PNAS Plus Significance Statements

Bacterial formate hydrogenlyase complex

Jennifer S. McDowall, Bonnie J. Murphy, Michael Haumann, Tracy Palmer, Fraser A. Armstrong, and Frank Sargent

The isolation of an active formate hydrogenlyase is a breakthrough in understanding the molecular basis of bacterial hydrogen production. For over 100 years, *Escherichia coli* has been known to evolve H_2 when cultured under fermentative conditions. Glucose is metabolized to formate, which is then oxidized to CO_2 with the concomitant reduction of protons to H_2 by a single complex called formate hydrogenlyase, which had been genetically, but never biochemically, characterized. In this study (pp. E3948–E3956), innovative molecular biology and electrochemical experiments reveal a hydrogenase enzyme with the unique ability to sustain H_2 production even under high partial pressures of H_2. Harnessing bacterial H_2 production offers the prospect of a source of fully renewable H_2 energy, freed from any dependence on fossil fuel.

Pleckstrin homology domain leucine-rich repeat protein phosphatases set the amplitude of receptor tyrosine kinase output

Gloria Reyes, Matt Niederst, Ksenya Cohen-Katsenelson, Joshua D. Stender, Maya T. Kunkel, Muhan Chen, John Brognard, Emma Sierecki, Tianyan Gao, Dawid G. Nowak, Lloyd C. Trotman, Christopher K. Glass, and Alexandra C. Newton

This work (pp. E3957–E3965) unveils a previously unidentified function of the tumor suppressor pleckstrin homology domain leucine-rich repeat protein phosphatase (PHLPP) in inhibiting oncogenic signaling by suppressing the steady-state levels of receptor tyrosine kinases such as the EGF receptor. Specifically, PHLPP modifies the histone code to control the transcription of receptor tyrosine kinases. This epigenetic function can account for the upregulation of receptor tyrosine kinases in the multiple cancer types where PHLPP function is compromised.

Aberrant calcium signaling by transglutaminase-mediated posttranslational modification of inositol 1,4,5-trisphosphate receptors

Kozo Hamada, Akiko Terauchi, Kyoko Nakamura, Takayasu Higo, Nobuyuki Nukina, Nagisa Matsumoto, Chihiro Hisatsune, Takeshi Nakamura, and Katsuhiko Mikoshiba

Reversible and repetitive structural changes are essential for ligand-gated ion channels to mediate biological signaling. The inositol 1,4,5-trisphosphate receptor (IP_3R) assembles ligand-gated ion channels that mediate calcium signaling. IP_3 activates channels at a distance by reversible allosteric changes in the IP_3R tetramer. Here (pp. E3966–E3975) we show a new mode of posttranslational modification that irreversibly blocks allosteric changes in the IP_3R. We identified an IP_3R-modifying enzyme as tissue transglutaminase that inhibits IP_3R function by locking subunit configurations. This modification chronically impaired calcium signaling and autophagy regulation in living cells, and up-regulated modification was observed in Huntington disease models. To our knowledge, this is the first demonstration of transglutaminase-catalyzed posttranslational modification in ligand-gated channel allostery and provides a new framework for enzymatic regulation of allostery.

Phosphatidylethanolamine deficiency disrupts α-synuclein homeostasis in yeast and worm models of Parkinson disease

Shaoxiao Wang, Siyuan Zhang, Liang-Chun Liou, Qun Ren, Zhaojie Zhang, Guy A. Caldwell, Kim A. Caldwell, and Stephan N. Witt

We tested the hypothesis that a form of mitochondrial dysfunction alters the homeostasis of the cytosolic Parkinson disease (PD)-associated protein α-synuclein (α-syn). Using yeast and worm models of PD, we show (pp. E3976–E3985) that low levels of phosphatidylethanolamine (PE), caused by the depletion of mitochondrial phosphatidylserine decarboxylase (psd), lead to decreased respiration, endoplasmic reticulum (ER) stress, high levels of α-syn and cytoplasmic α-syn foci, and slow growth. Ethanolamine, which replenishes PE through the Kennedy pathway, diminished ER stress, decreased the level of α-syn, eliminated foci, and restored growth of *psd1*Δ cells to near wild-type levels. A low level of mitochondrial PE disrupts the homeostasis of α-syn and leads to the accumulation of cytoplasmic foci of this protein.

Motor coupling through lipid membranes enhances transport velocities for ensembles of myosin Va

Shane R. Nelson, Kathleen M. Trybus, and David M. Warshaw

Intracellular cargo transport is carried out by ensembles of cytoskeleton-based molecular motors, such as myosin Va. Physiologically, motor molecules are bound to (and mechanically coupled through) the vesicular membrane, which is a fluid lipid bilayer. Utilizing a combination of experiment and computer simulation, we characterize the influence of three distinct aspects of the vesicular ensemble (vesicle size, membrane composition, and motor density) on cargo transport. We also demonstrate the presence of vesicle populations that travel at velocities up to twice the unloaded velocity of a single motor. These findings (pp. E3986–E3995) serve to bridge the gap between enhanced vesicular velocities measured in vivo and depressed velocities measured in vitro.

Increased Aurora B activity causes continuous disruption of kinetochore–microtubule attachments and spindle instability

Marta Muñoz-Barrera and Fernando Monje-Casas

During mitosis, Aurora B kinase plays a key role in ensuring that sister chromatids (each of the copies of a replicated chromosome) attach to different poles of the spindle to create a bipolar array of microtubules that allows correct distribution of the chromosomes.

Aurora B deficiency leads to massive defects in chromosome segregation. Surprisingly, an increase in Aurora B activity also is deleterious for the cells and has been associated with various cancers. Here (pp. E3996–E4005) we demonstrate that in yeast an increase in Aurora B activity causes defects in chromosome segregation and spindle-assembly checkpoint activation by erroneously destabilizing even correct attachments of the chromosomes to the spindle, and also promotes premature collapse of the spindle midzone.

Genetic, anatomic, and clinical determinants of human serum sterol and vitamin D levels

Ashlee R. Stiles, Julia Kozlitina, Bonne M. Thompson, Jeffrey G. McDonald, Kevin S. King, and David W. Russell

Cholesterol is the major sterol in blood and in excess causes cardiovascular disease. In addition to cholesterol, numerous other sterols of unknown function and pathogenicity circulate in the bloodstream. Here (pp. E4006–E4014), we use chemical methods to screen for over 60 different sterols and sterol derivatives in the sera of 3,230 clinically well-characterized individuals. Twenty-seven sterols and two sterol derivatives (vitamin D_2 and D_3) were routinely detected in vastly different amounts in a majority of individuals. Genes, ethnicity, gender, age, clinical phenotype, and anatomy were identified as significant sources of interindividual variation in these lipid metabolites.

Absence of SUN-domain protein Slp1 blocks karyogamy and switches meiotic recombination and synapsis from homologs to sister chromatids

Christelle Vasnier, Arnaud de Muyt, Liangran Zhang, Sophie Tessé, Nancy E. Kleckner, Denise Zickler, and Eric Espagne

Meiosis is the specialized cellular program that generates gametes for sexual reproduction. In the fungus Sordaria macrospora karyogamy is required to produce the diploid cell that enters the meiotic program. In absence of the mid-Sad1p, UNC-84–domain sun like protein 1, karyogamy does not occur. Meiosis nonetheless proceeds efficiently in the two haploid nuclei, but with the entire program of interhomolog events now occurring instead between sister chromatids, including spatially patterned recombination and synaptonemal complex formation. As a result, significant levels of gametes are still formed. In contrast, other cases of meiosis in haploid genome complements exhibit inefficient or aberrant chromosomal programs. We thus propose that Sordaria can sense the absence of karyogamy so as to trigger an appropriately regular response (pp. E4015–E4023).

Macrophage arginase-1 controls bacterial growth and pathology in hypoxic tuberculosis granulomas

María A. Duque-Correa, Anja A. Kühl, Paulo C. Rodriguez, Ulrike Zedler, Sandra Schommer-Leitner, Martin Rao, January Weiner III, Robert Hurwitz, Joseph E. Qualls, George A. Kosmiadi, Peter J. Murray, Stefan H. E. Kaufmann, and Stephen T. Reece

Tuberculosis (TB) granulomas represent sites of both bacterial containment and tissue pathology. Macrophage killing of *Mycobacterium tuberculosis* (*Mtb*) in granulomas to contain infection must be regulated to prevent collateral tissue damage. Nitric oxide synthase-2 (NOS2) and arginase-1 (Arg1), macrophage enzymes metabolizing L-arginine, play key roles in this process. NOS2 produces reactive nitrogen intermediates to kill *Mtb*, whereas Arg1 regulates NOS2 activity via substrate competition. Arg1 activity could predominate in hypoxic regions of granulomas where NOS2 activity likely is suboptimal. Here (pp. E4024–E4032) we show that Arg1 plays a central role in restricting bacterial growth and restraining tissue damage within granulomas in TB and other chronic inflammatory diseases. These findings point to the modulation of Arg1 activity as a potential host-directed therapy for TB.

Progressive increase in mtDNA 3243A>G heteroplasmy causes abrupt transcriptional reprogramming

Martin Picard, Jiangwen Zhang, Saege Hancock, Olga Derbeneva, Ryan Golhar, Pawel Golik, Sean O'Hearn, Shawn Levy, Prasanth Potluri, Maria Lvova, Antonio Davila, Chun Shi Lin, Juan Carlos Perin, Eric F. Rappaport, Hakon Hakonarson, Ian A. Trounce, Vincent Procaccio, and Douglas C. Wallace

Mitochondria generate signals that regulate nuclear gene expression via retrograde signaling, but this phenomenon is rendered more complex by the quantitative differences in the percentage of mutant and normal mtDNAs that can exist within patient cells. This study (pp. E4033–E4042) demonstrates that depending upon its relative cytoplasmic levels, a single mtDNA point mutation can cause a discrete set of cellular transcriptional responses within cells of the same nuclear background. This qualitative regulation of nuclear gene expression by quantitative changes in mtDNA mutant levels challenges the traditional "single mutation–single disease" concept and provides an alternative perspective on the molecular basis of complex metabolic and degenerative diseases, cancer, and aging.

Plasmid-based human norovirus reverse genetics system produces reporter-tagged progeny virus containing infectious genomic RNA

Kazuhiko Katayama, Kosuke Murakami, Tyler M. Sharp, Susana Guix, Tomoichiro Oka, Reiko Takai-Todaka, Akira Nakanishi, Sue E. Crawford, Robert L. Atmar, and Mary K. Estes

Human noroviruses are the predominant cause of acute gastroenteritis worldwide, but they remain noncultivatable. A tractable system is needed to understand the host restriction to cultivation. We established a reverse genetics system driven by a mammalian elongation factor-1α promoter without helper virus. This system (pp. E4043–E4052) supports genome replication, particle formation, and particles containing a GFP-marked genomic RNA. RNA from these particles is infectious. The system also produces infectious murine norovirus, confirming its broad applicability to other noroviruses.

Visual stimuli recruit intrinsically generated cortical ensembles

Jae-eun Kang Miller, Inbal Ayzenshtat, Luis Carrillo-Reid, and Rafael Yuste

This study demonstrates that neuronal groups or ensembles, rather than individual neurons, are emergent functional units of cortical activity. We show (pp. E4053–E4061) that in the presence and absence of visual stimulation, cortical activity is dominated by coactive groups of neurons forming ensembles. These ensembles are flexible and cannot be accounted for by the independent firing properties of neurons in isolation. Intrinsically generated ensembles and stimulus-evoked ensembles are similar, with one main difference: Whereas intrinsic ensembles recur at random time intervals, visually evoked ensembles are time-locked to stimuli. We propose that visual stimuli recruit endogenously generated ensembles to represent visual attributes.