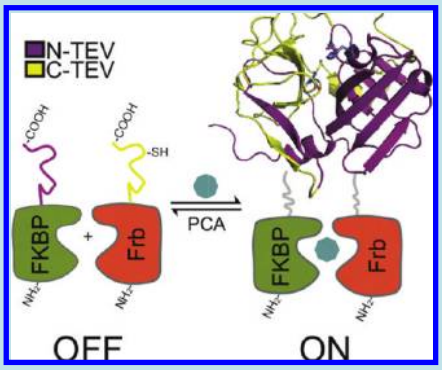
Spotlight

SNIPers and Executioners



Reprinted from *Cell*, *142*, Gray, D. C., *et al.*, Activation of Specific Apoptotic Caspases with an Engineered Small-Molecule-Activated Protease, 637–646, Copyright 2010, with permission from Elsevier.

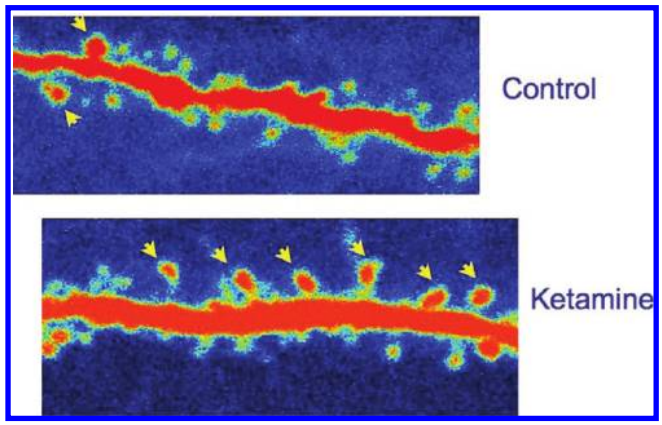
The executioner caspases are the final caspases activated during apoptosis, or programmed cell death. These activation events lead to the cleavage of nearly 1000 proteins, a process that propels the cell along its journey to the grave. However, deciphering the specific roles of each executioner caspase, caspase-3, -6, and -7, during the complex process of apoptosis is a formidable challenge. Now, Gray *et al.* (*Cell* 2010, *142*, 637–646) present the design of engineered executioner caspases under the control of a small-molecule activator and the use of this system to interrogate the individual functions of these important enzymes.

The design of the engineered caspase system relies on the activity of the NIa tobacco etch virus (TEV) protease and the expression of executioner caspases engineered to contain TEV cleavage sites. Building on a common method used for protein complementation assays, the TEV protease was split, with one portion attached to the protein FKBP and the other attached to FRB. In the presence of rapamycin, FKBP binds FRB, thus bringing the two portions of TEV together and reconstituting the active protease, a construct referred to as the SNIPer. When the engineered versions of caspase-3 or caspase-7 were expressed in a human kidney cell line expressing SNIPer and rapamycin was added to the cells, SNIPer promptly cleaved and activated the engineered caspase, and the cells rapidly underwent apoptosis. Remarkably, it was also found that the inclusion of proteasome inhibitors worked synergistically with caspase activation to promote apoptosis. Interestingly, however, when the engineered version of caspase-6 was activated, the cells underwent apoptosis only when proteasome inhibitors were also present. The authors propose an intriguing model of negative proteolytic regulation, in which the proteasome attempts to restrict caspase activity while caspases endeavor to dismantle the proteasome. This enticing link between the caspase and proteasome activity alludes to the possible therapeutic benefit of combined apoptosis activators and proteasome inhibitors. **Eva J. Gordon, Ph.D.**

The Actions of an Antidepressant

The depressing news about many antidepressants is that they can take weeks or even months to take effect, and many patients are resistant to them entirely. Ketamine, a nonselective N-methyl-D-aspartic acid (NMDA) receptor antagonist commonly used as an anesthetic, has recently been shown to exhibit antidepressant activity within a few hours, even in patients resistant to traditional medications. The mechanisms responsible for eliciting such a rapid response are not well understood but likely involve signaling cascades that mediate synaptic plasticity. Using a combination of biochemical, electrophysiological, and imaging methods, Li *et al.* (*Science* 2010, *329*, 959–964) explore the effects of ketamine administration on signaling pathways in the rat prefrontal cortex, protein synthesis in synapses, and behavioral models of depression.

In the rat prefrontal cortex, ketamine administration induced rapid activation of the mammalian target of rapamycin (mTOR) pathway or pathways linked to mTOR signaling. Specifically, increased



From Li, N., *et al.*, *Science*, 2010, *329*, 959. Reprinted with permission from AAAS.

levels of several phosphorylated and activated proteins, including mTOR, extracellular signal-regulated kinase (ERK), and protein kinase B (Akt), were observed. Supporting the involvement of these pathways in the antidepressant response, inhibitors of ERK or Akt were shown to block this phosphorylation induction of mTOR. Ex-

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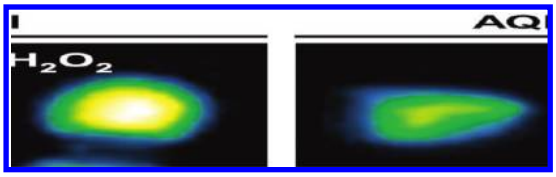
amination of protein synthesis in synapses revealed increased levels of pre- and postsynaptic proteins, which was blocked by the mTOR inhibitor rapamycin. The increase in synapse-associated protein synthesis suggests that spine number and morphology might also be affected. Indeed, two-photon imaging analysis and electrophysiological experiments in prefrontal cortex neurons provided evidence of a ketamine-dependent increase in spine density and maturation as well as enhanced synaptic strengthening. Finally, ketamine was shown to be effective in three distinct behavioral models related to depression, and the effects were blocked by exposure to inhibitors of mTOR, ERK, and Akt. Together, the results strongly implicate the mTOR signaling pathway in the antidepressant effects of ketamine, providing an exciting avenue for the development of novel antidepressant agents. **Eva J. Gordon, Ph.D.**

A Transport System for H_2O_2

The reactive oxygen species hydrogen peroxide (H_2O_2), notorious for its role in oxidative stress pathways that damage cells, has recently gained attention in a more favorable light as an important signaling molecule in essential cellular processes including growth, differentiation, and migration. While some members of the aquaporin (AQP) family of water channels have been shown transport H_2O_2 across the cell membrane in yeast and plants, a general lack of understanding of how cells compartmentalize the beneficial and deleterious activities of H_2O_2, coupled with challenges in the development of reliable, selective methods for detecting H_2O_2, have hampered delineation of H_2O_2 biology. Now, Miller *et al.* (*Proc. Natl. Acad. Sci. U.S.A.* 2010, *107*, 15681–15686) report the development of a new fluorescent indicator specific for H_2O_2, along with the identification of two membrane H_2O_2 transporters, to help elucidate the functions of this intriguing biomolecule.



Miller, E. W., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *107*, 15681–15686. Copyright 2010 National Academy of Sciences, U.S.A.

It was first investigated whether mammalian cells also utilize AQP family members as H_2O_2 transporters. A human kidney cell line was transfected with a select member from each of the three types of AQPs: the classical aquaporin AQP1, the aquaglyceroporin AQP3, and the unorthodox aquaporin AQP8. Using flow cytometry and the new fluorescent indicator PY1-ME, which relies on a selective reaction between a boronate moiety and H_2O_2, to generate a fluorescent signal, it was demonstrated that AQP3 and AQP8, but not AQP1, facilitate H_2O_2 uptake. The genetically encoded fluorescent H_2O_2 sensor HyPer was next used to confirm the AQP3-mediated uptake of exogenous H_2O_2 and to demonstrate that AQP3 mediates uptake of endogenously produced H_2O_2 as well. It was further shown that AQP3-mediated uptake of both exogenous and endogenous H_2O_2 affects H_2O_2 signaling pathways. Specifically, phosphorylation of the serine/threonine kinase Akt was enhanced in cells overexpressing AQP3, and AQP3 was shown to be linked to the transmission of signals from H_2O_2 expressed at the cell surface to Akt inside the cell. These intriguing findings offer compelling evidence that transport mechanisms for H_2O_2 exist as important components of H_2O_2 signaling pathways. **Eva J. Gordon, Ph.D.**

Avoiding the Death Dance

The therapeutic potential brewing inside human embryonic stem cells (hESCs) is the source of much excitement, though attempts to tap into this potential are hampered by our lack of understanding of the nature of pluripotent cells, *i.e.*, their ability to proliferate and to differentiate into all cell types. Interestingly, it has been observed that unlike mouse ESCs, hESCs must grow in clumps. Should they become dissociated, they immediately exhibit membrane blebbing (sometimes referred to as a "death dance"), which is an indication of hyperactivity within the actomyosin system, and shortly thereafter they undergo apoptosis (commit suicide). The reasons underling this dissociation-induced cell death are unclear. Now, two new reports by Ohgushi *et al.* (*Cell Stem Cell* 2010, *7*, 225–239) and Chen *et al.* (*Cell Stem Cell* 2010, *7*, 240–248) demonstrate that actomyosin hyperactivity plays a key role in the apoptosis associated with cell–cell dissociation.

Both the Ohgushi *et al.* and the Chen *et al.* studies clearly demonstrate that cell blebbing and apoptosis is initiated very shortly after hESC dissociation, that survival of hESCs increases with E-cadherin-dependent cell–cell contact, and that treatment with the myosin heavy chain ATPase inhibitor blebbistatin or inhibition of Rho-associated kinase (ROCK) activity prevents membrane blebbing, increases cell survival, and promotes colony formation.

Ohgushi *et al.* further explore the effects of cell dissociation by showing that loss of E-cadherin-mediated cell adhesion leads to actomyosin hyperactivation as well as activation of the G protein Rho. Notably, this phenomenon is also observed in mouse epiblast-derived pluripotent cells, but not in mouse ESCs, suggesting that it is the cell state, rather the species of origin, that determines the vulnerability of cells to dissociation-induced apoptosis. Employing a short-hairpin RNA screen, it was found that knockdown of Abr, a member of the Rho-GEF (guanine exchange factor) family, rescued hESCs from dissociation-induced apoptosis and is likely a key player in promoting myosin hyperactivation and apoptosis. Finally, it was also shown that the combination of high Rho activity and low activity of another G protein, Rac, is an important contributor to the induction of actomyosin hyperactivation. The authors propose a model

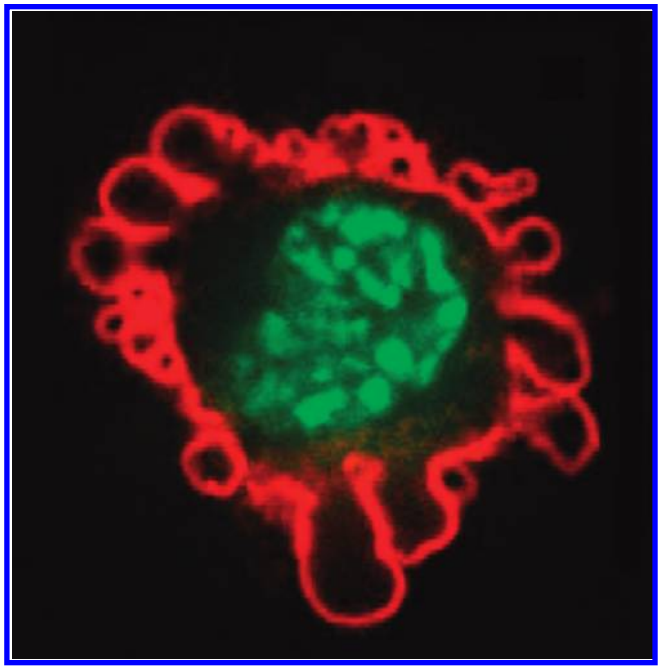


Image courtesy of Yoshiki Sasai.

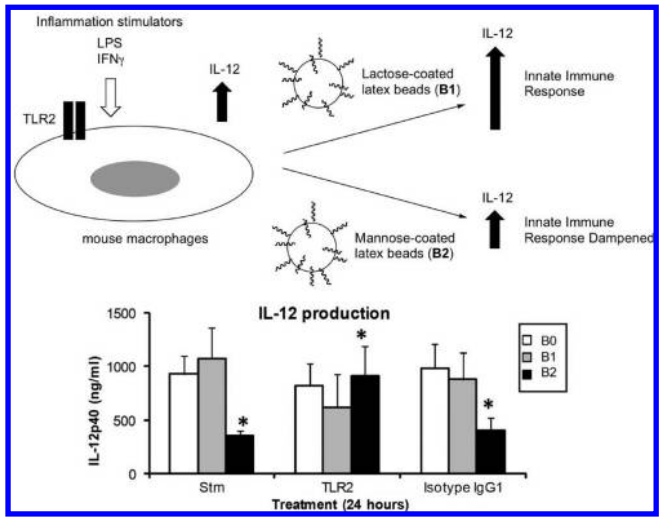
in which, upon cell dissociation, Abr is involved in activating the Rho/ROCK/myosin system, as well as suppressing the inhibitory actions of Rac, leading to myosin hyperactivation and eventual apoptosis.

Chen *et al.* probe the mechanism of dissociation-induced cell death by exploring the roles of key proteins involved in the cell blebbing and apoptotic processes. Small interfering RNA (siRNA) was used to knockdown the two most highly expressed nonmuscle myosin II heavy chains (*MYHs*) in hESCs, *MYH9* and *MYH10*, and all three myosin light chain (MLC) family members. It was demonstrated that *MYH9*, but not *MYH10*, is the major target for blebbistatin in hESCs, and that regulation of MYH motors by MLC is involved in dissociation-induced death. In addition, it was found that use of small molecule inhibitors to disrupt actin filaments, which are intimately involved in contraction of myosin motors and the cell blebbing process, also improved cell survival. Finally, siRNA experiments and use of small molecule ROCK inhibitors revealed that ROCKs regulate MYH function through the phosphorylation of MLC, offering further mechanistic insight into the role of ROCK in dissociation-induced apoptosis.

These remarkable studies point to a key role of the actin-myosin system in dissociation-induced death of hESCs. As the mechanisms of hESC proliferation and differentiation continue to be unraveled, clues provided by these and future studies will improve our ability to manipulate hESCs for numerous medical applications. **Eva J. Gordon, Ph.D.**

Sweet Damping of the Immune Response

The intracellular pathogens *Mycobacterium tuberculosis* and *Leishmania* persist in part because of their ability to hide in plain sight. These organisms evade the immune system by stowing away within macrophages, the agents that package and expel these infiltrating cells from the human body. This unique ability has made it particularly difficult to design treatments and vaccines for tuberculosis and leishmaniasis, diseases that remain major health problems in the developing world.



Reprinted with permission from Song, E.-H., *et al., J. Am Chem. Soc*, *132*, 11428−11430. Copyright 2010 American Chemical Society.

The cell walls of *M. tuberculosis* contain mannosylated lipoarabinomannan (ManLAM). This glycolipid plays a critical role in the reduced production of interleukin-12 (IL-12), the cytokine that recruits the immune response to the pathogens. The *Leishmania* parasite also has an outer lipophosphoglycan with an alpha-linked trimannose component. Zeroing in on this carbohydrate structure, Song *et al.* (*J. Am. Chem. Soc.*, 2010, *132*, 11428–11430) synthesized "artificial pathogens," beads decorated with mannose sugars found on these pathogens' surfaces. The researchers then demonstrated that a pathogen-like surface with multiple copies of mannose can suppress the immune response.

To mimic the surface and size of the pathogens, the researchers synthesized the alpha-linked trimannose structures and covalently linked those structures to 1-μm latex beads. Lactose-covered beads and unmodified beads served as controls. To test the inflammatory response, Song *et al.* stimulated mouse macrophages, incubated those cells with each type of latex bead, and measured IL-12 production after 24 h. Macrophages treated with mannose-covered beads showed significantly reduced IL-12 production when compared with those exposed to the unmodified and lactose-covered beads. Because the Toll-like receptor 2 (TLR2) plays a recognized role in innate immunity, the researchers blocked TLR2 to test the

mechanism of action. With TLR2 blocked, the immune response of macrophages treated with mannose-covered beads matched the response with uncoated beads, suggesting that these pathogens dampen the immune response through via a TLR2-mediated pathway. These results provide both a useful model system for studying the role of surface carbohydrates in immunity and valuable insights for the design of new vaccines and treatments for these diseases. **Sarah A. Webb, Ph.D.**

Genes, Sunscreens, and Imines

The sun enables life on Earth by warming our atmosphere and providing light for photosynthetic organisms, but with that tremendous energy comes a complicating consequence. Ultraviolet solar radiation is highly mutagenic to DNA, and as such, organisms have evolved DNA repair pathways or ultraviolet protection mechanisms to counteract the sun's effects. Mycosporine and mycosporine-related amino acids, or MAAs, are small molecule sunscreens produced by a variety of cyanobacteria, algae, and marine organisms such as coral. These UV-absorbing compounds are arranged such that natural amino acids are attached to a central cyclohexenone structure via imine linkages.

Image courtesy of Emily Balskus.

Though these natural sunscreens were uncovered almost 3 decades ago, the enzymes and exact chemical intermediates involved in producing mycosporine compounds remained elusive until now. Balskus and Walsh (*Science* 2010, *329*, 1653–1656) used a clue from a previous study regarding a putative biological route to mycosporines, the shikimate pathway, combined with the draft genome of the sea anemone, a creature known to harbor these natural sunscreens. Their search uncovered a gene cluster containing two biosynthetic genes that appeared to be strong candidates, a dehydroquinate synthase homologue and an O-methyltransferase. Searching other complete genomes revealed that a third conserved protein-coding gene of the ATP-grasp family also grouped with these two candidate genes. Previous studies implicated the ATP-grasp family in peptide bond formation, so this third gene made a perfect candidate for attaching amino acids to the core structure. With this knowledge in hand, the researchers turned to a cyanobacterium, *Anabaena*, where the identity of and structure of the main MAA, shinorine, was known. In *Anabaena*, the three genes were found together along with another peptide-bond-forming enzyme. After transferring the entire cluster of 4 genes into another host, the lab workhorse *E. coli*, remarkably, shinorine was found in the bacterial culture. Then, with purified enzymes in hand, the authors showed that while the proteins involved in installing amino acids onto the core structure display sequence properties of peptide bond forming enzymes, they actually both catalyze imine formation in an unprecedented fashion. This study displays how comparative genomics can empower the chemical biologist for mechanistic discoveries with widespread implications. **Jason G. Underwood, Ph.D.**