PNAS Plus Significance Statements

Stochastic initiation and termination of calcium-mediated triggered activity in cardiac myocytes

Zhen Song, Zhilin Qu, and Alain Karma

A normal heart cell behaves dynamically as an excitable element that generates a nonlinear electrical impulse in response to a suprathreshold current stimulus. However, under pathological conditions, cardiac myocytes can self-generate their own impulses after a normal excitation. This rogue behavior, known as triggered activity, can cause arrhythmias. However, how it is initiated and terminated is not fundamentally understood. We demonstrate computationally that initiation and termination are random events. Moreover, we elucidate the statistical properties of spontaneous Ca^{2+} release from intracellular stores that dictate the probability of those events and link fundamentally stochasticity at the ion channel and whole-cell levels. Our results provide mechanistic insights into cardiac arrhythmogenesis and highlight important differences between Ca^{2+} dynamics in cardiac myocytes and other eukaryotic cells. (See pp. E270–E279.)

Cell morphology drives spatial patterning in microbial communities

William P. J. Smith, Yohan Davit, James M. Osborne, Wook Kim, Kevin R. Foster, and Joe M. Pitt-Francis

Microbial communities contain cells of different shapes, and yet we know little about how these shapes affect community biology. We have developed a computational model to study the effects of microbial shape in communities. Our model predicts that shape will have strong effects on cells' positioning, and, consequently, their survival and reproduction. Rodshaped cells are better at colonizing the base of the community and its expanding edges, whereas round cells dominate the upper surface. We show that the same patterns occur in colonies of Escherichia coli, using strains with different shapes. Our work suggests that cell shape is a major determinant of patterning and evolutionary fitness within microbial communities. (See pp. E280–E286.)

Statistical mechanical model of gas adsorption in porous crystals with dynamic moieties

Cory M. Simon, Efrem Braun, Carlo Carraro, and Berend Smit

Some nanoporous, crystalline materials possess dynamic/flexible constituents, for example, a ligand that can rotate. Much like the induced-fit model of enzyme–substrate binding in biology, these dynamic moieties often change conformation when gas molecules adsorb. Such flexible constituents may endow nanoporous materials with enhanced properties for gas storage and separations, chemical sensing, drug delivery, and catalysis. We developed and solved a statistical mechanical model of gas adsorption in a porous material with a rotating ligand that is shared between cages. Our model contributes a more intimate understanding of gas adsorption in nanoporous materials with moving parts and lends insights into how to harness these dynamic constituents for adsorption-based processes. (See pp. E287–E296.)

Structural characterization of nonactive site, TrkA-selective kinase inhibitors

Hua-Poo Su, Keith Rickert, Christine Burlein, Kartik Narayan, Marina Bukhtiyarova, Danielle M. Hurzy, Craig A. Stump, Xufang Zhang, John Reid, Alicja Krasowska-Zoladek, Srivanya Tummala, Jennifer M. Shipman, Maria Kornienko, Peter A. Lemaire, Daniel Krosky, Amanda Heller, Abdelghani Achab, Chad Chamberlin, Peter Saradjian, Berengere Sauvagnat, Xianshu Yang, Michael R. Ziebell, Elliott Nickbarg, John M. Sanders, Mark T. Bilodeau, Steven S. Carroll, Kevin J. Lumb, Stephen M. Soisson, Darrell A. Henze, and Andrew J. Cooke

Signal transduction through Tropomyosin-related kinase A (TrkA), a receptor tyrosine kinase, is a target for inhibition of chronic pain and could lead to a new class of drugs against pain. Selectivity against kinases can be difficult to achieve, especially against members of the same kinase family. Structures of the compounds bound to TrkA show a binding site comprised of the kinase, which is conserved among the Trk family, and the juxtamembrane (JM), which is not well conserved. Depending on their chemical substructure, the region of the juxtamembrane that interacts with the compounds can be different, leading to differences in specificity. This study emphasizes the importance of including residues beyond the catalytic domain for small-molecule screening, importance of screening by affinity, and structural characterization to understand binding interactions. (See pp. E297–E306.)

SNX-1 and RME-8 oppose the assembly of HGRS-1/ESCRT-0 degradative microdomains on endosomes

Anne Norris, Prasad Tammineni, Simon Wang, Julianne Gerdes, Alexandra Murr, Kelvin Y. Kwan, Qian Cai, and Barth D. Grant

Endosomes are membrane-bound organelles that are important for nutrient uptake, protein and lipid sorting, and signal transduction. When integral membrane proteins have reached the endosomal system, they can be sent to the lysosome for degradation or

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recycled for reuse. Here we provide insight into how the machinery important for reuse controls the machinery that mediates degradation. We show that these opposing functions occupy physically distinct regions of the endosomes, termed microdomains, and that this separation is likely to provide a physical framework for a variety of sorting decisions. (See pp. E307–E316.)

Dual-specificity phosphatase 5 controls the localized inhibition, propagation, and transforming potential of ERK signaling

Andrew M. Kidger, Linda K. Rushworth, Julia Stellzig, Jane Davidson, Christopher J. Bryant, Cassidy Bayley, Edward Caddye, Tim Rogers, Stephen M. Keyse, and Christopher J. Caunt

The RAF-ERK kinase pathway drives cell proliferation and cancer growth. ERK kinase activity is terminated by dual-specificity MAPK phosphatases (MKP/DUSPs), which are often assumed to be tumor suppressors. We demonstrate that the MKP DUSP5 terminates nuclear ERK signaling but, surprisingly, promotes ERK activation in the cytoplasm by relieving feedback inhibition of upstream kinases. Cancer-causing RAF kinase mutations, which occur in ~8% of tumors and are refractory to feedback inhibition, reprogram DUSP5 to become a cell-wide attenuator of ERK signaling that prevents cellular senescence and promotes oncogenic transformation. Our results establish that interactions between feedback loops in the ERK cascade control localized signal promotion or suppression, which in turn govern cell proliferation and transformation. (See pp. E317–E326.)

Comprehensive population-based genome sequencing provides insight into hematopoietic regulatory mechanisms

Michael H. Guo, Satish K. Nandakumar, Jacob C. Ulirsch, Seyedeh M. Zekavat, Jason D. Buenrostro, Pradeep Natarajan, Rany M. Salem, Roberto Chiarle, Mario Mitt, Mart Kals, Kalle Pärn, Krista Fischer, Lili Milani, Reedik Mägi, Priit Palta, Stacey B. Gabriel, Andres Metspalu, Eric S. Lander, Sekar Kathiresan, Joel N. Hirschhorn, Tõnu Esko, and Vijay G. Sankaran

Human blood cell production is coordinated to ensure balanced levels of all lineages. The basis of this regulation remains poorly understood. Identification of genetic differences in human populations associated with blood cell measurements can shed light on such regulatory mechanisms. Here, we used whole-genome sequencing data to perform a genetic association study in a population-based biobank from Estonia. We identified a number of potential causal variants and underlying mechanisms. For example, we identified a regulatory element that is necessary for basophil production, which acts specifically during this process to regulate expression of the transcription factor CEBPA. We demonstrate how genome sequencing, genetic fine-mapping, and functional data can be integrated to gain important insight into blood cell production. (See pp. E327–E336.)

GAS6 is a key homeostatic immunological regulator of host–commensal interactions in the oral mucosa

Maria Nassar, Yaara Tabib, Tal Capucha, Gabriel Mizraji, Tsipora Nir, Meirav Pevsner-Fischer, Gili Zilberman-Schapira, Oded Heyman, Gabriel Nussbaum, Herve Bercovier, Asaf Wilensky, Eran Elinav, Tal Burstyn-Cohen, and Avi-Hai Hovav

Understanding the mechanisms by which the immune system and local microorganisms coexist in the oral cavity is important, as disruption of this delicate balance could cause oral and systemic diseases. We revealed that growth arrest specific 6 (GAS6), a ligand of the TYRO3–AXL–MERTK signaling system, plays a critical role in this process. Upon birth, microorganisms residing in the oral cavity induce expression of GAS6 in oral tissues; GAS6 in turn regulates antibacterial function in these tissues. We also found that GAS6 expressed by cells of the immune system further contributes to its regulatory role in oral tissues. Collectively, this work proposes that GAS6 restrains the immune response in the oral cavity to maintain coexistence with favorable microorganisms residing within the oral cavity. (See pp. E337–E346.)

Insights into the lifestyle of uncultured bacterial natural product factories associated with marine sponges

Gerald Lackner, Eike Edzard Peters, Eric J. N. Helfrich, and Jörn Piel

The candidate genus "*Candidatus* Entotheonella" belongs to a recently proposed bacterial candidate phylum with largely unknown properties due to the lack of cultivated members. Among the few known biological properties is an association of *Ca.* Entotheonella with marine sponges and an extraordinarily rich genomic potential for bioactive natural products with unique structures and unprecedented biosynthetic enzymology. Increasing evidence suggests that *Ca.* Entotheonella are widespread key producers of sponge natural products with a chemical richness comparable to soil actinomycetes. Given the unusual biology and exceptional pharmacological potential of *Ca.* Entotheonella, the bioinformatic and functional insights into their lifestyle presented here provide diverse avenues for marine natural product research, biotechnology, and microbial ecology. (See pp. E347–E356.)

Diverse evolutionary patterns of pneumococcal antigens identified by pangenome-wide immunological screening

Nicholas J. Croucher, Joseph J. Campo, Timothy Q. Le, Xiaowu Liang, Stephen D. Bentley, William P. Hanage, and Marc Lipsitch

The wealth of genomic data available for the respiratory pathogen *Streptococcus pneumoniae* enabled the design of a pangenome-wide proteome microarray. Of over 2,000 pneumococcal proteins, 208 strongly bound antibodies in adult human sera. The vast majority could be classified as either variants of four diverse loci or more conserved proteins involved in adhesion, enzymatic degradation, solute binding, or cell wall synthesis. Detailed analyses of the genomic data revealed some variable antigens rapidly diversified through mechanisms including homologous recombination, mobile genetic element transmission, and phase variation. Other antigens were conserved across the population and may be better candidates for simple vaccine formulations. This raises the question of what evolutionary advantage bacteria derive from altering only a subset of their antigenic loci. (See pp. E357–E366.)

Impact of short-chain galactooligosaccharides on the gut microbiome of lactose-intolerant individuals

M. Andrea Azcarate-Peril, Andrew J. Ritter, Dennis Savaiano, Andrea Monteagudo-Mera, Carlton Anderson, Scott T. Magness, and Todd R. Klaenhammer

Approximately 75% of the global human population are lactose malabsorbers. In a previous clinical trial, it was shown that feeding a high-purity galactooligosaccharide (>95% GOS) could improve symptoms of lactose-intolerant subjects, attaining lactose tolerance in a majority of subjects. To investigate the mechanism, we examined the microbiome of human subjects before and after GOS feeding. The results show a significant shift in the microbiome of responsive individuals, including lactose-fermenting microbes in their stools. The high-purity prebiotic GOS resulted in

adaptive shifts in the microbiome and correlated with improvement in clinical symptoms. (See pp. E367–E375.)

Characterization of cytopathic factors through genome-wide analysis of the Zika viral proteins in fission yeast

Ge Li, Melissa Poulsen, Csaba Fenyvuesvolgyi, Yoko Yashiroda, Minoru Yoshida, J. Marc Simard, Robert C. Gallo, and Richard Y. Zhao

The Zika virus (ZIKV) causes various neurologic defects including microcephaly and the Guillain-Barré syndrome. However, little is known about how ZIKV causes those diseases or which viral protein(s) is responsible for the observed cytopathic effects involved in restricted neuronal cellular growth, dysregulation of the cell cycle, and induction of cell hypertrophy or cell death. A genome-wide analysis of ZIKV proteins and peptides was conducted using fission yeast as a surrogate host. Seven ZIKV proteins conferred various cytopathic effects in which NS4A-induced cellular hypertrophy and growth restriction were mediated through the target of rapamycin (TOR) cellular stress-response pathway. These findings provide a foundation for identifying viral pathogenicity factors associated with the ZIKV diseases. (See pp. E376–E385.)

Genetic stability of genome-scale deoptimized RNA virus vaccine candidates under selective pressure

Cyril Le Nouën, Thomas McCarty, Michael Brown, Melissa Laird Smith, Roberto Lleras, Michael A. Dolan, Masfique Mehedi, Lijuan Yang, Cindy Luongo, Bo Liang, Shirin Munir, Joshua M. DiNapoli, Steffen Mueller, Eckard Wimmer, Peter L. Collins, and Ursula J. Buchholz

Recoding viral genomes by numerous synonymous substitutions provided live attenuated vaccine candidates predicted to have a low risk of reversion. However, their stability under selective pressure was largely unknown. We evaluated the phenotypic reversion of representative genome-scale deoptimized human respiratory syncytial virus (RSV) vaccine candidates in the context of strong selective pressure. We found that a virus bearing a deoptimized L-polymerase ORF evolved to escape temperature sensitivity restriction by mutations in L and multiple other proteins. Additional analysis revealed that single mutations in the M2-1 ORF were able to substantially escape the restriction imposed by the deoptimized polymerase. Based on this information, we generated a stable deoptimized RSV vaccine candidate with improved attenuation and immunogenicity suitable for additional development. (See pp. E386–E395.)

Astrocyte-derived interleukin-15 exacerbates ischemic brain injury via propagation of cellular immunity

Minshu Li, Zhiguo Li, Yang Yao, Wei-Na Jin, Kristofer Wood, Qiang Liu, Fu-Dong Shi, and Junwei Hao

Ischemic stroke is a leading cause of death and disability worldwide. Evidence indicates the detrimental effects of lymphocyte infiltration into the ischemic brain. However, a knowledge gap exists relating to the brain-specific cellular constituents and environmental factors that dictate the phenotype and function of infiltrating lymphocytes. Astrocytes bridge interactions between ischemic neurons and lymphocytes. We show that brain ischemia induces robust up-regulation of astrocytic interleukin-15 (IL-15). The present study was directed toward understanding the role of astrocyte-derived factors such as IL-15 in stroke. We discovered that astrocytic IL-15 is necessary and sufficient to amplify cell-mediated immune responses that promote ischemic brain injury. These results provide definitive evidence on the role of astrocyte-derived IL-15 in ischemic brain injury. (See pp. E396–E405.)

REST corepressors RCOR1 and RCOR2 and the repressor INSM1 regulate the proliferation–differentiation balance in the developing brain

Caitlin E. Monaghan, Tamilla Nechiporuk, Sophia Jeng, Shannon K. McWeeney, Jianxun Wang, Michael G. Rosenfeld, and Gail Mandel

Nervous system development involves a delicate balance between neural progenitor proliferation and neuronal differentiation. Repressors and corepressors each affect this balance, but in many cases, existence of the complexes in vivo has not been shown. Here, we identify a repressor/corepressor complex in embryonic brain consisting of Insulinoma-associated 1 (INSM1) and the RE1 Silencing Transcription factor (REST) corepressors RCOR1 and RCOR2. Elimination of RCOR1 and RCOR2, or INSM1, robustly promotes neural proliferation over neuronal and oligodendrocyte differentiation. Further, their elimination results in overexpression of REST, a direct target gene of INSM1. Normalizing REST levels in the RCOR1/2-deficient brain partially restores aberrant brain morphology. Our results identify a repressor complex required for critical events during normal brain development. (See pp. E406–E415.)

Tbx20 controls the expression of the *KCNH2* gene and of hERG channels

Ricardo Caballero, Raquel G. Utrilla, Irene Amorós, Marcos Matamoros, Marta Pérez-Hernández, David Tinaquero, Silvia Alfayate, Paloma Nieto-Marín, Guadalupe Guerrero-Serna, Qing-hua Liu, Roberto Ramos-Mondragón, Daniela Ponce-Balbuena, Todd Herron, Katherine F. Campbell, David Filgueiras-Rama, Rafael Peinado, José L. López-Sendón, José Jalife, Eva Delpón, and Juan Tamargo

Tbx20 is a transcription factor whose critical role in cardiogenesis is well-established. Here we functionally analyzed the electrophysiological effects produced by a mutation (p.R311C) in Tbx20 found in some affected individuals belonging to a family with long QT syndrome (an inherited cardiac arrhythmia due to delayed ventricular repolarization). We demonstrated that Tbx20 selectively increases the expression of *KCNH2*, which encodes for the channel Kv11.1 (hERG) that generates the main ventricular repolarizing current. Conversely, the p.R311C mutation disables the Tbx20 protranscriptional activity over *KCNH2*, leading to a decrease in the hERG current and a prolongation of the action potentials recorded in human induced pluripotent stem cell-derived cardiomyocytes. Therefore, we propose that Tbx20, besides its described role, regulates *KCNH2* expression. (See pp. E416–E425.)

ATG9 regulates autophagosome progression from the endoplasmic reticulum in *Arabidopsis*

Xiaohong Zhuang, Kin Pan Chung, Yong Cui, Weili Lin, Caiji Gao, Byung-Ho Kang, and Liwen Jiang

One fundamental question in the autophagy field is the membrane origin of the autophagosome. As the sole transmembrane autophagy-related (ATG) protein, ATG9 is conserved among eukaryotes and known to be important for autophagy, but its precise molecular function is still unknown. Through a combination of in vivo real-time imaging, 3D tomographic reconstruction, and genetic approaches, this study demonstrates that, in contrast to the *atg9* mutants characterized in yeast and animal, loss of ATG9 in *Arabidopsis* led to expanding autophagosome-related tubules connected to the endoplasmic reticulum during autophagy. This work thus provides functional evidence for a unique role of ATG9 in autophagosome progression from the endoplasmic reticulum in plant cells, shedding new light on the membrane origins of autophagosome in plants. (See pp. E426–E435.)