via interaction with co-

repressors, providing an additional level of transcriptional control in injured neurons. Future studies are needed to explore how the nuclear localization and activity of multiple HDACs is regulated in response to injury, which may in part underlies the epigenetic regenerative ability of central and peripheral neurons.

Sirtuins in neuronal development and regeneration

The sirtuins family of deacetylases also influences neuronal development and repair. During neurogenesis, the Notch pathway represses neuron-specific genes [59] and hence impairs the transition of neuronal progenitors to neurons. The oncogene BCL6 allows epigenetic silencing of Hes5 and hence neuronal differentiation despite active Notch signaling by recruiting Sirt1 and excluding the co-activator Mastermind-like 1 at *Hes* promoter region [60].

During neurite formation, Sirt2 controls the levels of tubulin acetylation. Sirt2 overexpression reduces the levels of acetylated, stable, microtubules and strongly impairs neurite formation in cultured hippocampal neurons [61]. In contrast to Sirt2, up-regulation of Sirt1 promotes the formation and elongation of axons, whereas inhibition of Sirt1 retards axonal development in hippocampal neurons [62]. Sirt1's effect in axon development relies on its deacetylase activity towards Akt and thereby activating the Akt/GSK3 pathway [62].

Sirt1 also plays a role in injured axons to stimulate axon regeneration. Sirt1 is the target of miR-138 in DRG neurons and down-regulation of miR-138 after axon injury correlates with an increase in Sirt1 levels, which is required for axon regeneration [63]. The precise mechanism by which Sirt1 regulates axon regeneration awaits further clarifications, but given the role of Sirt1 in deacetylating cortactin [64], Sirt1 may increase actin-dependent motility [29].

Conclusion

The complexity of HDACs' functions during development and in mature neurons is rapidly coming to light. It is clear that HDAC family members have very specific functions and that studies using HDAC inhibitors need careful interpretation. It is also clear that different roles of HDACs are isoform-specific, rather than class-specific. Furthermore, a given HDAC can also play different roles depending on the developmental stage. The ability of HDACs to influences other histone modifications will also have to be considered, since HDACs regulate other histone post-translational modifications, i.e. by interacting with DNA methyltransferases [65]. Given that epigenetic regulation affects globally, yet specifically, an ensemble of genes, targeting specific HDAC pathways represent an ideal strategy to promote neural repair. Therefore, identifying the HDAC-dependent epigenetic signaling mechanisms involved in neuronal development and repair will be critical to our understanding and approaches to the treatment of injuries in the nervous system. The emerging functions of HDACs as regulatory enzymes in the cytoplasm will also need to be carefully investigated and may lead to new strategies to improve axon growth and repair following damage.

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Highlights

1. HDACs function in the differentiation of neuronal progenitor cells to neurons during development as well as in the adult brain.
2. HDAC family members have very specific functions: different roles of HDACs are isoform-specific rather than class-specific.
3. In injured neurons, HDACs function locally at the site of injury to control cytoskeleton dynamics and in the nucleus to activate a pro-regenerative program.
4. HDAC family members emerge as regulatory enzymes in the cytoplasm that can also function independently of their deacetylase activity.

Class	Domain organization	Localization in neurons	Lethality In KO	Nervous system phenotypes	Ref.
I	HDAC1	Nucleus > Axon	E9.5-10.5	Brain structure abnormalities	Montgomery et al., PNAS 2009 Kim et al., Nat Neurosci 2010
	HDAC2	Nucleus	P1		
	HDAC3	Nucleus > Axon	E9.5	Enhanced long-term memory (focal homozygous deletion)	Susan et al., J Neurosci 2011 Soriano et al., PLOS One 2011 Cho et al., Cell 2013
	HDAC8	Nucleus > Cytoplasm	P1	Intracranial hemorrhage	Haberland et al., Genes Dev 2009
IIa	HDAC4	Nucleus > Axon	P7-P14	Exencephaly, Learning/LTP defect	Chawla et al., J Neurochem 2003 Vega et al., Cell 2004
	HDAC5	Nucleus > Axon	Viable	Delayed axon regeneration	Kim et al., J Neurosci 2012 Chang et al., Mol Cell Biol 2003 Cho and Cavalli, EMBO J 2012 Cho et al., Cell 2013
	HDAC7	Nucleus > Cytoplasm*	E11	-	Fischle et al., J Biol Chem 2001 Chang et al., Cell 2006
	HDAC9	Nucleus > Cytoplasm*	Viable	-	Fischle 2001, Chang 2003, 2007
IIb	HDAC6	Nucleus < Axon	Viable	Hyperactive, anxiolytic	Hubbert et al., Nature 2002 Fukada et al., PLOS One 2012
	HDAC10	Nucleus < Cytoplasm	-	-	Kao et al., J Biol Chem 2002
III	sirtuins	-	-	-	
IV	HDAC11	-	-	-	Gao et al., J Biol Chem 2002

Figure 1. Comparison of classes I, II, III and IV HDAC protein structure and subcellular localization

All HDACs contain a highly conserved catalytic domain known as zinc-dependent histone deacetylases. Class IIa enzymes are characterized by an N-terminal extension not found in other classes I. The sirtuins represents class III but are functionally unrelated to HDACs as their deacetylase activity depends on the co-factor NAD⁺. HDAC6 is the only HDAC that contains an identical duplication of two catalytic domains. It was traditionally thought that class I HDACs are located in the nucleus, whereas class II HDACs can shuttle between the nucleus and the cytoplasm. However, many studies have shown that specific signal transduction pathways can regulate the cellular localization of various HDACs, including class I HDACs. The localization of HDACs known from studies using neuronal cells, the lethality in total knockout mice and the neuronal phenotypes in loss-of-function mutant is indicated.

Figure 2. The roles of HDACs in neuron development

The differentiation of neuronal progenitor cells to neurons requires the transduction of signals to the genome to de-repress neuron-specific genes. The nuclear export of HDAC3, HDAC5 and HDAC9 induces neurogenesis and differentiation by activating target genes. HDAC1 and HDAC2 are required for this process as well, in part by silencing progenitor transcripts and by promoting the expression of a neurogenic program. The development of axon and dendrites also depends upon HDACs function. In addition to a traditional role of HDACs in regulating gene expression, HDACs also influence dendrite and axon development through their action on the cytoskeleton (HDAC6) and signaling pathways (Sirt1).

Figure 3. The roles of HDACs in axon regeneration

Axon injury triggers multiple events that engage several HDACs locally at the site of injury as well as distantly in the cell soma. In the injured axon, HDAC5 and HDAC6 regulate the necessary changes in the microtubule cytoskeleton to optimize growth cone dynamics and axon re-growth. Whereas HDAC5 activity is required for re-growth on permissive substrates, HDAC6 prevents growth on inhibitory substrates mimicking the environment of the injured CNS. In the cell soma, injury-induced nuclear export of HDAC5 and HDAC3 elicits changes in the epigenetic landscape that are required to activate a pro-regenerative gene expression program.