

CLASPs, enters a limited subset of clathrin-coated pits. Instead the authors claim that the LDL receptor is targeted uniformly to all clathrin-coated pits within hepatocytes (Keyel et al., 2006).

As always, many open questions remain. Is there a common mechanistic basis underlying the formation of distinct clathrin-coated pit subpopulations caused by different cargo proteins and their corresponding CLASPs? Does functional specialization of the early endocytic pathway also account for the spatial and temporal regulation of early endocytic membrane traffic in polar-

ized cells and tissues including neurons? For example, synaptic vesicles need to maintain their compositional identity during repetitive cycles of exocytosis and endocytosis, perhaps via CLASPs such as stonin2 (Diril et al., 2006). A thorough mechanistic understanding of early endocytic specialization may also enable us to eventually identify compounds that selectively target endocytic delivery of select signaling receptors.

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## Driving Actin Dynamics under the Influence of Alcohol

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The actin cytoskeleton plays a pivotal role in regulating neuronal development and activity, and dysregulation of actin dynamics has been linked to impaired cognitive function. In this issue of *Cell*, Rothenfluh et al. (2006) and Offenhäuser et al. (2006) show that actin dynamics can also affect the cellular and behavioral responses of flies and mice to alcohol.

The consumption of moderate amounts of alcohol is a pleasurable pastime for many and a popular means to facilitate social interaction. It is not by chance that the ancient Greeks had a god, Dionysus, to consecrate the pleasures of libation. However, the consumption of alcohol can also lead to dependence, and so determining what factors make a person susceptible to alcoholism is an important area of research.

Several reports have indicated that a low-level response to the acute intoxicating effects of alcohol correlates with an increased risk for

alcoholism, and that this response is genetically influenced in both humans and animals (Enoch and Goldman, 1999). Based on these findings, animal models have been developed to define the genetic components and signaling pathways underlying alcohol resistance. In this issue, Rothenfluh et al. (2006) and Offenhäuser et al. (2006) report that the dynamic regulation of the actin cytoskeleton in flies and mice, respectively, influences cellular and behavioral responses to alcohol.

Behaviors elicited by ethanol exposure are remarkably similar

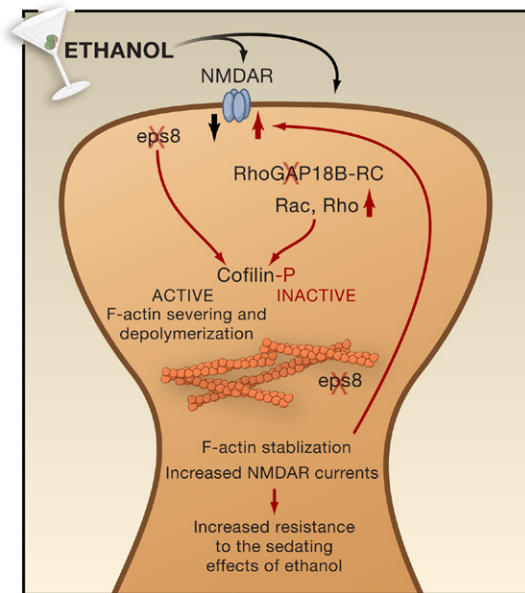
in animals and humans. Studies in both the fruit fly *Drosophila* and mice have consistently suggested that deregulation of biochemical and neurobiological determinants can lead to differences in alcohol/ethanol sensitivity and levels of alcohol consumption. More specifically, these studies have identified proteins involved in alcohol metabolism (for example, alcohol dehydrogenase and acetaldehyde dehydrogenase) and in neuronal signaling pathways (such as the N-methyl-D-aspartate receptor [NMDAR], the  $\gamma$ -aminobutyric acid type A [GABA<sub>A</sub>] receptor,

protein kinase A, calcium/calmodulin-sensitive adenylylase cyclases, and the serotonin receptor) as key players in alcohol dependency (Dick and Bierut, 2006; Wernicke et al., 2003). Notably, the NMDA receptor and the GABA<sub>A</sub> receptor are also key targets of ethanol in the brain, with ethanol potentiating GABA<sub>A</sub>-receptor-gated ion currents and inhibiting those mediated by NMDA receptors.

By performing a genetic screen in the fruit fly *Drosophila*, Rothenfluh et al. (2006) show that mutations in the *RhoGAP18B* gene lead to resistance to the sedating effects of ethanol (Figure 1). RhoGAPs (GTPase-activating proteins) negatively control Rho small GTPases by increasing their intrinsic GTPase activity. Rho GTPases switch from an inactive GDP-bound conformation to an active form when bound to GTP. In the GTP-bound state, they can interact with effector proteins that in turn mediate their biological activities. Rho GTPases were first identified as major regulators of the actin cytoskeleton (Govek et al., 2005). Consistent with the biochemical function of RhoGAP18B

as a RhoGAP, the investigators found that modulation of the activity of Rho GTPases was sufficient to affect the flies' response to acute ethanol consumption. Specifically, they determined that an increase in Rho or Rac activity, a condition expected to mimic loss of RhoGAP, decreased the sedating effects of ethanol.

Interestingly, the authors also demonstrate that the *RhoGAP18B* gene has a number of different isoforms (*RA–RD*) generated by alternative splicing, and that these isoforms have distinct biological activities. The *RA* transcript regulates the stimulatory effects of ethanol, whereas the *RC* transcript mediates alcohol's sedating effects. Because the two isoforms share only the GAP domain,



**Figure 1. Regulators of the Actin Cytoskeleton Influence Responses to Ethanol**

High doses of ethanol are sedating and induce inhibition of NMDA-gated ion currents and depolymerization of the actin cytoskeleton. Loss of *RhoGAP18B* and *Eps8* renders flies and mice, respectively, more resistant to the intoxicating effects of ethanol, a risk factor for addiction. Evidence suggests that the increased resistance to ethanol is at least in part due to altered actin dynamics (Rothenfluh et al., 2006; Offenhäuser et al., 2006). In the absence of Eps8, NMDA-induced dephosphorylation/activation of cofilin, an actin-filament depolymerizing/severing factor, is reduced in cerebellar granule neurons in mice. This results in more phosphorylated inactive cofilin molecules and hence increased actin stability. In flies, loss of *RhoGAP18B* leads to increased Rho GTPase activity, which may also trigger phosphorylation and inactivation of cofilin, leading to F-actin stabilization. The increased stability of F-actin renders cells more resistant to the effects of alcohol on actin depolymerization, which in turn could alleviate the inhibitory effects of ethanol on NMDA-gated ion currents.

their distinct biological activities may be due to interactions (mediated through their unique amino-terminal domains) with different cellular components that could change their subcellular localization, stability, or biochemical properties. Alternatively, the amino-terminal moiety of the RhoGAP18B isoform could by itself affect the rate of GAP activity and change the specificity of the isoform to Rho family members.

The cellular processes on which RhoGAP18B activity impinges remain to be defined. However, the fact that discrete changes in the activation of small GTPases can result in a dramatic behavioral change is particularly intriguing and is consistent with accumulating work showing that mutations

in regulators and effectors of small GTPases play an important role in cognitive disorders (Govek et al., 2005). Although many details regarding the specific roles of Rho GTPases in the development of the central nervous system (CNS) and in disorders affecting the CNS still remain to be elucidated, it is now well established that they are critically involved in various neuronal processes through modulation of the actin cytoskeleton, such as neurite/axon outgrowth, dendritic branching, and dendritic-spine morphogenesis (Govek et al., 2005). Dendritic spines serve as postsynaptic compartments for the majority of excitatory synapses in the brain, and spine formation and elimination are thought to reflect structural reorganization of synaptic connections in the brain. The shape, motility, and growth of spines are determined by the architecture of their actin cytoskeleton. Abundant evidence supports the idea that spine morphology is associated with synapse strength, thus linking synapse formation and strength with actin organization and dynamics.

Synaptic activity and glutamate receptors have been shown to influence actin dynamics and spine morphology. Also, a role for actin in maintaining synaptic receptors and in long-term potentiation (which contributes to memory formation) has been documented (Toda and Sheng, 2006). These findings raise the intriguing possibility that RhoGAP18B, a regulator of Rho GTPases, may influence ethanol resistance by modulating the actin cytoskeleton in neuronal structures of the fly brain.

Interestingly, the work of Offenhäuser et al. (2006) also suggests that changes in actin dynamics can modify the response of neurons to ethanol exposure. Offenhäuser et al. (2006) analyzed the behavioral response of mice lacking Eps8, a

key regulator of actin dynamics, to ethanol exposure. They found that these mice are more resistant to the sedative effects of ethanol and show increased ethanol consumption compared to wild-type mice. The expression of *Eps8* was observed in some mouse brain areas implicated in ethanol tolerance, including the hippocampus, which is central to learning and memory, and the cerebellum, which is involved in sensory perception and motor output. In particular, the authors showed that *Eps8* is located together with the NMDAR complex at postsynaptic structures of cerebellar granule neurons (CGNs), where its loss of expression leads to increased NMDA-gated ion currents and decreased sensitivity of the NMDAR to the inhibitory effects of ethanol. The increased NMDAR currents in neurons lacking *Eps8* could be reversed by drugs that depolymerize actin, suggesting that *Eps8* could potentially influence NMDAR currents by stabilizing actin. Consistent with this notion, CGNs lacking *Eps8* are more resistant to the actin-remodeling activities of NMDA and ethanol. In these cells, NMDA-elicited dephosphorylation/activation of cofilin, an actin-filament depolymerizing/severing factor, is dramatically reduced, thereby decreasing actin dynamics. It should be pointed out that ethanol does not affect the status of cofilin phosphorylation or the interaction between *Eps8* and actin. This observation implies that the increased actin stability caused by loss of *Eps8* renders the cells more resistant to the actin-depolymerizing effects of ethanol, which in turn appears to alleviate ethanol-induced inhibition of NMDAR ion currents.

Based on these results, the authors infer that changes in actin dynamics in the CGNs could explain the resistance of the *Eps8*-deficient mice to some of the intoxicating effects of ethanol. Specifically, compared to wild-type mice,

the *Eps8*-deficient mice exhibited greater motor coordination and a quicker righting reflex (the time it takes a mouse to put its paws on the ground after being placed on its back) following alcohol administration. Further elucidation of the contribution of actin dynamics to the response of mice to ethanol will require experiments with cultured neurons or brain slices that investigate changes in actin dynamics by, for example, FRET-based imaging of the equilibrium between F-actin (polymerized actin) and G-actin (actin monomers). Also, additional work will be required to understand the precise mode of action of *Eps8* in neurons at a molecular level.

In fibroblasts, *Eps8* regulates the actin cytoskeleton by contributing to the activation of Rac and capping the barbed ends of actin filaments. Whether loss of *Eps8* results in decreased Rac activity in CGNs remains to be seen. However, such a finding would not evidently tie in with the results of the Rothenfluh et al. (2006) study, in which increased Rac activity renders flies more resistant to ethanol, and reports showing that subsequent activation of Lim kinase triggers cofilin phosphorylation (Kuhn et al., 2000). On the other hand, as Rac activation inhibits RhoA activity in some neuronal cell types, it is possible that loss of *Eps8* could result in decreased Rac activity, leading to enhanced RhoA activity. Fly mutants that overexpress activated Rac or Rho exhibit ethanol resistance.

The two new studies support the notion that genetic mutations can modify our responses to alcohol. More specifically, they show that discrete changes in the actin cytoskeleton in certain neuronal cell types can alter the cellular and behavioral response to alcohol. This finding fits with the notion that mutations in genes encoding regulators and effectors of small GTPases may contribute to certain neurological disorders.

Interestingly, the large number of RhoGAPs (66), RhoGEFs (84), and Rho GTPase effectors (161) in the human genome compared to the more restricted number of Rho small GTPases (18) also suggests that the pleiotropic activity of Rho GTPases became tightly regulated and spatiotemporally restricted during evolution (Bernards, 2003). Lastly, the finding that loss of *Eps8* and *RhoGAP18B* in mice and flies, respectively, leads to an increased tolerance to alcohol exposure raises the potential that selective allelic variants of these two genes in humans may contribute to alcoholism. Unfortunately, based on protein sequence similarity, it is not obvious which of the many human RhoGAPs represents the true *RhoGAP18B* ortholog. It would therefore be interesting to determine whether loss of a specific RhoGAP protein in humans and mice has effects on the response to alcohol similar to the loss of *RhoGAP18B* in flies.

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