PNAS Plus Significance Statements

Effect of cholesterol on the molecular structure and transitions in a clinical-grade lung surfactant extract

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Cholesterol is currently removed from most lung surfactant extract preparations used in clinical applications. Cholesteroldepleted samples are also used in most in vitro studies of the lung surfactant layer. In our study we have performed a detailed molecular characterization of the structure of the lung surfactant with regard to cholesterol content and temperature. We show that cholesterol has a strong impact on the phase behavior, structure, and dynamics of the lung surfactant system even at low concentrations. We emphasize the importance of controlling the cholesterol content of the lung surfactant system studied to better mimic the endogenous lung surfactant and develop better preparations for clinical treatments. (See pp. E3592–E3601.)

SNAT7 is the primary lysosomal glutamine exporter required for extracellular protein-dependent growth of cancer cells

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Lysosomes are degradative intracellular organelles essential to cell maintenance and homeostasis. Although their degradative function is well documented, the proteins responsible for the efflux, and reuse, of lysosomal degradation products remain largely unknown. In this study, we identify the transporter responsible for lysosomal efflux of glutamine, an amino acid central to several key metabolic pathways. This central role of glutamine is exploited by several types of cancer cells with increased consumption of glutamine. Interestingly, genetic inactivation of the transporter impairs their growth under conditions of limited glutamine availability when internalized extracellular proteins are used as an alternative source of amino acids, suggesting novel approaches for anticancer therapies. (See pp. E3602–E3611.)

Using microsecond single-molecule FRET to determine the assembly pathways of T4 ssDNA binding protein onto model DNA replication forks

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A microsecond-resolved single-molecule FRET method was used to monitor the binding and unbinding of the ssDNA binding protein (gene product 32) of the T4 bacteriophage replication complex to biologically relevant primer-template DNA constructs. A unique multitime correlation function analysis was applied to the resulting sparse data, which permitted the investigation of the kinetics and mechanisms of noncooperative and cooperative protein binding, unbinding, and "sliding." Our results indicate that noncooperatively bound monomer proteins dissociate on the timescale of tens of milliseconds, which is consistent with the known rate of nucleotide addition during DNA replication. The rapid dissociation of the monomer suggests that sliding is a much more likely mechanism for translocation of cooperatively bound clusters of indeterminate size. (See pp. E3612–E3621.)

Structural features and lipid binding domain of tubulin on biomimetic mitochondrial membranes

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Tubulin has emerged as a highly unexpected component of mitochondrial membranes involved in regulation of membrane permeability. This discovery has reawakened interest in the nature of the tubulin–membrane interaction to answer a new question: How does tubulin, a cytosolic protein famous for its role in microtubule structure and dynamics, come to target mitochondrial membranes? Here, using a combination of five biophysical methods, we study peripheral binding of tubulin to biomimetic membranes of different lipid compositions. We conclude that tubulin distinguishes between lamellar and nonlamellar lipids through a highly conserved amphipathic binding motif. Specifically, α-tubulin targets cell and organelle membranes by sensing lipid-packing defects via an amphipathic α-helix, with broad consequences for both normal cellular function and disease. (See pp. E3622–E3631.)

NGF-TrkA signaling in sensory nerves is required for skeletal adaptation to mechanical loads in mice

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Peripheral sensory nerves expressing TrkA, the high-affinity receptor for NGF, densely innervate the surfaces of long bones, a privileged location for the regulation of biomechanical signaling. In this study,

we used several genetically engineered mouse models to examine the role of NGF-TrkA signaling in skeletal adaptation to mechanical loads. Our results support a model in which mechanical signals up-regulate the expression of NGF in osteoblasts on the bone surface that, in turn, activates TrkA sensory nerves, leading to the release of osteogenic cues that modulate osteocytic Wnt/β-catenin signaling and support bone formation. (See pp. E3632–E3641.)

Drebrin restricts rotavirus entry by inhibiting dynamin-mediated endocytosis

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Many clinically significant human viral and bacterial pathogens use dynamin-dependent endocytosis to initiate infection or deliver toxin into host cells. Owing to the complex nature of this cellular process, the molecular mechanisms that regulate this pathway remain to be fully elucidated. Here, we use rotavirus (RV) as a model and identify drebrin as a regulatory protein that restricts the cell entry of multiple viruses. We demonstrate that genetic depletion or chemical inhibition of drebrin leads to enhanced RV infection in vitro and increased diarrhea incidence and virus shedding in vivo. Our current study provides insights into endocytosis regulation in general and highlights the potential broad application of blocking drebrin to augment the uptake of viruses and other dynamin-mediated cargo. (See pp. E3642–E3651.)

Discovery of chemoautotrophic symbiosis in the giant shipworm *Kuphus polythalamia* (Bivalvia: Teredinidae) extends wooden-steps theory

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Certain marine invertebrates harbor chemosynthetic bacterial symbionts, giving them the remarkable ability to consume inorganic chemicals such as hydrogen sulfide (H_2S) rather than organic matter as food. These chemosynthetic animals are found near geochemical (e.g., hydrothermal vents) or biological (e.g., decaying wood or large animal carcasses) sources of H_2S on the seafloor. Although many such symbioses have been discovered, little is known about how or where they originated. Here, we demonstrate a new chemosynthetic symbiosis in the giant teredinid bivalve (shipworm) *Kuphus polythalamia* and show that this symbiosis arose in a wood-eating ancestor via the displacement of ancestral cellulolytic symbionts by sulfur-oxidizing invaders. Here, wood served as an evolutionary stepping stone for a dramatic transition from heterotrophy to chemoautotrophy. (See pp. E3652–E3658.)

Single-cell analysis of HIV-1 transcriptional activity reveals expression of proviruses in expanded clones during ART

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Previously, we showed that the virus that persists in human immunodeficiency virus (HIV)-infected individuals on antiretroviral therapy (ART) is derived from cells infected prior to initiating treatment. We also showed that HIV-infected cells can undergo cellular proliferation during ART. However, it is not known what fraction of infected cells that persist during ART are latent and what fraction are actively producing HIV RNA. The method described here was developed to determine the fraction of infected cells that produce HIV RNA and the levels of HIV RNA in single cells, including cells that have undergone cellular proliferation. Additionally, the method can be used to identify the sources of rebound virus after stopping ART and the efficacy of experimental interventions designed to cure HIV infection. (See pp. E3659–E3668.)

Neurophysiological dynamics of phrase-structure building during sentence processing

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According to most linguists, the syntactic structure of sentences involves a tree-like hierarchy of nested phrases, as in the sentence [happy linguists] [draw [a diagram]]. Here, we searched for the neural implementation of this hypothetical construct. Epileptic patients volunteered to perform a language task while implanted with intracranial electrodes for clinical purposes. While patients read sentences one word at a time, neural activation in left-hemisphere language areas increased with each successive word but decreased suddenly whenever words could be merged into a phrase. This may be the neural footprint of "merge," a fundamental tree-building operation that has been hypothesized to allow for the recursive properties of human language. (See pp. E3669–E3678.)

Defective synaptic connectivity and axonal neuropathology in a human iPSC-based model of familial Parkinson's disease

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Parkinson's disease (PD) is an incurable neurodegenerative disorder characterized by motor and nonmotor deficits, including cognitive decline and dementia. The protein αSyn is strongly associated with PD pathogenesis, whereas αSyn mutations, such as p.A53T, cause familial forms of PD. Animal models are crucial for understanding PD pathogenesis, but there are limitations in the extent to which these models reproduce faithfully the human disease. Cell-reprogramming technologies allow the generation of human neurons from patients with PD, but it has proven difficult to identify cellular pathologies in induced pluripotent stem cell–derived neurons. In this study, we created a robust p.A53T patient–derived model of PD that captures disease-related phenotypes under basal conditions, thus providing a unique system for studies of disease mechanisms and development of therapeutics. (See pp. E3679–E3688.)

Selective in vivo removal of pathogenic anti-MAG autoantibodies, an antigen-specific treatment option for anti-MAG neuropathy

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Anti-MAG (myelin-associated glycoprotein) neuropathy is a rare but disabling autoimmune disorder affecting the peripheral nervous system. The pathogenicity of anti-MAG IgM autoantibodies

that target the HNK-1 glycoepitope is well established. Current therapies are mostly immunosuppressive but so far are neither approved nor sufficiently effective. Therefore we designed a glycopolymer that acts as an autoantibody scavenger by mimicking the natural HNK-1 glycoepitope and demonstrated that the glycopolymer neutralizes disease-causing antibodies in patient sera. Moreover, pathogenic antibodies were removed efficiently in an immunological mouse model of anti-MAG neuropathy. Because clinical improvement of patients' neuropathic symptoms correlates with reduced serum levels of anti-MAG antibodies, the glycopolymer represents a promising antigen-specific therapeutic option for the treatment of this neuropathy. (See pp. E3689–E3698.)

Dissociation of *Per1* and *Bmal1* circadian rhythms in the suprachiasmatic nucleus in parallel with behavioral outputs

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The circadian clock in the suprachiasmatic nucleus (SCN) regulates seasonality in physiology and behavior, which is best characterized by the change in the activity time of behavioral rhythms. In nocturnal rodents, the activity time was shortened in long summer days and lengthened in short winter days because of the change in the phase relationship of activity onset and offset, for which different circadian oscillators are predicted. Taking advantage of in vivo monitoring of clock gene expression in freely moving mice, we demonstrated that the circadian rhythms of *Per1* and *Bmal1* in the SCN are associated differentially with the phase shifts of activity onset and offset, respectively, suggesting the existence of two oscillations with different molecular mechanisms in timing of circadian behavior. (See pp. E3699–E3708.)

Adult enteric nervous system in health is maintained by a dynamic balance between neuronal apoptosis and neurogenesis

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The demonstration of a robust neurogenesis program in the adult gut and the existence of an enteric neural precursor cell (ENPC) responsible for the same has profound biological and clinical implications. This demonstrates the presence of robust adult neurogenesis outside of the CNS, and indicates the vulnerability of the enteric nervous system to exogenous influences, even in adults. As an example, it is possible that acquired diseases of the enteric nervous system, such as achalasia, may result from a loss of ENPC, analogous to congenital disorders, such as Hirschsprung's. The ability to identify the adult ENPC will therefore enable a new understanding of the pathogenesis of enteric neuromuscular diseases as well as the development of novel regenerative therapies. (See pp. E3709–E3718.)

Immunomodulation-accelerated neuronal regeneration following selective rod photoreceptor cell ablation in the zebrafish retina

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Recent evidence suggests human retinal Müller glia retain a potential for neuronal regeneration. Defining the mechanisms governing retinal repair in robustly regenerative species may provide insights for harnessing this potential therapeutically. Here, we investigated roles of the innate immune system during rod photoreceptor regeneration in zebrafish. Our data establish a role for retinal microglia, the tissue-resident macrophage of the retina, in regulating retinal Müller glia responsiveness to cell death, and thereby controlling photoreceptor regeneration kinetics. Further, we show that immunosuppression can either inhibit or accelerate photoreceptor regeneration kinetics depending on the timing of treatment. We conclude that modulation of immune cell responses to retinal neuron cell death stands as a promising strategy for promoting repair of the human eye. (See pp. E3719–E3728.)

Hippo pathway mediates resistance to cytotoxic drugs

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Cytotoxic therapy is still the backbone of effective chemotherapy, although most current pharmaceutical interest is in targeted therapy. Our findings concern a general adaptive response of cells that causes resistance to gemcitabine and 15 other FDA-approved cytotoxic drugs when cells are grown at high density. Although on the surface cell confluence seems like it could be an irrelevant property of cells in culture, our work shows that it is very relevant to tumors in mice and retrospectively to the success of chemotherapy in humans. On a fundamental cell biological level, these studies identify a previously unappreciated function of the enigmatic Hippo pathway, which controls this response. "Switching-off" this pathway could present an opportunity to overcome drug resistance in pancreatic cancer. (See pp. E3729–E3738.)

Knockout of the LRRC26 subunit reveals a primary role of LRRC26-containing BK channels in secretory epithelial cells

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Ca^{2+}- and voltage-regulated K^+ channels (termed BK channels) are expressed in a diverse variety of cells, playing distinct physiological roles often defined by cell-specific regulatory subunits. Here, genetic deletion of one particular regulatory subunit, LRRC26, reveals that LRRC26-containing BK channels are found, perhaps exclusively, in secretory epithelial cells, including salivary glands, airways, and gastrointestinal tract. Such cells mediate fluid, peptide, and mucus secretion, influencing digestion, airway function, gut resistance to infection, and lactation. The absence of LRRC26 in secretory epithelial cells renders BK channels inactive during normal physiological conditions and alters ion efflux from salivary gland. LRRC26-containing BK channels are critical for normal ionic flux in secretory epithelial cells, likely impacting on a variety of epithelial cell pathologies. (See pp. E3739–E3747.)