PNAS Plus Significance Statements

Identification of molecular hinge points mediating alternating access in the vesicular monoamine transporter VMAT2

Dana Yaffe, Sebastian Radestock, Yonatan Shuster, Lucy R. Forrest, and Shimon Schuldiner

Regulated release of neurotransmitters is made possible by activity of transporters that mediate their accumulation into synaptic vesicles. Vesicular monoamine transporter (VMAT), a member of the largest superfamily of transporters, mediates transport of monoamines. One of the structural features of these transporters is the pseudo symmetry between the first and the second half of the protein. We demonstrate (pp. E1332–E1341) the importance of two anchor points between these two domains that provide hinge points about which the two halves of the protein flex and straighten to open and close the translocation pathway, a process that enables alternating exposure of the substrate-binding site.

Interchangeable adaptors regulate mitochondrial dynamin assembly for membrane scission

Sajjan Koirala, Qian Guo, Raghav Kalia, Huyen T. Bui, Debra M. Eckert, Adam Frost, and Janet M. Shaw

Mitochondrial fission is critical for mammalian cell division, mitophagy, and development. Fission initiates via recruitment of dynamin-related GTPases to the mitochondrial surface. In yeast and human, the recruitment utilizes adaptors that differ in sequence and predicted structure. Key unresolved issues are whether these adaptors function independently in membrane recruitment and whether a single adaptor and GTPase are sufficient to catalyze scission. We show (pp. E1342–E1351) that three human adaptors work interchangeably with a single mitochondrial dynamin to accomplish fission. We also show that an adaptor alters the architecture of the dynamin polymer in a manner that could facilitate membrane constriction and severing.

Hypoxia induces a phase transition within a kinase signaling network in cancer cells

Wei Wei, Qihui Shi, Francoise Remacle, Lidong Qin, David B. Shackelford, Young Shik Shin, Paul S. Mischel, R. D. Levine, and James R. Heath

Reduced oxygen supply—hypoxia—is a near-universal feature of solid tumors that can alter how tumors respond to therapies. We investigated (pp. E1352–E1360) the transition from normoxia to hypoxia in model brain cancer systems, using single-cell proteomics and data analysis tools based on physicochemical concepts. This approach permits the simplification of otherwise complex biology. We find a hypoxia-induced switch within a mammalian target of rapamycin (mTOR) signaling network. At the switching point, mTOR is predicted, and then shown by experiment, to be unresponsive to inhibition. These results may help explain the undistinguished performance of mTOR inhibitors in certain clinical trials.

Integrin-dependent force transmission to the extracellular matrix by α-actinin triggers adhesion maturation

Pere Roca-Cusachs, Armando del Rio, Eileen Puklin-Faucher, Nils C. Gauthier, Nicolas Biais, and Michael P. Sheetz

Mechanical forces transmitted between a cell and its surrounding extracellular matrix determine functions like proliferation or differentiation, and drive processes in development, tumorigenesis, and wound healing. However, the molecules involved in this force transmission remain unclear. Here (pp. E1361–E1370) we show that forces exerted by cells are transmitted to the extracellular matrix through α-actinin molecules via the transmembrane protein integrins. Furthermore, this transmission enables the growth and maturation of adhesion sites to the matrix, and takes place in competition with another molecule submitted to force, talin. This force regulation mechanism may help us understand the role of force in different biological scenarios.

Viral infection modulation and neutralization by camelid nanobodies

Aline Desmyter, Carine Farenc, Jennifer Mahony, Silvia Spinelli, Cecilia Bebeacua, Stéphanie Blangy, David Veesler, Douwe van Sinderen, and Christian Cambillau

Lactococcal siphophages infect *Lactococcus lactis*, a Gram-positive bacterium used in commercial dairy fermentations. The phage TP901-1 baseplate (BP) recognizes and binds specifically to polysaccharides covering the host cell. We raised (pp. E1371–E1379) llama nanobodies against the BP as tools to dissect the molecular determinants of phage infection. Using complementary techniques, we identified BP binders and determined their affinity and epitopes for their targets and their impact on phage infectivity. X-ray structures revealed that two nanobodies block the BP saccharide binding site, and viral infection assays showed that they neutralize infection, a possible way to circumvent phages detrimental effect on dairy fermentation.

www.pnas.org/cgi/doi/10.1073/pnas.ss11015

Enterocyte loss of polarity and gut wound healing rely upon the F-actin–severing function of villin

Florent Ubelmann, Mathias Chamaillard, Fatima El-Marjou, Anthony Simon, Jeanne Netter, Danijela Vignjevic, Buford L. Nichols, Roberto Quezada-Calvillo, Teddy Grandjean, Daniel Louvard, Céline Revenu, and Sylvie Robine

Intestinal epithelium damage is common but becomes recurrent in chronic intestinal disorders. Healing implies cell migration, which necessitates extensive cellular reorganization. We demonstrate (pp. E1380–E1389) that intestinal epithelial cells completely disassemble their apical actin-based microvilli upon migration, and we identify the protein villin and its actin-severing function as responsible for this physiological process. We show that this apical pole effacement is required for the acquisition of a motile phenotype and efficient wound healing. These findings demonstrate how intestinal epithelial cells acquired a mechanism at the level of the actin cytoskeleton to convert efficiently from a highly differentiated to a motile polarity.

Cell-free study of F plasmid partition provides evidence for cargo transport by a diffusion-ratchet mechanism

Anthony G. Vecchiarelli, Ling Chin Hwang, and Kiyoshi Mizuuchi

ParA-type partition systems self-organize and pattern the bacterial nucleoid to organize plasmids, chromosomes, and protein machinery spatially. To study how protein patterns generate cargo movement, we reconstituted and visualized the partition system of F plasmid using a DNA-carpeted flowcell as an artificial nucleoid surface. We found (pp. E1390–E1397) that the partition proteins could bridge plasmid to the DNA carpet dynamically and mediate plasmid motion. Our data favor a diffusion-ratchet mechanism inherently different from classical motor protein or actin/microtubule filament-based transport. We expect surface-mediated patterning to become increasingly recognized as a means of intracellular transport in all kingdoms of life.

New World cattle show ancestry from multiple independent domestication events

Emily Jane McTavish, Jared E. Decker, Robert D. Schnabel, Jeremy F. Taylor, and David M. Hillis

Cattle were independently domesticated from the aurochs, a wild bovine species, in the vicinity of the current countries of Turkey and Pakistan ~10,000 y ago. Cattle have since spread with humans across the world, including to regions where these two distinct lineages have hybridized. Using genomic tools, we investigated (pp. E1398–E1406) the ancestry of cattle from across the world. We determined that the descendants of the cattle brought to the New World by the Spanish in the late 1400s show ancestry from multiple domesticated lineages. This pattern resulted from pre-Columbian introgression of genes from African cattle into southern Europe.

Functional link between bone morphogenetic proteins and insulin-like peptide 3 signaling in modulating ovarian androgen production

Claire Glister, Leanne Satchell, Ross A. D. Bathgate, John D. Wade, Yanzhenzi Dai, Richard Ivell, Ravinder Anand-Ivell, Raymond J. Rodgers, and Philip G. Knight

Ovarian androgen synthesis is essential for normal ovarian follicle development and female fertility in animals and humans. However, ovarian androgen excess, a feature of the widespread polycystic ovarian syndrome in women, is detrimental to fertility and has other pathophysiological consequences. Our findings (pp. E1426–E1435) reveal the importance of the intraovarian growth factor insulin-like peptide 3 signaling for maintaining androgen production by ovarian theca cells and show that the suppressive action of bone morphogenetic proteins on androgen production is linked to their inhibitory effect on insulin-like peptide 3 signaling, likely mediated via down-regulation of the nuclear transcription factor steroidogenic factor-1.