Renegade nuclear enzymes disrupt axonal integrity

Robert H Miller

How does axonal loss, a hallmark of multiple sclerosis, occur? A study in this issue implicates histone deacetylase 1 (HDAC1), better known for regulating DNA transcription, in such axon degeneration. HDAC1 is exported from the nucleus and interacts with motor proteins, blocking axonal transport and leading to axon loss.

Axonal loss is a hallmark of chronic lesions in autoimmune demyelinating diseases such as multiple sclerosis. This occurs relatively early in the process of demyelination and researchers have developed a plethora of hypotheses to explain this phenomenon. One possibility is that demyelination itself leaves axons vulnerable to attack from immune cells and the initiation of subsequent axonopathy. Alternatively, demyelination may eliminate critical survival factors for axons, resulting in axonal transection and ultimately neuronal loss. Indeed, axons may be the direct target of pathological stimuli and axonal loss may precede frank demyelination. Defining the mechanisms by which inflammatory responses and/or demyelination lead to axonal loss is fundamental to understanding the pathology of the disease, particularly as the long term functional deficits observed in individuals with chronic multiple sclerosis are largely a consequence of axonal loss. In this issue, Kim et al. offer a critical insight into the mechanism of perturbation of axon integrity in response to immunological insults. Their data provide compelling evidence in support of the hypothesis that, in response to such insults, histone deacetylase 1 (HDAC1) is exported from the nucleus and interacts competitively with motor proteins. This blocks the efficiency of axonal transport and leads to perturbations in axonal integrity (Fig. 1).

Members of the HDAC family of nuclear enzymes are best known for their capacity to remove acetyl groups from histones, resulting in compaction of chromatin and repression of transcription. Surprisingly, these same enzymes have also been implicated in neuronal damage and axon perturbation in a variety of different pathological conditions. In the case of cytosolic HDAC6, this is thought to result from modification of the levels of acetylation of α-tubulin, a critical cytoskeletal element that regulates cell morphology and vesicle transport. To determine whether the axon loss seen in autoimmune demyelinating diseases such as multiple sclerosis could be attributed to the presence of cytosolic HDACs, Kim et al. examined pathological material from individuals with multiple sclerosis. Using antibody labeling, the authors found that HDAC1 was localized to the cytoplasm in tissue samples from multiple sclerosis patients only in regions of demyelination. To interrogate an experimental model, the authors utilized the mouse model of cuprizone-induced demyelination. This model consists of feeding animals a diet containing the copper chelator cuprizone, which results in localized demyelination in the corpus callosum. Demyelinated axons of the cuprizone-treated animals also showed morphological perturbation and a cytoplasmic localization of HDAC1. The expression of other HDACs was not correlated with axonal damage, suggesting that there is...
a functional correlation between cytoplasmic expression of HDAC1 and axonal loss.

The authors resorted to an in vitro approach to address the function of HDAC1 in axonal degeneration. Exposure of primary embryonic cultures of hippocampus or cortex to toxic concentrations of TNFα or glutamate resulted in rapid neuritic beading, interpreted as an initiation of axonal damage. Time-lapse studies confirmed subsequent axonal transection and neuronal loss in treated cultures. In control cultures, labeling with antibodies to HDAC1 showed clear nuclear compartmentalization that rapidly became cytoplasmic as a consequence of exposure to glutamate or TNFα, consistent with the hypothesis that nuclear export of HDAC1 contributed to neuronal damage. Furthermore, time-lapse studies revealed a direct correlation between HDAC1 localization and the regulation of mitochondrial transport. In the presence of HDAC1, mitochondrial transport was maintained in the cytoplasm of the cell soma, but not in neurites. However as HDAC1 moved into neurites, mitochondrial transport was substantially reduced, suggesting that HDAC1 directly influences mitochondrial transport. Pre-treatment of cultures with calcium chelators such as EDTA blocked both the induction of neuritic beading and the release of HDAC1 from the nucleus, suggesting that increased cytosolic calcium promotes the nuclear release of HDAC1.

The shuttling of other HDACs between the nuclear and cytoplasmic compartments has been reported to depend on nuclear exporting C signals that engage the Exportin 1 receptor (CRM1). Sequence analysis identified an appropriate motif in HDAC1 and western blot analysis confirmed the formation of complexes specifically between CRM1 and HDAC1 in stimulated cultures. The selective inhibitor LMB blocked the release of HDAC1 and attenuated the effects of cytokine stimulation on mitochondrial transport and neuritic beading.

These observations suggest that the localized presence of HDAC1 in the cytoplasm of neuronal processes perturbs mitochondrial transport. The direct linkage between inhibition of fast axonal transport of mitochondria and the presence of localized, cytosolic HDAC1 was illuminated through a series of analyses that identified the formation of a complex between HDAC1, α-tubulin and members of the kinesin family. Characterization of the motility complex suggested that HDAC1 interacts specifically with motor proteins KIF2A and KIF5, but not with the cargo-associated protein dynamin. In the absence of HDAC1, members of the kinesin family associate with dynamin and facilitate cargo transport. In contrast, cytosolic HDAC1 competes with dynamin and, consequently, kinesin molecules preferentially form a complex with HDAC1 rather than dynamin, thereby blocking the formation of a functionally active motor complex. The competition of distinct kinesins away from dynamin provides an elegant mechanistic explanation for the inhibition of mitochondrial trafficking and subsequent axonal damage.

The results of Kim et al. provide critical insights into the induction of axonopathies and raise important issues. The mechanisms of mitochondrial trafficking in neurons have been extensively studied. As the primary generators of ATP and regulators of intracellular calcium levels, the positioning and continued transport of mitochondria are clearly critical for maintaining axonal and neuronal health. However, the means by which blocking mitochondrial transport leads to their dysfunction is less obvious. It may simply be energy deprivation to distal processes, but this would be expected to lead to dying back pathology. The beading phenomena suggest a local effect, perhaps resulting from localized calcium dysregulation and mitochondrial swelling. In multiple sclerosis research, there is an emerging hypothesis that mitochondrial failure underlies much of the axonal degeneration seen in the chronic disease. Therefore, current and future investigations into the mechanism of mitochondrial dysfunction will likely yield important insights into multiple sclerosis. Attention is currently focused on regulation of calcium, free radical production and axonal transport. The timely analysis by Kim et al. gives us the first mechanistic framework in which to consider the mitochondrial hypothesis of axonal degeneration in multiple sclerosis. This study highlights the issue of specificity. It is unclear why HDAC1, but not other HDAC family members, aggregates the motility complex. More importantly, why neurons are specifically susceptible to induction of cytosolic HDAC1 is not well understood. The inhibition of mitochondrial transport appears to be dependent on local HDAC1; how HDAC1 distributes along axons is also unclear. Diffusion may facilitate short-distance dispersal in vitro, but in the human brain, axonal damage occurs at sites distal to the cell nucleus. Whether there are active transport processes that transport HDAC1 along axons is certainly worthy of further investigation. Finally, whether HDAC1 mobilization is unique to immune-mediated cytokine stimulation or reflects a more general injury response remains to be resolved.

A general characteristic of biological systems is that similar molecular motifs are used in different settings with different endpoints. Provided temporal or spatial segregation is maintained, the chances of inappropriate or undesirable use are remote. On occasion, however, such segregation breaks down as a consequence of pathology or aberrant stimulation, resulting in unexpected, and often unwelcome, new functions for a previously well-defined molecule. This study by Kim et al. represents just such an example. The authors describe the consequence of transport of a nuclear enzyme into axons of neurons in the CNS. The aberrant placement of those enzymes and their surreptitious ability to outcompete native cytosolic proteins leads to perturbation of active mitochondrial transport and subsequent axonal loss. Such studies may identify new therapeutic targets for demyelinating diseases such as multiple sclerosis. Indeed, it is rapidly becoming apparent that the perturbation of fast axonal transport is a characteristic of many neurodegenerative diseases. Studies of Huntington’s disease suggest that alterations in fast axonal transport result in the direct influence of the pathogenic huntingtin protein on signaling cascades targeted to motor proteins. There are clear similarities in the overall principles regulating perturbation of intracellular trafficking by huntingtin and mitochondrial transport by HDAC1, as described by Kim et al., suggesting a fundamental mechanistic basis for neurodegenerative disease.