PNAS Plus Significance Statements

Improving the lens design and performance of a contemporary electromagnetic shock wave lithotripter

Andreas Neisius, Nathan B. Smith, Georgy Sankin, Nicholas John Kuntz, John Francis Madden, Daniel E. Fovargue, Sorin Mitran, Michael Eric Lipkin, Walter Neal Simmons, Glenn M. Preminger, and Pei Zhong

Electromagnetic (EM) shock wave lithotripters are widely used for noninvasive treatment of kidney stone patients. Here (pp. E1167–E1175), we report the design of a new acoustic lens to rectify three fundamental drawbacks in contemporary EM lithotripters, based on in situ pulse superposition, leading to significantly improved stone comminution both in vitro and in vivo with minimal tissue injury. The new lens design improves the pressure distribution around the lithotripter focus with better alignment of the peak pressure and cavitation activities with the kidney stones under clinically relevant treatment conditions. The general principle of the new lens design is applicable to different lenses or reflectors and with further optimizations may enhance the performance and safety of contemporary EM lithotripters.

SpyLigase peptide–peptide ligation polymerizes affibodies to enhance magnetic cancer cell capture

Jacob O. Fierer, Gianluca Veggiani, and Mark Howarth

Building proteins into assemblies faces challenges in specificity and stability of the connections. Proteins are ideally connected via peptide tags for minimal disruption of function (pp. E1176–E1181). *Streptococcus pyogenes* contains a protein that locks itself together. After genetic dissection, we created a protein (SpyLigase) that locks two peptide tags together. With tags on opposite ends of an antibody or affibody (an antibody-like scaffold), SpyLigase assembled the proteins into polyantibody or polyaffibody chains. Magnetic beads can isolate specific cell types, but the small area of bead-to-cell contact means that abundant cell-specific target is required for cell capture. Polymerization of affibodies enabled capture of cancerous cells expressing less cancer marker and should enhance sensitivity of cell isolation for various research and clinical applications.

Flying *Drosophila* stabilize their vision-based velocity controller by sensing wind with their antennae

Sawyer Buckminster Fuller, Andrew D. Straw, Martin Y. Peek, Richard M. Murray, and Michael H. Dickinson

Insects are widely appreciated for their aerial agility, but the organization of their control system is not well understood. In particular, it is not known how they rapidly integrate information from different sensory systems—such as their eyes and antennae—to regulate flight speed. Although vision may provide an estimate of the true groundspeed in the presence of wind (pp. E1182–E1191), delays inherent in visual processing compromise the performance of the flight speed regulator and make the animal unstable. Mechanoreceptors on the antennae of flies cannot measure groundspeed directly, but can detect changes in airspeed more quickly. By integrating information from both senses, flies achieve stable regulation of flight speed that is robust to perturbations such as gusts of wind.

MinCDE exploits the dynamic nature of FtsZ filaments for its spatial regulation

Senthil Arumugam, Zdeněk Petrášek, and Petra Schwille

Although the mechanisms of microtubule depolymerization are relatively well understood, those of the tubulin homologue FtsZ have been difficult to understand owing to differences in its filament architecture and dynamics compared with those of microtubules. MinC, an important negative regulator of FtsZ and a component of the Min oscillatory system in *Escherichia coli*, positions the Z-ring to the midcell (pp. E1192–E1200). With single-molecule fluorescence imaging in a cell-free minimal system on supported lipid bilayers, in which a network of FtsZ bundles assemble in a chemically well-defined system, the dynamic nature of the FtsZ bundles and the mechanism of disassembly by MinC is elucidated.

Mapping protein conformational heterogeneity under pressure with site-directed spin labeling and double electron–electron resonance

Michael T. Lerch, Zhongyu Yang, Evan K. Brooks, and Wayne L. Hubbell

Excited states of proteins play functional roles, but their low population and conformational flexibility pose a challenge for characterization by most spectroscopic techniques. Here (pp. E1201–E1210), this challenge is met by combining high hydrostatic pressure, which reversibly populates excited states, and site-directed spin labeling with double electron–electron resonance (DEER) spectroscopy, which resolves distinct conformational substates of proteins by measuring distances between spin-labeled pairs. We present a method for trapping high-pressure equilibria of proteins by rapid freezing under pressure, followed by depressurization and acquisition of DEER data at atmospheric pressure. The methodology is applied to myoglobin, revealing unique information on the length scale of helical fluctuations in the pressure-populated as compared with the pH-populated molten globule states of the apo-protein.

M2 macrophages promote beta-cell proliferation by up-regulation of SMAD7

Xiangwei Xiao, Iljana Gaffar, Ping Guo, John Wiersch, Shane Fischbach, Lauren Peirish, Zewen Song, Yousef El-Gohary, Krishna Prasadan, Chiyo Shiota, and George K. Gittes

Here (pp. E1211–E1220), we show how, mechanistically, inflammation-recruited macrophages may stimulate beta-cell proliferation in the pancreas, and specifically identify that TGFβ1 and EGF, which are secreted by M2 macrophages, induce SMAD7 expression in beta cells. SMAD7 not only activates cell cycle activators but also induces the nuclear exclusion of cell cycle inhibitors to promote beta-cell replication. Our study thus reveals a molecular pathway to induce beta-cell proliferation through enhanced SMAD7 activity specifically in beta cells.

RSPO–LGR4 functions via IQGAP1 to potentiate Wnt signaling

Kendra S. Carmon, Xing Gong, Jing Yi, Anthony Thomas, and Qingyun Liu

R-spondins (RSPOs) and LGR4 emerged as a major ligand–receptor system in the regulation of Wnt signaling as manifested in their pleotropic roles in development and survival of adult stem cells. The mechanism of how RSPO–LGR4 interacts with the Wnt signaling system remains poorly understood. In this work, we describe the identification of IQGAP1 as the first intracellular signaling partner of RSPO–LGR4 and the delineation of IQGAP1's roles and mechanism in mediating RSPO–LGR4-induced potentiation of Wnt signaling. We also elucidate the relationship between the RSPO–LGR4–IQGAP1 pathway and the function of RSPO–LGR4 in inhibiting RNF43/ZNRF3. The findings (pp. E1221–E1229) uncovered a unique mechanism of RSPO–LGR4 signaling and provide a mechanistic basis for the pleiotropic functions of RSPO–LGR4 signaling in normal and pathological processes.

Aβ-induced Golgi fragmentation in Alzheimer's disease enhances Aβ production

Gunjan Joshi, Youjian Chi, Zheping Huang, and Yanzhuang Wang

In Alzheimer's disease (AD), formation of the Aβ aggregates occurs by the cleavage of the amyloid precursor protein (APP) during its trafficking inside the nerve cells. The Golgi apparatus plays a critical role in APP trafficking; fragmentation of the normally highly ordered Golgi structure occurs in nerve cells of AD patients. Here (pp. E1230–E1239) we report that Aβ accumulation triggers Golgi fragmentation by activating cyclin-dependent kinase-5 (cdk5), which phosphorylates Golgi structural proteins such as GRASP65. Rescue of Golgi structure by inhibiting cdk5 or by expressing nonphosphorylatable GRASP65 mutants reduced Aβ secretion. Our study provides a molecular mechanism for Golgi fragmentation and its effects on APP trafficking and processing, suggesting Golgi as a potential drug target for AD treatment.

Role of BRCA1 in brain development

Gerald M. Pao, Quan Zhu, Carlos G. Perez-Garcia, Shen-Ju Chou, Hoonkyo Suh, Fred H. Gage, Dennis D. M. O'Leary, and Inder M. Verma

The developing brain is highly sensitive to ionizing radiation and DNA damage. Here we report that tumor suppressor breast cancer susceptibility gene 1 (*BRCA1*) plays a novel role in regulating the embryonic brain development and postnatal brain size. We found (pp. E1240–E1248) that loss of *BRCA1* induces p53-dependent proapoptotic pathways in the CNS. *BRCA1* possibly functions as a centrosomal factor in establishing the cellular polarity of the neural progenitors through the DNA damage sensor kinase ATM. Our data provide new insight in understanding the control of DNA damage sensitivity and brain size during development and evolution.

Embryonic thermosensitive TRPA1 determines transgenerational diapause phenotype of the silkworm, *Bombyx mori*

Azusa Sato, Takaaki Sokabe, Makiko Kashio, Yuji Yasukochi, Makoto Tominaga, and Kunihiro Shiomi

Diapause has evolved as a specific subtype of dormancy in most insect species and as a seasonal polyphenism that ensures survival under unfavorable environmental conditions and synchronizes populations. In *Bombyx mori*, embryonic diapause is induced transgenerationally as a maternal effect. However, the molecular mechanisms involved in the perception of environmental temperature and in linking thermal information to neuroendocrine functions are still unknown. Here (pp. E1249–E1255), we show that the *Bombyx* transient receptor potential A1 (TRPA1) could be thermally activated during embryogenesis, and an unknown signaling pathway linked to the release of diapause hormone may then be activated to affect the induction of diapause in progeny. The *Bombyx* TRPA1 acts as a molecular switch for the development of an alternative phenotype in an animal with seasonal polyphenism.

Therapeutic vaccine against DPP4 improves glucose metabolism in mice

Zhengda Pang, Hironori Nakagami, Mariana K. Osako, Hiroshi Koriyama, Futoshi Nakagami, Hideki Tomioka, Munehisa Shimamura, Hitomi Kurinami, Yoichi Takami, Ryuichi Morishita, and Hiromi Rakugi

Type 2 diabetes mellitus (T2DM) has become a common disease, and long-term effective drugs have become a necessity. In recent years, dipeptidyl peptidase 4 (DPP4) inhibitors have been commercialized due to its ability to inhibit glucagon-like peptide 1 degradation, a hormone important for enhancing insulin secretion. Despite the availability of efficient drugs, the success of treatment is limited by patients' inconsistent drug intake and the economic burden included in a lifelong treatment required for T2DM. To alleviate these limitations, in this study (pp. E1256–E1263) an affordable and effective immunotherapeutic method was developed and assessed for T2DM treatment. We selected and designed the appropriate peptide sequences that induce the anti-DPP4 antibody that effectively improves the diabetic phenotype without an adverse autoimmune response.

Complete replication of hepatitis B virus and hepatitis C virus in a newly developed hepatoma cell line

Darong Yang, Chaohui Zuo, Xiaohong Wang, Xianghe Meng, Binbin Xue, Nianli Liu, Rong Yu, Yuwen Qin, Yimin Gao, Qiuping Wang, Jun Hu, Ling Wang, Zebin Zhou, Bing Liu, Deming Tan, Yang Guan, and Haizhen Zhu

More than 500 million people are persistently infected with hepatitis B virus (HBV) and/or hepatitis C virus (HCV) and are at a risk of developing chronic hepatitis, cirrhosis, and liver cancer. The absence of robust cell culture systems for both viral infections limits the understanding of the virus lifecycle and pathogenesis required for the development of vaccine and antivirals. We have established (pp. E1264–E1273) a novel human hepatoma cell line termed "HLCZ01" that supports the entire lifecycle of both HBV and HCV produced both in cell culture and clinically. This cell line provides a powerful tool for addressing the virus lifecycle and the development of antivirals and vaccines.

Modulation of synaptic function through the α-neurexin–specific ligand neurexophilin-1

Gesche Born, Dorothee Breuer, Shaopeng Wang, Astrid Rohlmann, Philippe Coulon, Puja Vakili, Carsten Reissner, Friedemann Kiefer, Martin Heine, Hans-Christian Pape, and Markus Missler

Communication between neurons via synapses is essential for information processing and cognitive function in our brains and is found impaired in many neuropsychiatric disorders. Synaptic transmission is remarkably variable in strength, and cell-adhesion molecules as neurexins and their binding partners are candidates to regulate neurotransmission. This study (pp. E1274–E1283) changes our understanding of how neurotransmission can be adapted to local demands by investigating the previously undescribed functions of neurexophilins, arguably the most elusive ligands of α-neurexins. Neurexophilins are expressed only in subpopulations of synapses, and their presence is able to change short-term plasticity and molecular composition at these terminals.

PDF and cAMP enhance PER stability in *Drosophila* clock neurons

Yue Li, Fang Guo, James Shen, and Michael Rosbash

This work describes an advance in the understanding of how the important circadian neuropeptide PDF contributes to the function of the molecular clock in *Drosophila* neurons. The famous per^S allele of *period* significantly improves the rhythmicity of flies missing PDF. The per^S gene product degrades rapidly, suggesting that PDF-mediated cAMP affects PER stability. Indeed, increasing cAMP levels and cAMP-mediated protein kinase A (PKA) activity stabilizes PER, in tissue culture cells and in circadian neurons. PDF addition to fly brains in vitro has a similar effect. Our observations (pp. E1284–E1290) taken together indicate that PDF contributes to clock neuron function by activating PKA, stabilizing PER, and thereby slowing the pace of clock neurons that respond to PDF.

Cartography of neurexin alternative splicing mapped by single-molecule long-read mRNA sequencing

Barbara Treutlein, Ozgun Gokce, Stephen R. Quake, and Thomas C. Südhof

Neurexins are presynaptic cell-adhesion molecules that are essential for synapse formation and synaptic transmission. Extensive alternative splicing of neurexin transcripts may generate thousands of isoforms, but it is unclear how many distinct neurexins are physiologically produced. We used unbiased long-read sequencing of full-length neurexin mRNAs to systematically assess the alternative splicing of neurexins in prefrontal cortex. We identified (pp. E1291–E1299) a novel, abundantly used alternatively spliced exon of neurexins, and found that the different events of alternative splicing of neurexins appear to be independent of each other. Our data suggest that thousands of neurexin isoforms are physiologically generated, consistent with the notion that neurexins represent transsynaptic protein-interaction scaffolds that mediate diverse functions and are regulated by alternative splicing at multiple independent sites.

Periodic root branching in *Arabidopsis* requires synthesis of an uncharacterized carotenoid derivative

Jaimie M. Van Norman, Jingyuan Zhang, Christopher I. Cazzonelli, Barry J. Pogson, Peter J. Harrison, Timothy D. H. Bugg, Kai Xun Chan, Andrew J. Thompson, and Philip N. Benfey

A fundamental question in developmental biology is how patterns are established in space and time. In plants, key differences in root system architecture are attributed to the spatial distribution pattern of lateral roots (LRs), yet how the pattern of LRs is established is only beginning to be understood. We demonstrate (pp. E1300–E1309) that the establishment of sites competent to form LRs roots requires carotenoid biosynthesis. Furthermore, our results implicate an uncharacterized carotenoid-derived molecule that functions non–cell-autonomously, specifically in LR formation. The results of this study reveal novel aspects of carotenoid biology and expand the roles of carotenoid-derived molecules into root developmental patterning.

Suppressing unwanted memories reduces their unconscious influence via targeted cortical inhibition

Pierre Gagnepain, Richard N. Henson, and Michael C. Anderson

After a trauma, people often suppress intrusive visual memories. We used functional MRI to understand how healthy participants suppress the visual content of memories to overcome intrusions, and whether suppressed content continues to exert unconscious influences. Effective connectivity, representational similarity, and computational analyses revealed a frontally mediated mechanism that suppresses intrusive visual memories by reducing activity in the visual cortex. This reduction disrupted neural and behavioral expressions of implicit memory during a later perception test. Thus, our findings (pp. E1310–E1319) indicate that motivated forgetting mechanisms, known to disrupt conscious retention, also reduce unconscious expressions of memory, pointing to a neurobiological model of this process.