PNAS Plus Significance Statements

Autophagy and ubiquitin–proteasome system contribute to sperm mitophagy after mammalian fertilization

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Maternal inheritance of mitochondria and mitochondrial genes is a major developmental paradigm in mammals. Propagation of paternal, sperm-contributed mitochondrial genes, resulting in heteroplasmy, is seldom observed in mammals, due to postfertilization targeting and degradation of sperm mitochondria, referred to as "sperm mitophagy." Our and others' recent results suggest that postfertilization sperm mitophagy is mediated by the ubiquitin–proteasome system, the major protein-turnover pathway that degrades proteins and the autophagic pathway. Here we demonstrate that the co-inhibition of the ubiquitin-binding autophagy receptors, sequestosome 1 (SQSTM1) and gamma-aminobutyric acid receptor-associated protein (GABARAP), and the ubiquitinated protein dislocase valosin-containing protein (VCP)-dependent pathways delayed postfertilization sperm mitophagy. Our findings provide the mechanisms guiding sperm mitochondrion recognition and disposal during preimplantation embryo development, which prevents a potentially detrimental effect of heteroplasmy. (See pp. E5261–E5270.)

2-Sulfonylpyrimidines: Mild alkylating agents with anticancer activity toward p53-compromised cells

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Cancers with mutant p53 often show increased metastasis, genomic instability, and higher chemoresistance. The development of drugs targeting tumors with mutant p53 background is a current strategy for anticancer therapy. We found that certain activated electrophilic 2-sulfonylpyrimidines are a new class of thiol-reactive anticancer agents. These agents are especially effective in killing cancer cells with mutant or inactivated p53 or impaired reactive oxygen species detoxification and have relatively low cytotoxicity toward normal cells; they are mild electrophiles, some of which will, for example, stabilize mutant p53 by selective targeting of its thiol groups and have little general alkylating reactivity. (See pp. E5271–E5280.)

Impact of membrane lipid composition on the structure and stability of the transmembrane domain of amyloid precursor protein

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Aggregation of proteins of known sequence is linked to a variety of neurodegenerative disorders. Familial mutations in the amyloid precursor protein (APP), from which the amyloid β (Aβ) protein is excised, are associated with early onset of Alzheimer's disease. The structures of APP-C99 dimers and the associated stability as well as the monomer–dimer equilibrium are critically influenced by membrane composition. Using a multiscale modeling approach, we have investigated the influence of varying lipid composition on the structure of homodimers of an APP-C99 congener peptide. Besides resolving contradicting experimental results, we demonstrate that membrane lipid composition dramatically influences the relative populations of competing homodimer structures in a way that is linked to the recognition and processing of APP-C99 by γ-secretase. (See pp. E5281–E5287.)

Emergence of ion channel modal gating from independent subunit kinetics

Brendan A. Bicknell and Geoffrey J. Goodhill

Many key features of the behavior of cells are controlled by ion channels—pores in cell membranes that are sometimes open and sometimes closed. It is therefore critically important to understand what controls these opening and closing events. This is challenging because ion channels exhibit stochastic dynamics over several timescales, from the rapid kinetics of a single opening to the slow switching between distinct levels of activity known as modal gating. By mathematically modeling the basic biophysical events that control ion channel opening, we introduce a new principle for understanding the origin of modal gating. Although we focus on the inositol 1,4,5-trisphosphate receptor channel, the framework can be applied more generally to other ion channels. (See pp. E5288–E5297.)

Paradoxical signaling regulates structural plasticity in dendritic spines

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The basis for learning and memory formation is in tiny (1 – 2 μm) membranous protrusions along short branched extensions (dendrites) of nerve cells (neurons) called dendritic spines. Recently, common themes have begun to emerge in identifying the cellular basis of many conditions associated with learning and memory, mainly through the observation of dynamic changes to the dendritic spine. The ability of the dendritic spine to grow, shrink, and change shape has long been associated with synaptic plasticity, learning, and memory. We develop a mathematical model that couples the biochemical signaling machinery and the actin remodeling events to

provide insight into the dynamics of the dendritic spine. (See pp. E5298–E5307.)

Binding of EBP50 to Nox organizing subunit p47^{phox} is pivotal to cellular reactive species generation and altered vascular phenotype

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Our findings identify a previously unidentified role for scaffolding protein ezrin-radixin-moesin-binding phosphoprotein 50 (EBP50; aka NHERF1) in the activation of NADPH oxidases (Nox), a family of professional reactive oxygen species (ROS) producing enzymes implicated in numerous pathologies. We demonstrate that EBP50 is critical for agonist-induced production of ROS superoxide anion ($O_2^{\bullet-}$), and that it directly associates with the Nox organizing subunit p47^{phox}. EBP50 deletion abolishes angiotensin II-induced cellular hypertrophy and resistance artery vasoconstriction. Given the wide array of EBP50 cellular interactions and the ubiquity of Nox, the current findings support a broader, more complex orchestration of Nox regulation than is currently hypothesized. The findings could augment future strategies targeting this interaction in disease involving aberrant ROS, tissue remodeling, and/or smooth muscle constriction. (See pp. E5308–E5317.)

Rab5 and its effector FHF contribute to neuronal polarity through dynein-dependent retrieval of somatodendritic proteins from the axon

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Ras-related proteins in brain (Rab) GTPases are general regulators of intracellular traffic in eukaryotic cells, but their specialized roles in neurons are poorly understood. Here we report that Rab5 contributes to the somatodendritic polarity of various surface receptors by mediating their retrieval from the axon. This retrieval is dependent on the Rab5 effector Fused Toes (FTS)–Hook–FTS and Hook-interacting protein (FHIP) (FHF), which in turn interacts with the minus-end–directed microtubule motor dynein–dynactin to drive retrograde transport of receptor-containing carriers from the axon to the soma. These findings reveal a mechanism for coupling axonal retrograde carriers to dynein–dynactin and demonstrate that the somatodendritic polarity of various receptors results from a combination of biosynthetic sorting, the barrier function of the axon initial segment, and retrieval from the axon. (See pp. E5318–E5327.)

Combination therapy with BPTES nanoparticles and metformin targets the metabolic heterogeneity of pancreatic cancer

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There are no effective therapies currently available for advanced pancreatic cancer. We show that there are two populations of cancer cells within a pancreatic tumor that require targeting by different metabolic inhibitors for effective tumor control. Rapidly dividing cells use glutamine, and can be effectively killed by administration of a nanoparticle containing an inhibitor of glutamine metabolism. Hypoxic cells, which are slowly dividing cells, metabolize glucose and can be targeted by metformin, a drug used for the treatment of diabetes. Clinical trials are needed to determine whether combination therapy, with drugs that effectively block the metabolism of glutamine and glucose, improves the survival of patients with pancreatic cancer. (See pp. E5328–E5336.)

Vibrio cholerae biofilm growth program and architecture revealed by single-cell live imaging

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Biofilms are surface-associated bacterial communities embedded in an extracellular matrix. Connections between biofilm architectural, material, and mechanical features have never been systematically studied at the individual cell level due to inadequate optical resolution. Here, we develop imaging, experimental, and modeling tools to analyze living, growing bacterial biofilms at single-cell resolution. We discover that *Vibrio cholerae* biofilms undergo a 2D-to-3D transition as a consequence of directional cell division and anisotropic pressure caused by cell-to-surface adhesion. Moreover, deletion of a single gene responsible for cell-to-cell adhesion changes the biofilm growth mode from directional cell growth to expansion caused by the extracellular matrix. The technology reported here enables future studies of single-cell gene expression in bacterial communities. (See pp. E5337–E5343.)

Stromal uptake and transmission of acid is a pathway for venting cancer cell-generated acid

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Metabolism energizes cancer growth, but only if its end product, acid, is removed effectively. A bottleneck for acid handling is slow diffusion across the underperfused extracellular milieu of hypoxic tumors. Here, we characterize the acid-handling mechanisms operating in stromal myofibroblasts that can improve the flow of acid through tumors. We show that myofibroblasts are high-capacity reservoirs that absorb excess extracellular acidity, via the AE2 transporter, and transmit this acid load across a syncytium fused by channels, such as connexin-43. Furthermore, the cytokine TGFβ1, which orchestrates many cancer–stromal interactions, can stimulate acid uptake and transmission in stromal cells with lower baseline activities. Because many colorectal cancer cells do not express AE2 and connexin-43, acid traffic would be routed preferentially through the stromal compartment of tumors. (See pp. E5344–E5353.)

pH determines the energetic efficiency of the cyanobacterial CO_2 concentrating mechanism

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Cyanobacteria are responsible for roughly 10% of global photosynthetic primary production of reduced carbon. Although cyanobacteria are incredibly diverse, all known species contain a complex protein system called the CO_2 concentrating mechanism (CCM), which enables rapid growth even in environments with extremely limited CO_2. The CCM enables cyanobacteria to accumulate HCO_3^- and convert this inorganic carbon pool to utilizable CO_2. We demonstrate here that a quantitative description of the CCM must include the effect of pH on the abundance of HCO_3^- and H_2CO_3. This pH-dependent description is consistent with cyanobacterial physiology. Furthermore, the model predicts that alkaline cytosolic pH reduces the energetic cost of the CCM, consistent with pH measurements of photosynthesizing cyanobacteria. (See pp. E5354–E5362.)