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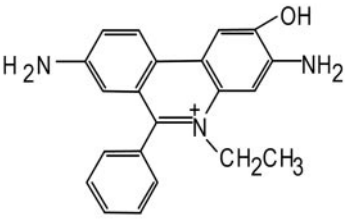
BIOPHYSICS

Quantifying intracellular superoxide

According to Hongtao Zhao *et al.*, HPLC and fluorescence using hydroethidine (HE) as a probe can detect and quantify intracellular superoxide anions (O_2^-), which is essential for testing and screening drug compounds. Prior research by the team showed that both chemically and enzymatically generated superoxide can react with HE to form a specific fluorescent product different from E^+, 2-hydroxyethidium (2-OH-E^+). Zhao *et al.* treated animal cells with menadione, a quinone known to generate intracellular superoxide, followed by incubation with HE. The authors found that the peak height of 2-OH-E^+ was nearly 3-fold greater than that of E^+ over a range of menadione concentrations. Extracellular 2-OH-E^+ and E^+ levels were slightly elevated by menadione treatment, and superoxide dismutase blocked the formation of 2-OH-E^+ without affecting intracellular levels. The researchers used the spin trap, 5-tert-butoxycarbonyl-5-methyl 1-pyrroline *N*-oxide (BMPO), in addition to menadione and HE, and observed a significant reduction in the amount of both intra- and extracellular 2-OH-E^+. When ceramide, a synergistic inducer of intracellular reactive oxygen species, was added to the cells, the authors observed an increase in 2-OH-E^+.



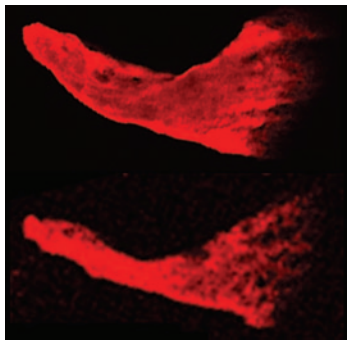
2-Hydroxyethidium (2-OH-E^+).

"Detection and characterization of the product of hydroethidine and intracellular superoxide by HPLC and limitations of fluorescence" by Hongtao Zhao, Joy Joseph, Henry M. Fales, Edward A. Sokoloski, Rodney L. Levine, Jeannette Vasquez-Vivar, and B. Kalyanaraman (see pages 5727–5732)

EVOLUTION

Few genes may trigger evolution of bony fish

To study the role of skeletal morphology changes in vertebrate evolution, Charles Kimmel *et al.* examined variations in a facial bone of the Alaskan threespine stickleback fish. The authors analyzed the size, shape, and development of the opercle, a large facial bone that supports the gill covers, in oceanic (ancestral) and lake (derived) sticklebacks. When normalized to body length, the opercles of oceanic sticklebacks were larger and more elongated in the dorsal–ventral axis than those from lake sticklebacks. Both fish showed the first signs of mineralization 7 days after fertilization (shortly after hatching), but the opercle in oceanic fish began laying down more bone by 9 days after fertilization. Quantitative trait loci analysis detected a strong genetic linkage between these morphological differences and linkage group 19. These results suggest that the skeletal differences between oceanic and lake sticklebacks are established early, within 7–9 days after fertilization. According to the authors, an early developmental event, possibly involving the genetic loci identified in this study, likely set up the different developmental trajectories for these two populations of fish.



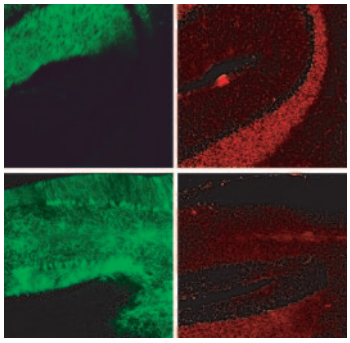
Oceanic (*Upper*) and lake (*Lower*) stickleback bone, 9 days after fertilization.

"Evolution and development of facial bone morphology in threespine sticklebacks" by Charles B. Kimmel, Bonnie Ullmann, Charline Walker, Catherine Wilson, Mark Currey, Patrick C. Phillips, Michael A. Bell, John H. Postlethwait, and William A. Cresko (see pages 5791–5796)

MEDICAL SCIENCES

RNA interference for Huntington's disease therapy

RNA interference (RNAi), induced by short hairpin RNAs, may prove useful in treating Huntington's disease (HD), according to Scott Harper *et al.* HD develops as a result of a polyglutamine repeat expansion and subsequent toxic gain of function of the protein encoded by the huntingtin (htt) gene. Although therapies targeting downstream and possibly indirect effects of disease allele expression have been tested, no therapies that directly reduce mutant htt gene expression have yet been described. Harper *et al.* investigated the utility of RNAi in a transgenic mouse model of HD and found that RNAi directed against the mutant human htt gene reduced htt mRNA and protein expression in cell culture and in HD mouse brain. Additionally, htt gene silencing improved behavioral and neuropathological abnormalities associated with HD. Because RNAi decreased HD symptoms in a mouse model, the authors suggest that these data support the further development of RNAi for HD therapy.



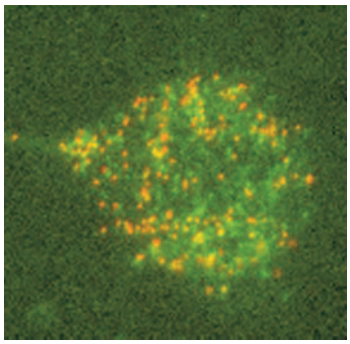
RNAi reduces htt immunoreactivity in Huntington's disease mouse model.

"RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model" by Scott Q. Harper, Patrick D. Staber, Xiaohua He, Steven L. Eliason, Inês H. Martins, Qinwen Mao, Linda Yang, Robert M. Kotin, Henry L. Paulson, and Beverly L. Davidson (see pages 5820–5825)

MICROBIOLOGY

Tegument proteins help route herpesvirus

Tegument proteins, which lie between the viral capsid and membrane envelope, route herpesviruses to either the cell bodies or axon terminals of neurons, according to Gant Luxton *et al*. The α-herpesviruses, which cause cold sores and shingles, enter sensory neurons, where they take up lifelong residence. When the viruses become reactivated, the progeny virus particles travel down axons to the periphery, resulting in physical symptoms. The viral proteins associated with the microtubule motors that allow this transport remain unknown. To determine which viral proteins were involved in trafficking, Luxton *et al.* used correlative motion analysis to simultaneously track fluorescently labeled capsid and tegument proteins in living neurons. The researchers found that the tegument proteins are the key components of the capsid transport complex; when tegument proteins were associated with the capsid, the viral particles moved toward the axon (anterograde motion). Conversely, when tegument proteins were removed, viral particles moved toward the cell body (retrograde motion). Identifying tegument proteins as an important component of the capsid transport complex reveals a key mechanistic step in the infectious cycle of human herpesvirus.



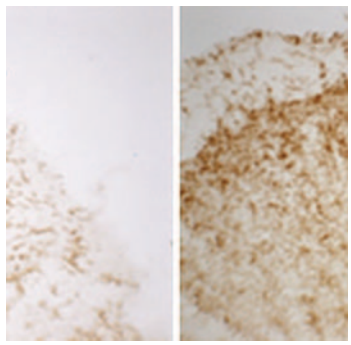
Viral tegument proteins at axon terminals.

"Targeting of herpesvirus capsid transport in axons is coupled to association with specific sets of tegument proteins" by G. W. Gant Luxton, Sarah Haverlock, Kelly Elizabeth Coller, Sarah Elizabeth Antinone, Andrew Pincetic, and Gregory Allan Smith (see pages 5832–5837)

NEUROSCIENCE

Reducing the Toll of neuropathic pain

Flobert Tanga *et al.* report that Toll-like receptor 4 (TLR4) plays a critical role in inducing neuropathic pain. Expressed exclusively by microglia, TLR4 has been implicated in behavioral hypersensitivity, a model of neuropathic pain whereby the CNS overreacts to sensory input. To determine whether TLR4 directly contributes to neuropathic pain, Tanga *et al.* transected the lumbar 5 spinal nerve in mice lacking a functional TLR4. To assess hypersensitivity, the researchers measured how the mice responded when their hindpaws were exposed to tactile or thermal stimulation. The authors observed that hypersensitivity was attenuated in TLR4-deficient mice compared with controls, and TLR4-deficient mice exhibited decreased expression of proinflammatory cytokines and markers for activated microglia. These results demonstrate that activation of microglial TLR4 contributes to the initiation of the CNS immune response, leading to proinflammatory cytokine release and behavioral hypersensitivity. Further understanding of TLR4-induced hypersensitivity may provide an opportunity to regulate microglial activation and alleviate chronic pain caused by nerve damage.



Microglial activation reduced in TLR4-knockout (*Left*) vs. wild-type (*Right*) mice.

"The CNS role of Toll-like receptor 4 in innate neuroimmunity and painful neuropathy" by Flobert Y. Tanga, Nancy Nutile-McMenemy, and Joyce A. DeLeo (see pages 5856–5861)