Microtubule deacetylation sets the stage for successful axon regeneration

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Successful axon regeneration relies on a sophisticated balance between stable and dynamic microtubules. While it has been established that moderate microtubule stabilization improves regenerative performance, little is known how microtubules are normally regulated during regeneration. A new study by Cho and Cavalli (2012) uncovers an early role for local microtubule deacetylation that seems important for converting a cut axon stump to a regenerating axon. The authors demonstrate that histone deacetylase 5 is responsible for local deacetylation, and that it is locally activated by PKC downstream of axotomy-induced calcium influx.

Axons in the peripheral nervous system (PNS) possess a robust ability to regrow after axotomy, whereas regeneration frequently fails in the central nervous system (CNS). Figuring out why PNS neurons regenerate so much better than CNS neurons may suggest strategies to improve CNS regeneration. Two key types of differences seem to be important: (1) a growth inhibitory environment in the CNS, and (2) a low intrinsic capacity to make a new axon in CNS neurons (Liu *et al*, 2011). Cho and Cavalli (2012) focus on a relatively uncharacterized aspect of the intrinsic growth response.

For sustained axon regrowth, an injury signal needs to be sent back to the cell body and converted to a transcriptional response (Abe and Cavalli, 2008; Rishal and Fainzilber, 2010). However, before this happens, successful regeneration is presaged by specific local responses at the cut axon tip (Bradke *et al*, 2012). Shortly after axotomy, peripheral axon tips transform into growth cones consisting of organized microtubule bundles. In contrast, injured central axons swell into retraction bulbs enriched with destabilized

microtubules (Erturk *et al*, 2007). Moreover, moderate microtubule stabilization helps CNS axons regenerate (Hellal *et al*, 2011). These results suggest that local control

Figure 1 A model for HDAC5 activity after axon injury is shown. In step 1, a calcium wave is triggered by the initial axon severing event. As it travels towards the cell body it activates PKC in its wake (step 2). Activated PKC phosphorylates HDAC5, which is found throughout the axon. Once phosphorylated, HDAC5 binds to kinesin, which transports it towards the injury site along microtubules oriented with plus ends away from the cell body (step 3). As phosphorylated HDAC5 accumulates near the injury site, it deacetylates microtubules near the cut axon tip (step 4). Microtubule deacetylation seems somehow to facilitate initiation of axon outgrowth (step 5), perhaps by promoting microtubule dynamics.

of microtubule stability is different in CNS and PNS axons after injury, and that this difference is relevant to regeneration. Cho and Cavalli (2012) have found one player that could be important for this difference.

To start to pin down how microtubules are reorganized to support growth after axon injury, Cho and Cavalli (2012) assessed tubulin posttranslational modifications (PTMs) using an *in vivo* mouse model. Injury in peripheral, but not central, axons increased the amount of dynamic microtubules within 24 h, as revealed by a significant decrease in tubulin acetylation together with increased tyrosination. Interestingly, deacetylation was very prominent on the proximal side of the injury site and decreased with distance from the cut tip. Since this change in PTMs seemed to distinguish the PNS injury response from that in CNS neurons, the authors dug deeper into its significance.

Tubulin deacetylation can be mediated by histone deacetylase 6 (HDAC6) and sirtuin 2 (Janke and Bulinski, 2011), and is inhibited by drugs including scriptaid (Su *et al*, 2000). Cho and Cavalli (2012) treated injured neurons with scriptaid *in vitro* and *in vivo*, and found that the drug reduced the changes in acetylation, axon outgrowth, and even functional recovery. As HDACs have major roles in the nucleus, a key experiment was to add scriptaid to neurons cultured in compartmentalized chambers. Importantly, addition of the drug to the axon-containing compartment reduced outgrowth of dorsal root ganglion neurons, supporting the idea that HDAC activity in the axon can influence growth.

Having gained support for the functional importance of HDAC activity for regeneration, the authors set out to track down the key regulator of injury-induced microtubule deacetylation. An obvious candidate was the known tubulin deacetylase, HDAC6. Indeed, RNAi knockdown of HDAC6 reduced deacetylation after axotomy. Surprisingly, however, knockdown of HDAC5, which had not been linked to microtubule deacetylation, had an even stronger effect. As this result was unexpected, the authors confirmed that HDAC5 could deacetylate microtubules *in vitro*. Thus, either HDAC5 or HDAC6, or both, could be responsible for the change in microtubule acetylation after injury. To sort out these possibilities, the authors looked at the position of the two HDACs after axotomy. HDAC5 did something quite striking. In injured neurons, it moved towards the cut site such that a gradient of HDAC5 was formed with high levels near the injured end where tubulin was deacetylated the most. In contrast, the behaviour of HDAC6 after injury was not spectacular. It remained at fairly constant levels in the axon. These data, in conjunction with some additional experiments, led to a model in which HDAC6 controls normal levels of tubulin acetylation in the axon and HDAC5 is specifically activated by injury to deacetylate microtubules in preparation for growth. In support of this model, the authors showed that knockdown of HDAC5 in cultured neurons blocked regrowth after axotomy while HDAC6 knockdown had no effect.

If HDAC5 is the key injury-regulated deacetylase, this begs the question: how is it regulated? The authors identify two types of regulatory mechanisms, and provide experimental support for both. Since the overall localization of HDAC5 was changed by axon injury, this suggested that a motor protein might be involved in repositioning it. Indeed, Cho and Cavalli

(2012) found that in cultured dorsal root ganglion neurons, HDAC5 co-immunoprecipitated with the motor protein kinesin-1. In addition to localization, the authors provide strong evidence that HDAC5 is regulated by phosphorylation.

To understand the model Cho and Cavalli (2012) propose for HDAC5 regulation, let us start at the moment of injury. In other systems, including *Caenorhabditis elegans* (Ghosh-Roy *et al*, 2010), axon severing triggers immediate influx of calcium and a calcium wave that travels back to the cell body. Although this calcium wave seems to be important for starting the process of regeneration, it is not exactly clear what its targets are. A second type of retrograde injury signalling involves motor-mediated transport of proteins, including JNK and importin, from the injured axon to the cell body (Abe and Cavalli, 2008; Rishal and Fainzilber, 2010). The transcriptional response that supports regeneration seems to depend on this second motor-mediated transport that takes place on a much slower time scale than the original calcium wave.

Cho and Cavalli (2012) demonstrate that a calcium wave similar to that seen in invertebrate neurons after injury occurs in injured dorsal root ganglion neurons. Importantly, they also show that tubulin deacetylation does not occur when calcium entry is blocked. Thus perhaps regulation of HDAC5 is a key early event regulated by this first rapid injury signal.

To link calcium entry and HDAC regulation, the authors turn to PKC. HDAC5 has previously been shown to be regulated by PKC in another context (Vega *et al*, 2004), and PKC is a major target of increased intracellular calcium. Indeed phospho-HDAC5 levels were increased in axons after injury, and this increase was blocked by a PKC inhibitor. Moreover, HDAC5 phosphorylation increased HDAC5 deacetylation activity *in vitro* and increased binding to kinesin-1. To further test the link between PKC and microtubules, a PKC inhibitor was added to an *in vivo* model where it blocked deacetylation after sciatic nerve injury.

Taken together, the authors generate an intriguing model for early modifications of microtubules after axon injury. First, calcium enters the axon as a direct result of axon injury. The calcium activates PKC as it travels back to the cell body. PKC turns on HDAC5 throughout the axon, and potentially in the cell body. Phospho-PKC grabs onto kinesin-1 and heads towards the injury site, where it accumulates and locally deacetylates tubulin. Deacetylated tubulin then supports dynamic microtubules and regenerative axon outgrowth (Figure 1). Presumably in order for this initial priming of growth capacity to transform into longer-term regeneration, it needs to be supported by transcriptional changes induced by the second motor-mediated retrograde injury signal.

This model is particularly interesting for two reasons: (1) It provides a cytoskeletal regulatory molecular target for the calcium wave that initiates directly from axon injury, and (2) it suggests that the presence of HDAC5 in axons may be a key difference in the intrinsic regenerative potential of PNS and CNS neurons. It will be interesting to determine in future studies whether HDAC5 also sets the stage for the nuclear response to axon injury.

Conflict of interest

The authors declare that they have no conflict of interest.

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