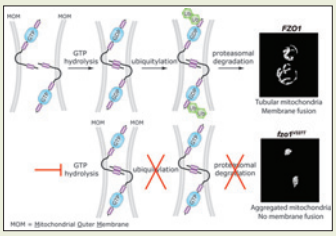
MBC Editor's Note

The December 2009 issues of *Molecular Biology of the Cell* include even more than the usual number of fine papers. Thus this edition of InCytes from *MBC* highlights eight papers instead of the usual four. The first four have clear relevance to disease and serve to illustrate the potential contribution of cell biology to human health. The second four papers are excellent contributions to basic research.

Beginning with the next issue of the *ASCB Newsletter,* InCytes from *MBC* will be replaced by Highlights from *MBoC.* Highlights from *MBoC* will include shorter descriptions of more articles. Incoming Editor-in-Chief David Drubin has instituted this change to better publicize the many excellent papers in the journal.

—Sandra L. Schmid, Editor-in-Chief
Molecular Biology of the Cell



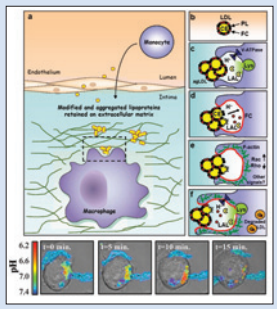
A Mutation Associated with CMT2A Neuropathy Causes Defects in Fzo1 GTP Hydrolysis, Ubiquitylation, and Protein Turnover

Elizabeth A. Amiott, Mickael M. J. Cohen, Yann Saint-Georges, Allan M. Weissman, and Janet M. Shaw

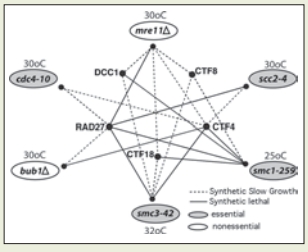
Mutation of the human mitochondrial fusion GTPase *MFN2* causes Charcot-Marie-Tooth neuropathy (CMT2A) by an unknown mechanism. The authors used the yeast ortholog *FZO1* to investigate molecular defects associated with disease-linked mutations. Each CMT2A mutation produced different in vivo and in vitro effects. The most severe GTPase domain mutation (V327T) caused loss of mitochondrial fusion and GTPase activity. The authors show that loss of GTPase activity leads to reduced Fzo1 ubiquitylation and degradation, causing stabilization of the mutant proteins. Importantly, the V327T disease mutation is not dominant negative. Complexes containing wild-type Fzo1 and V327T are GTPase active, and restore mutant protein ubiquitylation and turnover. Loss of the F-box protein Mdm30 does not affect GTPase activity, placing GTP hydrolysis upstream of Fzo1 ubiquitylation. These studies link Fzo1 GTP hydrolysis to ubiquitylation and turnover. They also raise the possibility that mitofusin ubiquitylation and degradation play a role in the etiology of CMT2A.

Macrophages Create an Acidic Extracellular Hydrolytic Compartment to Digest Aggregated Lipoproteins

Abigail S. Haka, Inna Grosheva, Ethan Chiang, Adina R. Buxbaum, Barbara A. Baird, Lynda M. Pierini, and Frederick R. Maxfield



A critical event in atherosclerosis is the interaction of vessel wall macrophages with aggregates of low density lipoprotein (LDL). LDL aggregates are formed by the action of lipases and other chemical modifications and bound to extracellular matrix components. Previous data suggested some hydrolysis of cholesteryl esters by macrophages while the bulk of aggregated LDL is outside the cell. This hydrolysis required lysosomal acid lipase and thus it was surmised that the cholesteryl ester hydrolysis occurred in lysosomes. In this study, the authors show that macrophages create an extracellular, acidic compartment where the cells contact the aggregated LDL. Lysosomal contents are delivered to these compartments, thereby forming an extracellular hydrolytic compartment—a lysosomal synapse. The catabolism of aggregates was observed by timelapse imaging. An increase in free cholesterol was seen in aggregates in these compartments. This cholesterol can be delivered to the cell, initiating the process of macrophage cholesterol loading.



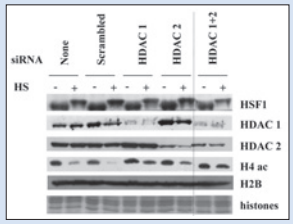
Synthetic Lethal Genetic Interactions That Decrease Somatic Cell Proliferation in *Caenorhabditis elegans* Identify the Alternative RFC^{CTF18} as a Candidate Cancer Drug Target

Jessica McLellan, Nigel O'Neil, Sanja Tarailo, Jan Stoepel, Jennifer Bryan, Ann Rose, and Philip Hieter

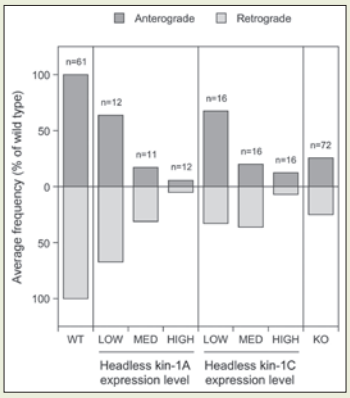
Somatic mutations causing chromosome instability (CIN) in tumors are an Achilles' heel that can be exploited for selective killing of tumor cells by knockdown of second-site genes that cause synthetic lethality. Cancer CIN gene mutations often cause aneuploidy at levels that are tolerated in somatic cells but not tolerated in the developing embryo. The authors have developed an assay system in the multicellular animal *Caenorhabditis elegans* that uses the post-embryonic vulval cell lineage as a readout to test genetic interactions causing synthetic lethality (SL) in proliferating somatic cells. This approach was used to validate, in *C. elegans*, yeast SL genetic interactions involving members of the cohesin complex known to be mutated in colon tumors. The authors identified conserved SL interactions with *CTF4*, *RAD27*, and components of the alternative RFC^{CTF18} complex. This cross-species SL with CIN approach using the *C. elegans* assay system has strong potential to identify new cancer therapeutic targets.

Heat Shock Factor 1 Controls Genome-wide Acetylation in Heat-shocked Cells

Sabrina Fritah, Edwige Col, Cyril Boyault, Jérôme Govin, Karine Sadoul, Susanna Chiocca, Elisabeth Christians, Saadi Khochbin, Caroline Jolly, and Claire Vourc'h



The cellular response to heat shock is characterized, at the transcriptional level, by the activation of hsp genes, which correlates with a global repression of most cellular genes. Here, the authors show that heat shock factor 1 (HSF1), the key transactivator of hsp genes, also plays a major role in the global shutdown of transcription. They show that global deacetylation of core histones, driven by histone deacetylases HDAC1 and 2, necessitates the presence of active HSF1 and its interaction with HDAC1 and 2. This work thus brings to light a much wider role for HSF1 than initially assigned. HSF1 now appears to be a master regulator of global chromatin acetylation in both unstressed and heat-shocked cells.



Tight Functional Coupling of Kinesin-1A and Dynein Motors in the Bidirectional Transport of Neurofilaments

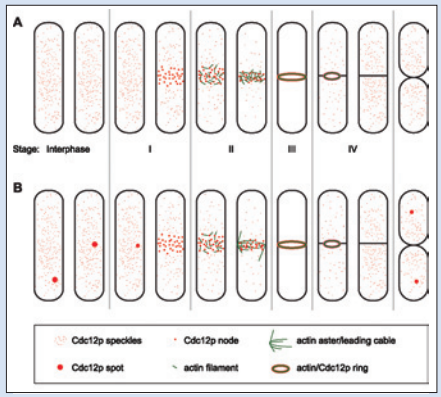
Atsuko Uchida, Nael H. Alami, and Anthony Brown

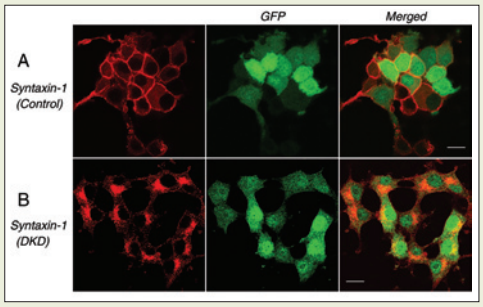
In axons, neurofilaments are transported along microtubule tracks both towards and away from the axon tip (anterograde and retrograde, respectively). These movements are thought to be powered by dynein and kinesin-1A motors, respectively. Consistent with this, the authors show that neurofilament transport is impaired in cultured neurons from kinesin-1A knockout mice and that this impairment can be rescued by full-length kinesin-1A. Neurofilament transport is also impaired by a dominant negative kinesin-1A construct and by disruption of dynein function using RNAi, function-blocking antibody, and dominant negative dynactin constructs. However, all of these perturbations block both anterograde and retrograde neurofilament movement to a similar extent. These data support the hypothesis that kinesin-1A and dynein are neurofilament motors. Importantly, they also indicate that the activities of the anterograde and retrograde neurofilament motors are tightly coupled.

Roles of Formin Nodes and Myosin Motor Activity in Mid1p-dependent Contractile-ring Assembly during Fission Yeast Cytokinesis

Valerie C. Coffman, Aaron H. Nile, I-Ju Lee, Huayang Liu, and Jian-Qiu Wu

During cytokinesis an actomyosin contractile ring assembles and constricts in coordination with mitosis to properly segregate genetic materials into two daughter cells. The molecular mechanism of contractile-ring assembly remains poorly understood and controversial. The authors test several assumptions of the two prevailing models for contractile-ring assembly during cytokinesis in the fission yeast *Schizosaccharomyces pombe*: the spot/leading cable model and the search, capture, pull, and release (SCPR) model. The two models differ in their predictions for the number of initiation sites of actin assembly and in the role of myosin-II. Live microscopy of cells expressing formin Cdc12p fluorescent fusion proteins shows that Cdc12p localizes to a broad band of 30 to 50 dynamic nodes, where actin filaments are nucleated in random directions. Perturbations of myosin-II motor activity demonstrated that it is required to condense the nodes into a contractile ring. Taken together, these data provide strong support for the stochastic SCPR model of contractile-ring formation in cytokinesis.





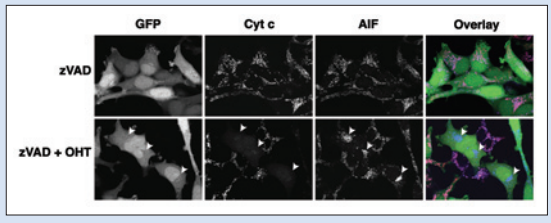
Rescue of Munc18-1 and -2 Double Knockdown Reveals the Essential Functions of Interaction between Munc18 and Closed Syntaxin in PC12 Cells

Liping Han, Tiandan Jiang, Gayoung A. Han, Nancy T. Malintan, Li Xie, Li Wang, Frederic W. Tse, Herbert Y. Gaisano, Brett M. Collins, Frederic A. Meunier, and Shuzo Sugita

The Sec1-Munc18 (SM) proteins play essential roles in secretion and specifically bind to cognate syntaxin isoforms via two distinct modes, "closed" conformation and N-terminus binding. To precisely define the contributions of each binding mode, the authors use lentiviral vectors to engineer Munc18-1/-2 double knockdown neurosecretory cells and show that expression not only of syntaxin-1 but also of syntaxin-2 and -3 is significantly reduced as a result of this double knockdown. Syntaxin-1 is mislocalized, and regulated secretion is abolished. The authors next perform rescue experiments of these knockdown cells using a number of site-specific mutants of Munc18-1 that specifically inhibit each of the two syntaxin interaction modes. Their results suggest that Munc18's binding to closed syntaxin is essential, enabling Munc18-1 to stabilize and deliver syntaxin-1 to the plasma membrane and restore secretion defects. Moreover, the interaction with the syntaxin N-terminus may be largely dispensable for neurosecretion.

Caspase-independent Mitochondrial Cell Death Results from Loss of Respiration, Not Cytotoxic Protein Release

Lydia Lartigue, Yulia Kushnareva, Youngmo Seong, Helen Lin, Benjamin Faustin, and Donald D. Newmeyer



Cells undergoing apoptosis through the typical mitochondria-driven pathway normally activate effector caspases, proteases that cleave and activate key death-promoting proteins. However, even when these caspases are inactive, the cells die. One possible explanation was that mitochondrial outer membrane permeabilization (MOMP), an early apoptotic event, allows the release of non-caspase cytotoxic proteins like AIF and EndoG. However, here the authors show that cell death is caused instead by a progressive caspase-independent loss of mitochondrial function following MOMP. Surprisingly, cells continue to divide for ~48 h, but show a slow decline in ATP levels and DNA replication rate over 48–72 h, leading to delayed proliferation arrest. The earliest defects, seen at 4–8 h after MOMP, are the complete loss of respiratory complex I and partial loss of complex IV activities. Much later, mitochondria undergo more profound degradative effects. Thus MOMP leads to progressive and probably irreversible mitochondrial damage, independent of effector caspases. ■