In This Issue

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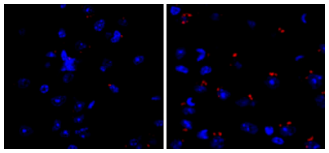
AMPK amplifies Huntington's disease



Injection of an AMPK activator (right) increased the number of apoptotic neurons (red) in the striatum of mice with HD.

Ju et al. describe how hyperactivation of AMP-activated protein kinase (AMPK) promotes neurodegeneration in Huntington's disease (HD).

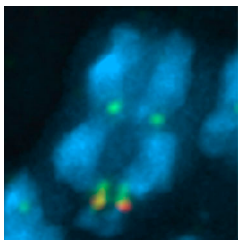
The aggregation of mutant Huntingtin protein in HD disrupts many cellular processes, including metabolism. AMPK—a protein that maintains energy homeostasis—is abnormally active in the brains of mice with HD, but whether the kinase protects neurons from the metabolic imbalances associated with HD or whether AMPK contributes to neuronal death is unknown.

Ju et al. determined that the α1 isoform of AMPK was specifically activated and translocated into the nuclei of neurons in a mouse model of HD, whereas AMPK-α2 was unaffected. An inhibitor of Ca^{2+}/calmodulin-dependent protein kinase II reduced AMPK activity, suggesting that AMPK-α1 is activated by this kinase, probably because Ca^{2+} signaling is disrupted in HD neurons. Further stimulation of AMPK by injection of the AMPK-activating drug AICAR increased neuronal death and decreased the lifespan of HD mice. AICAR also promoted the death of neuronal cell lines, an effect reversed by an AMPK inhibitor. Active, nuclear AMPK-α1 promoted neuronal apoptosis by reducing expression of the survival factor Bcl2. Bcl2 levels and cell survival were restored by CGS21680, a drug that alleviates the symptoms of HD mice.

AMPK was also hyperactivated in the brains of human HD patients, suggesting that the kinase could be a therapeutic target. Senior author Yijuang Chern now wants to investigate how AMPK-α1 and -α2 isoforms are differentially regulated in neuronal tissue.

Ju, T.-C., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201105010.

HJURP puts the centromere in place



The kinetochore protein NDC80 (green) is recruited to an ectopic location by the N terminus of HJURP fused to LacI (red).

The histone chaperone HJURP directs the formation of functional centromeres by assembling the histone variant CENP-A into chromatin, Barnhart et al. reveal.

CENP-A is a specialized version of histone H3 that marks the position of centromeres. A protein called HJURP helps deliver CENP-A to centromeric chromatin, but whether HJURP simply protects CENP-A until the histone is incorporated or whether the protein actively assembles CENP-A into nucleosomes is unclear.

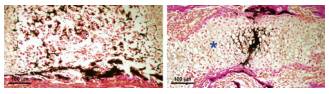
Barnhart et al. targeted HJURP to noncentromeric chromatin by tagging it with the Lac repressor protein LacI and expressing this fusion protein in cells carrying an array of LacI-binding DNA sequences on one of their chromosomes. LacI-HJURP bound to this array and stably incorporated CENP-A into the underlying chromatin. Moreover, additional centromeric and kinetochore proteins were also recruited to the array, allowing it to stably attach to spindle microtubules during mitosis.

A short N-terminal fragment of HJURP fused to LacI was sufficient to assemble these ectopic kinetochores. This same fragment—which contains the Scm3 domain conserved in yeast homologues of HJURP—could also assemble CENP-A into DNA-bound nucleosomes in vitro. Centromeric nucleosomes have been proposed to differ in size and structure from regular nucleosomes, but the CENP-A nucleosomes formed by HJURP in vitro were octameric and contained negatively supercoiled DNA, just like canonical nucleosomes.

Barnhart et al. found that HJURP normally requires the centromeric protein complex Mis18 to localize to centromeres. Senior author Daniel Foltz now wants to investigate how the Mis18 complex associates with centromeres and how it recruits HJURP and CENP-A.

Barnhart, M.C., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201012017.

Sphingomyelinase helps bones get their minerals



Compared to wild type (left), the absence of black staining indicates the reduced mineralization of bones lacking nSMase2 (right).

Khavandgar et al. demonstrate that bone-forming osteoblasts require the enzyme neutral sphingomyelinase 2 (nSMase2) to mineralize the extracellular matrix during skeletal development.

nSMase2 cleaves sphingomyelin to generate ceramide and other bioactive lipids. Mice lacking nSMase2 have severe skeletal abnormalities such as shortened and bent limb bones. The precise nature of these skeletal defects has remained unclear, however, as has the site of nSMase2's action. Some studies have suggested that nSMase2 acts in the brain to regulate endocrine signals controlling bone development.

Khavandgar et al. analyzed mice lacking nSMase2 activity due to a chemically induced mutation called *fragilitas ossium* (*fro*) and found that although their osteoblasts differentiated and secreted collagen matrix as normal, they failed to mineralize this matrix with calcium and inorganic phosphate. Mutant osteoblasts also failed to mineralize in culture. In addition, the long bones of *fro/fro* embryos contained increased numbers of hypertrophic chondrocyte-like cells that normally die during long bone growth. Restoring wild-type nSMase2 expression to the osteoblasts of *fro/fro* mice rescued the bone mineralization and skeletal defects of these animals. Osteoblast-specific nSMase2 expression failed to boost hypertrophic chondrocyte apoptosis, however, indicating that nSMase2 has tissue-specific functions during skeletal development.

The results may explain why some osteogenesis imperfecta patients have bone mineralization defects despite having intact collagen genes and normal levels of mineral ions in their serum. Senior author Monzur Murshed now wants to investigate how nSMase2 promotes mineralization. The enzyme may regulate the release of specialized matrix vesicles from osteoblasts that initiate the mineralization process.

Khavandgar, Z., et al. 2011. *J. Cell Biol.* doi:10.1083/jcb.201102051.