BIOCHEMISTRY

High-throughput identification of kinase phosphorylation targets

Combining chemical genetic and proteomic techniques, Noah Dephoure et al. developed a high-throughput method for identifying substrates of yeast protein kinases. Kinases are highly conserved and constitute one of the largest gene families, but the identification of kinase phosphorylation substrates, which is essential for understanding the enzyme’s cellular role, has been labor intensive. To simplify this task, Dephoure et al. made use of a collection of yeast strains in which each strain produced a single, epitope-tagged protein expressed from its native chromosomal locus, under the control of its endogenous promoter. Extracts were generated from these strains, and a mutant, analog-specific kinase utilizing radiolabeled ATP in the phosphorylation reaction was added. In a screen for substrates of the yeast cyclin-dependent kinase Pho85, 34 candidate proteins were isolated from 4,250 yeast strains. Further proteolysis techniques allowed the authors to identify 24 protein targets that Pho85 directly phosphorylates. These 24 substrates included the known Pho85 substrate Rvs167, cell maintenance proteins, cell growth proteins, and proteins that direct yeast budding. — F.A.

"Combining chemical genetics and proteomics to identify protein kinase substrates" by Noah Dephoure, Russell W. Howson, Justin D. Blethrow, Kevan M. Shokat, and Erin K. O’Shea (see pages 17940–17945)

CELL BIOLOGY

Mitochondrial DNA role in aging without free radicals

Aleksandra Trifunovic et al. show that mice with acquired mutations in their mitochondrial DNA (mtDNA) do not overproduce reactive oxygen species (ROS), although the animals do experience a reduction in lifespan. The free radical theory of aging suggests that intracellular ROS, such as superoxide and hydrogen peroxide, can cause age-associated cellular damage. Previous animal research has correlated the rate of ROS formation with maximal lifespan. Trifunovic et al. utilized a homozygous knock-in mouse line expressing an error-prone version of mtDNA polymerase γ (PolgAmut). These mice accumulated somatic mtDNA mutations and aged prematurely. Measuring oxygen consumption, the respiration of embryonic fibroblast cells from PolgAmut mice was <5% of wild-type cells. No differences in the superoxide and hydrogen peroxide levels in wild-type and mutant fibroblasts were observed. Also, no differences in hydrogen peroxide-induced necrosis or apoptosis of the cells were observed. The activity of mitochondrial aconitase, an enzyme used as a marker of oxidative damage, and the levels of superoxide dismutase, an antioxidant defense enzyme induced in response to increased ROS levels, were found to be normal in the mitochondria of the mutant animals. — F.A.

"Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production" by Aleksandra Trifunovic, Anna Hansson, Anna Wredeenberg, Anja T. Rovio, Eric Dufour, Ivan Khvorostov, Johannes N. Spelbrink, Rolf Wibom, Howard T. Jacobs, and Nils-Göran Larsson (see pages 17993–17998)
**MICROBIOLOGY**

**Avian influenza vaccination in chickens**

J. A. van der Goot et al. report that vaccination of poultry may be an effective tool to prevent the spread of highly pathogenic avian influenza (HPAI) viruses. Although it has been shown that vaccination can protect chickens from overt disease and mortality, the effectiveness of vaccination in preventing transmission from animal to animal has been unclear. The authors studied the effect of vaccination on the transmission characteristics of the virus HPAI A/Chicken/Netherlands/03 H7N7 in chickens. Infected and uninfected chickens were housed together, and the chain of infection was monitored by virus isolation and serology. The authors’ analysis was based on a stochastic SEIR (susceptible, latently infected, infectious, recovered) epidemic model. Vaccination was found to reduce the transmission level to such an extent that a major outbreak was prevented, with important variables between the type of vaccine used and the time of challenge after vaccination. One week after vaccination, the H7N1 vaccine was more effective than the H7N3 vaccine in reducing spread of the H7N7 virus. Two weeks after vaccination, both vaccines tested were shown to completely block transmission. — R.N.

“Quantification of the effect of vaccination on transmission of avian influenza (H7N7) in chickens” by J. A. van der Goot, G. Koch, M. C. M. de Jong, and M. van Boven (see pages 18141–18146)

**NEUROSCIENCE**

**Bone marrow stem cells promote neural growth**

James Munoz et al. demonstrate the proliferation of endogenous neural stem cells (NSCs) in mice after implantation of human bone marrow stem cells (MSCs). Previous research has shown that NSCs in the adult brain can differentiate in response to central nervous system injury, but this neurogenesis does not fully compensate for the neural loss. Munoz et al. surgically implanted human MSCs into the dentate gyrus of the hippocampus of adult immunodeficient mice, and found that up to 26% of the implanted cells survived at 3 days after implantation. Marker immunostaining showed little proliferation of the MSCs but showed increased proliferation of endogenous mouse cells in the hippocampus. Proliferating NSCs began to migrate and differentiate 7 days after implantation and were found to express doublecortin (marker for migrating neural cells), nestin (neural progenitor marker), GFAP (astrocyte marker), and NG2 (oligodendrocyte progenitor marker). By 30 days after implantation, the cells ceased the production of these markers and began expressing the markers of mature neurons. The cells at this stage exhibited elaborate processes immunoreactive for nerve growth factor and vascular endothelial growth factor. In addition, the human MSCs promoted the expression of neurotrophins by endogenous cells, which promote neurogenesis. — F.A.

“Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice” by James R. Munoz, Brooke R. Stoutenger, Andrew P. Robinson, Jeffrey L. Spees, and Darwin J. Prockop (see pages 18171–18176)

**PSYCHOLOGY**

**Off-line learning during sleep and wake**

Daniel Cohen et al. report that the memory of the movement component of a task is consolidated while awake, whereas the goal is consolidated during sleep, lending support to the notion that the brain continues to process memories for skills “off-line.” Learning a new skill requires the brain to process the movement and goal aspects of a task separately. Cohen et al. trained a group of 40 right-handed individuals in a sequence learning task, testing the participants’ performance, and then retested that performance after a 12-h interval to measure improvement. The intervals were either during the day (8:00 a.m. to 8:00 p.m.) or over a night of sleep (8:00 p.m. to 8:00 a.m.). Significant improvements in movement only occurred during the day, whereas goal-based improvements developed only during the night. The authors concluded that the exact time of when practicing a task takes place dramatically affects the nature of the off-line skill improvements. These observations demonstrate that sleep and wake play distinct and complementary roles during consolidation, with each enhancing different aspects of a skill memory. — B.T.