The study by Kitajiri et al. not only reveals how TRIOBP densely packs actin filaments into the rootlets of stereocilia but also paves the way to a better understanding of the role of the rootlets in hair bundle micromechanics. The widespread expression of TRIOBP protein isoforms in mice (Riazuddin et al., 2006) suggests that TRIOBP-mediated F-actin bundling might be an important new mechanism for building highly elastic and robust F-actin structures in cells.

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For Synapses, It's Depression Not Death

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Long-term depression (LTD) of synaptic strength is an activity-dependent process in neurons that may be important for learning and memory. Li et al. (2010) now reveal a new apoptosis-independent role for mitochondrial-activated caspases in LTD suggesting that neurons have co-opted the canonical cell death pathway to perform a specialized function at synapses.

Many forms of learning and memory are thought to require experience-dependent adjustments to the properties of synapses in a specialized region of the brain named the hippocampus. Such modulation can be induced by specific patterns of neuronal activity that lead to long-lasting changes in the number of glutamate receptors at the synapse. For example, an increase in the number of AMPA-type glutamate receptors is often triggered when action potentials in a pair of connected neurons are temporally correlated, whereas a decrease in the number of these receptors is triggered by decorrelated activity. Since the discovery of these processes, known respectively as synaptic long-term potentiation (LTP) and long-term depression (LTD), there has been a big effort to determine the molecular players involved (Malenka and Bear, 2004).

The study by Li et al. (2010) in this issue of *Cell* demonstrates an intriguing and perhaps unexpected central role for the mitochondrial proapoptotic signaling pathway in LTD. LTD is known to require the ubiquitin/proteasome system as well as clathrin-mediated endocytosis, supporting the idea that proteins need to be removed from the synapse and turned over in order to establish LTD (Malenka and Bear, 2004). Furthermore, previous work by Li and colleagues (Li et al., 2004) established that motile mitochondria in the dendrites of neurons translocate rapidly to active synapses, but a specific function for mitochondria in synaptic plasticity has remained elusive.

In their new study, Li et al. (2010) discover that the induction of synaptic depression requires mitochondrial-activated caspase-3 (a proapoptotic signaling molecule) and is prevented by overexpression of antiapoptotic proteins (Figure 1). Notably, the authors find that caspase-3 is activated in healthy neurons, does not induce cell death, and is not necessary for other synaptic processes such as the maintenance of basal synaptic transmission or the induction of LTP. These findings are yet another example of the frugality of the nervous system, which has co-opted signaling pathways that regulate apoptosis, growth, or cell division in non-neuronal cells to regulate uniquely neuronal processes such as synaptic transmission and plasticity. Examples of pathways that are "reused" in neurons include the mTOR cascade, which has been traditionally studied in the context of cell growth, division, and cancer but is also a synaptic regulator (Kelleher and Bear, 2008). Other such

pathways include the classical complement cascade, which mediates phagocytosis of foreign cells as part of the innate immune system but also may induce removal of unwanted synapses (Stevens et al., 2007), and MHC molecules, which recruit T cells as part of adaptive immunity but are transcriptionally induced in the nervous system in an activity-dependent manner in order to modulate synapse refinement (Shatz, 2009).

Related to this theme, Li and coworkers find that inhibition or genetic deletion of caspase-3 blocks the induction of LTD at the CA3-CA1 synapses of hippocampal neurons. The loss of LTD is selectively caused by disruption of caspase-3 and caspase-9 as inhibitors of other caspases did not affect synaptic plasticity. In a complementary set of experiments in hippocampal slice cultures, Li et al. also find that overexpression of the mitochondrial antiapoptotic factor Bcl-xL and the caspase inhibitor protein XIAP blocks LTD without disrupting LTP.

These electrophysiology studies demonstrate a requirement for caspase signaling in the induction of LTD, but does this pathway also regulate AMPA receptor internalization, the final manifestation of LTD? In a key set of experiments, the authors demonstrate that activation of another class of glutamate receptor, the calcium ion-permeable NMDA receptor, which induces a chemical form of LTD, causes release of cytochrome *c* from the mitochondria and activates both caspase-3 and caspase-9. Importantly, the activation of caspases by the opening of NMDA receptor channels and calcium ion influx is quantitatively less than that observed with an inducer of neuronal apoptosis and insufficient to induce neuronal death. In contrast, NMDA receptor stimulation does induce clathrin-dependent internalization of AMPA receptors. Indeed, Li et al. demonstrate that this process is blocked by peptide inhibitors of caspase-3 and caspase-9, overexpression of Bcl-xL or XIAP, or genetic deletion of caspase-3.

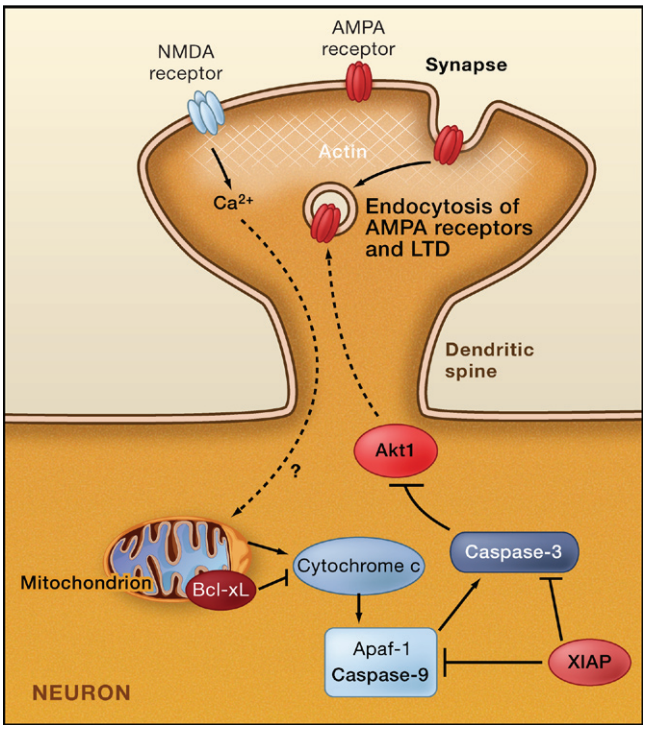

Figure 1. Mitochondrial-Activated Caspases in Synaptic LTD
A dendritic spine of a neuron showing AMPA glutamate receptors (red) and NMDA glutamate receptors (blue) in a region of the synapse called the postsynaptic density. In response to neuronal activity, NMDA receptors open and calcium ions enter the spine. Through a series of intermediate signaling steps that include the activation of protein phosphatases, this NMDA receptor-calcium ion signal results in clathrin-mediated endocytosis of AMPA receptors at the synapse and a reduction in synaptic responses. The signaling pathway required for this synaptic long-term depression (LTD) depends upon activation of the proapoptotic protease caspase-3 (Li et al., 2010). In this scenario, release of cytochrome *c* from the mitochondria, activation of caspase-3 and caspase-9, and cleavage of the protein kinase Akt1 are required for the induction of LTD. Overexpression of the antiapoptotic factors Bcl-xL or XIAP or a cleavage-resistant form of Akt1 disrupts LTD. Whether these signaling events occur locally at activated synapses, how the NMDA receptor-calcium ion signal regulates mitochondria, and how caspase-3 activation leads to the endocytosis of AMPA receptors remain to be elucidated.

This study presents intriguing evidence for a nonapoptotic role for caspase signaling in mature neurons and opens exciting new avenues for further research. The challenge now is to link the caspases and mitochondria with other known components of the LTD induction and expression pathway. The authors take a first step in this direction and propose that the protein kinase Akt1, which is cleaved by caspase-3 in other cell types and is linked to LTD via its regulation of GSK3β, is the relevant caspase substrate in neurons for LTD induction. The authors perform an extensive set of biochemical experiments to generate and validate an enzymatically active Akt1 mutant that is resistant to caspase cleavage. They find that overexpression of this mutant protein kinase selectively prevents LTD without affecting LTP.

Despite a clear electrophysiological effect, the authors were unable to detect cleavage or loss of endogenous Akt1 in cultured neurons following NMDA receptor activation. They suggest that perhaps only a small fraction of Akt1 is cleaved by caspase-3. This could be the case if NMDA-receptor-mediated caspase activation and proteolysis are spatially restricted, occurring at only a subset of synapses undergoing LTD. Such a mechanism could explain why caspase activation does not lead to cell death in this context. To address this interesting possibility, it would be useful to visualize in real time the activation of caspase-3 and caspase-mediated cleavage of targets in the dendrites and spines of live neurons. This type of analysis could be achieved with fluorescence lifetime imaging microscopy (FLIM) using tools like those developed to study the dynamic activation and localization of the synaptic signaling molecule Ras (Yasuda et al., 2006). Alternatively, imaging the translocation of mitochondria to activated dendritic spines during LTD stimulation could provide interesting insights into the spatiotemporal dynamics of this mechanism.

Further studies are needed to determine the molecular targets that link caspase signaling with the effectors of AMPA receptor internalization (Figure 1). Although AMPA receptors themselves can be targets of caspase-3 activity in vitro (Lu et al., 2002), another possibility is that the elements of the actin cytoskeleton, which provide structure and stability to dendritic spines, are cleaved by caspases possibly through an indirect mechanism involving the caspase-3

substrate gelsolin (Furukawa et al., 1997; Kothakota et al., 1997). Such a mechanism might contribute to the shrinkage or loss of dendritic spines in response to LTD-inducing stimuli. The study by Li et al. introduces a new set of molecules at the center of the pathway underlying synaptic LTD and further demonstrates the resourcefulness of postmitotic neurons in borrowing signaling pathways traditionally associated with cell growth, differentiation, and death in dividing cells to perform specialized functions at synapses.

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A Bone to Pick with Compulsive Behavior

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Mice with mutations in the *Hoxb8* gene exhibit compulsive grooming behavior. Chen et al. (2010) now report that this behavior stems from *Hoxb8* deficiency in microglia, a type of immune cell in the brain derived from bone marrow. These findings provide intriguing connections between immune dysfunction and neuropsychiatric disorders.

Mice with mutations in the *Hoxb8* gene groom themselves at about twice the frequency of wild-type mice, resulting in hair loss and open skin lesions (Greer and Capecchi, 2002). The *Hoxb8* mutant mouse has been proposed as a model for a human behavioral disorder, trichotillomania (compulsive hair pulling), which may be related to obsessive-compulsive disorder (OCD; Chamberlain et al., 2006). Reporting in this issue, Chen et al. (2010) now investigate the cellular basis of the overgrooming behavior in *Hoxb8* mutant mice. Reasoning that lack of *Hoxb8* expression in the brain should play an important role in the behavioral phenotype, the authors ask what cell types in the brain normally express *Hoxb8*. They find that within the mouse brain the only *Hoxb8*-expressing cells are microglia that originate in bone marrow and migrate into the brain. To test whether bone marrow-derived microglia lacking *Hoxb8* are responsible for compulsive grooming behavior, the authors carry out bone marrow transplants. Strikingly, transplant of wild-type bone marrow into *Hoxb8* mutants restored normal, noncompulsive grooming behavior within a timeframe consistent with the migration of new microglia into the brain. These findings provide important new evidence that abnormalities of the immune system can produce compulsive behavior and strengthen the case that microglia play an important role in modulation of nervous system function.

Behaviors are said to be compulsive if they continue despite causing significant harm or distress. Compulsions are thought to result from abnormal functioning of neural circuits that connect the cerebral cortex and the striatum, a component of the basal ganglia (Figure 1). Parallel loops run from diverse regions of the cerebral cortex to the striatum, then, by way of the thalamus, back to prefrontal regions of the cerebral cortex. These loops facilitate the consolidation of repeated sequences of movements or thoughts into highly efficient modules that can be replayed automatically, that is, without conscious supervision (Graybiel, 2008). Depending on the precise movements or thoughts in the sequences, these automatic behaviors may range from skilled performances to habits or rituals. It is thought that when corticostriatal loops that control habitual behaviors become dysfunctional, compulsions can result.

How might mutations in the widely expressed Hox gene family member *Hoxb8* lead to compulsive grooming behavior in mice? The possible mechanism is not as straightforward as it would be if the gene encoded a synaptic protein such as the SAP90/PSD95-Associated Protein 3 (SAPAP3), which has been implicated in compulsive grooming (Welch et al., 2007). In the latter case,