Published Online: 12 May, 2003 | Supp Info: http://doi.org/10.1083/jcb1613iti5

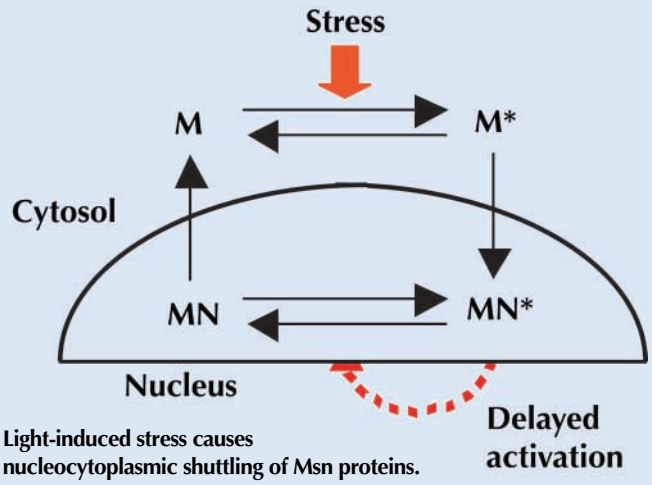
In This Issue

Stressed cells dance on a bright stage

While studying the stress response of yeast cells, Jacquet et al. (page 497) discovered a new type of oscillatory process that can control gene expression. In addition to creating a computational model that should help to direct future studies of cell stress, the authors identified a sort of biological Heisenberg effect, in which the process of observing certain cells under the microscope could significantly influence their physiology.

In the yeast *Saccharomyces cerevisiae,* two related transactivators, Msn2 and Msn4, translocate from the cytoplasm to the nucleus in response to a wide variety of stresses. Using high resolution time-lapse video microscopy, Jacquet et al. examined the translocation of an Msn2-GFP hybrid protein in single cells. Under the bright light of the fluorescence microscope, Msn2 migrates to the nucleus, indicating that light generates a stress response in GFP-expressing cells.

Rather than simply translocating to the nucleus, Msn2 and Msn4 display an unexpected oscillatory pattern in the light-exposed cells, synchronously shuttling into and out of the nucleus with a periodicity of a few minutes. The oscillations only occur at intermediate stress levels; high stress causes Msn2 and Msn4 to remain in the nucleus, whereas at low stress levels the proteins remain in the cytoplasm. The oscillatory behavior varies between individual cells and does not require new protein synthesis.



Light-induced stress causes nucleocytoplasmic shuttling of Msn proteins.

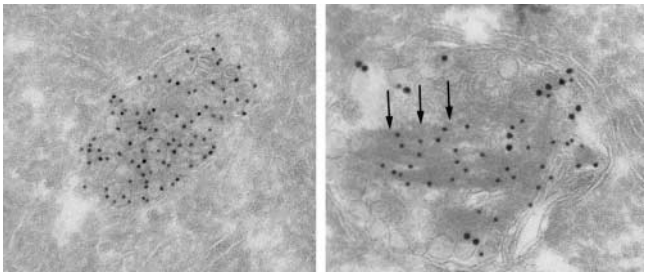
A computational model of the stress response predicts that one or more additional components make up an autoregulatory loop that primes Msn2 and Msn4 for export from the nucleus. Similar autoregulatory models explain oscillatory phenomena like calcium waves and biological clocks. Because it does not require new protein synthesis, though, the oscillation of Msn2 and Msn4 constitutes a new class of periodic process. The authors are now searching for additional components of the autoregulatory loop in yeast. ■

Cut protein to boost fibers

In a new analysis of melanosome biogenesis, Berson et al. (page 521) demonstrate that the proteolytic cleavage of a glycoprotein drives the formation of the characteristic fibrous striations seen in these organelles. The work uncovers a general mechanism that may regulate the development of lysosome-related organelles in a variety of cell types, and also shows a striking parallel between the experimentally tractable melanocyte system and the complex pathogenesis of amyloid diseases.

Melanosomes, specialized organelles that store melanin pigments, develop intralumenal fibrils superficially similar to those seen in amyloid diseases, but little is known about how these fibrils form. Previous work identified an apparent paradox, suggesting that the melanosome fibrils do not contain membrane, but do contain the integral membrane glycoprotein Pmel17.

The new work resolves this issue, showing that Pmel17 must be cleaved by proprotein convertases to initiate fibril formation. A cleavage product is eventually released into the lumen of the melanosome and incorporated into fibrils. Since many cell types develop specialized lysosome-related organelles, proprotein convertases or other proteases may be general initiators of similar morphogenetic processes for a wide range of organelles.



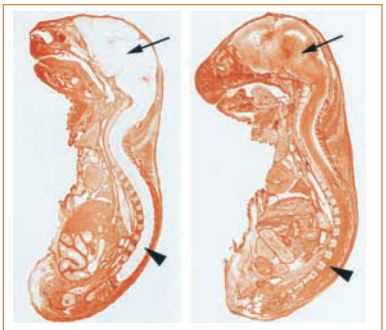
Cleavage site deficient Pme17 localizes to MVBs (left) but fails to form fibers formed by wild type (arrows, right).

Besides illuminating a previously obscure aspect of organelle biogenesis, the work suggests a strong similarity between normal melanosome fibril formation and the pathogenic fibril formation that occurs in amyloid diseases. Some pathogenic amyloid proteins are specifically cleaved by proprotein convertases, and proteolytic processing is also a general feature of Alzheimer's disease and prion diseases. Berson et al. propose that proteolytic maturation is a normal step in lysosome-related organelle biogenesis, and pathogenic variations in the process may drive inherited organelle defects as well as amyloid diseases. The authors are now trying to reproduce melanosome fibril formation in vitro, and hope to use the system as a model for understanding both normal and pathogenic fibril formation. ■

Text By Alan W. Dove
alanwdove@earthlink.net

Breaking down barriers

On page 653, Nitta et al. show that removal of the tight junction component claudin-5 makes the murine blood–brain barrier (BBB) selectively permeable to small molecules. Although considerably more work is needed before this approach can be applied clinically, the finding could be a boon for drug delivery to the central nervous system.



Impairment of the BBB in cluadin-5 knock-out mice (right).

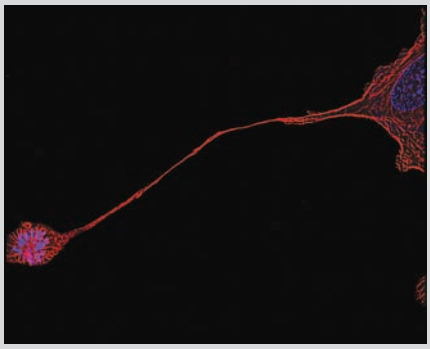
Although it was first described over a century ago, it has been shown more recently that the BBB consists of intercellular tight junctions, which prevent most molecules in the bloodstream from reaching the brain. In the new work, the authors found that the tight junctions of the BBB are primarily composed of the membrane proteins claudin-5 and claudin-12. Mice with a homozygous deletion in the claudin-5 gene appear to develop normally, and do not show signs of bleeding or edema, but do exhibit a striking abnormality in tracer experiments. Although the BBB of the knockout animals still blocks large molecules, it permits molecules smaller than ~800 D to pass into the central nervous system.

Unfortunately, all of the claudin-5 knockout mice died within ten hours of birth for unknown reasons. The authors are now trying to generate conditional deletions to characterize the functions of claudin-5 and claudin-12 in more detail. ■

Getting a handle on centrosomes

Centrosomes are required for cytokinesis and are important in cell cycle progression—but how are centrosomes connected to these essential cellular functions at the molecular level? On page 535, Gromley et al. describe the maternal centriole protein centriolin, the first integral centrosome protein linked to both cytokinesis and cell cycle progression in vertebrate cells.

Overexpression, siRNA silencing, or antibody inhibition of centriolin causes an unusual cytokinesis defect, in which cells remain connected by long strands of cytoplasm and form syncytia. Some of the cells later undergo cell cycle arrest in G1/G0, and some undergo apoptosis. The cytokinesis defect is caused by a domain in centriolin that shares homology with yeast regulatory proteins in the MEN/SIN pathway, which controls yeast mitotic exit and cytokinesis.



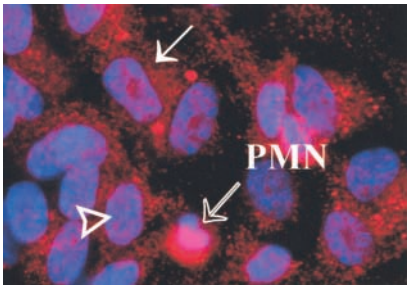
Centriolin deficiency disrupts cytokinesis.

The results suggest that centriolin links centrosomes to a critical cytokinesis regulatory system and possibly to a cell cycle checkpoint. Screening work is now uncovering additional members of the cytokinesis pathway. Centriolin also contains domains with homology to proteins implicated in human tumorigenesis. Since centrosome defects would cause aneuploidy, a hallmark of cancer, the authors are now trying to determine whether centriolin has oncogenic functions. ■

The neutrophil as firefighter

Severe bacterial infection or trauma frequently leads to a systemic inflammatory response, a self-reinforcing activation of neutrophils and vascular endothelial cells that can be deadly. On page 641, Cepinskas et al. describe a neutrophil-mediated signaling mechanism that inhibits inflammation. The findings demonstrate a novel function for neutrophils and a previously unknown form of immunological tolerance, and they identify a promising target for new anti-inflammatory drugs.

In systemic inflammation, circulating cytokines cause the transcription factor NFκB to translocate from the cytoplasm to the nucleus of vascular endothelial cells, where it induces the transcription of pro-inflammatory genes. Using a cell culture model of inflammation, the authors found that the migration of neutrophils across a monolayer of cytokine-activated endothelial cells causes NFκB levels in the endothelial cell nuclei to drop. Cross-linking the adhesion molecule PECAM-1 on the surface of the endothelial cells produces the same effect, suggesting that the neutrophils send anti-inflammatory signals to the endothelium through PECAM-1. Exposing the neutrophil-calmed endothelial cells to a second round of cytokine activation results in even further reduction of the pro-inflammatory response. The authors are now trying to determine what controls this novel type of induced tolerance at the molecular level. ■



Transendothelial migration of neutrophils affects the localization of NFκB in endothelial cells.