PNAS Plus Significance Statements

Panoramic view of a superfamily of phosphatases through substrate profiling

Hua Huang, Chetanya Pandya, Chunliang Liu, Nawar F. Al-Obaidi, Min Wang, Li Zheng, Sarah Toews Keating, Miyuki Aono, James D. Love, Brandon Evans, Ronald D. Seidel, Brandan S. Hillerich, Scott J. Garforth, Steven C. Almo, Patrick S. Mariano, Debra Dunaway-Mariano, Karen N. Allen, and Jeremiah D. Farelli

Here (pp. E1974–E1983), we examine the activity profile of the haloalkanoic acid dehalogenase (HAD) superfamily by screening a customized library against >200 enzymes from a broad sampling of the superfamily. From this dataset, we can infer the function of nearly 35% of the superfamily. Overall, the superfamily was found to show high substrate ambiguity, with 75% of the superfamily utilizing greater than five substrates. In addition, the HAD members with the least amount of structural accessorization of the Rossmann fold were found to be the most specific, suggesting that elaboration of the core domain may have led to increased substrate range of the superfamily.

Productive mRNA stem loop-mediated transcriptional slippage: Crucial features in common with intrinsic terminators

Christophe Penno, Virag Sharma, Arthur Coakley, Mary O'Connell Motherway, Douwe van Sinderen, Lucyna Lubkowska, Maria L. Kireeva, Mikhail Kashlev, Pavel V. Baranov, and John F. Atkins

Perturbation of transcription register by RNA polymerase, transcription slippage, is used to yield additional protein products. Known functionally important cases involve a small number of short sequences without secondary structure. The discovery reported here of the dependence of a newly identified motif on nascent RNA forming a stem loop structure within the RNA exit channel of the polymerase greatly extends the potential for a broad variety of putative slippage sequences, especially as the phenomenon has been observed with both bacterial and eukaryotic RNA polymerases. Characterization of the mechanism involved shows similarities with, and differences from, intrinsic transcription termination, which also depends on formation of RNA stem loop structures. Our findings (pp. E1984–E1993) reveal novel insights to RNA polymerase versatility and functioning.

Structural characterization of toxic oligomers that are kinetically trapped during α-synuclein fibril formation

Serene W. Chen, Srdja Drakulic, Emma Deas, Myriam Ouberai, Francesco A. Aprile, Rocío Arranz, Samuel Ness, Cintia Roodveldt, Tim Guilliams, Erwin J. De-Genst, David Klenerman, Nicholas W. Wood, Tuomas P.J. Knowles, Carlos Alfonso, Germán Rivas, Andrey Y. Abramov, José María Valpuesta, Christopher M. Dobson, and Nunilo Cremades

Certain oligomeric species generated during the self-assembly of specific proteins into ordered fibrillar aggregates are likely to be key players in the initiation and spreading of neurodegenerative diseases. We have purified stable toxic oligomeric species of α-synuclein and defined and minimized their degree of heterogeneity, which has allowed us to identify distinct subgroups of oligomers and determine their structural properties and three-dimensional molecular architectures (pp. E1994–E2003). All the oligomeric subgroups possess approximately cylindrical architectures with marked similarities to amyloid fibrils, suggesting that these types of oligomers are kinetically trapped during protein self-assembly. The relative stabilities and inherent pathological roles of different amyloid oligomers are likely to result from the multiplicity of pathways of the misfolding process and the remarkably slow rates of structural conversions.

Three-dimensional architecture of extended synaptotagmin-mediated endoplasmic reticulum–plasma membrane contact sites

Rubén Fernández-Busnadiego, Yasunori Saheki, and Pietro De Camilli

Membrane contact sites, the sites of close physical proximity between intracellular membranes, are emerging as key players in multiple cellular processes. In particular, endoplasmic reticulum (ER)–plasma membrane (PM) contact sites play important roles in calcium homeostasis, signaling, and lipid regulation/exchange. However, the architecture of these contact sites remains poorly understood. Here (pp. E2004–E2013) we study the 3D architecture of ER–PM contact sites at molecular resolution using cryo-electron tomography. We define the structural signature of the ER–PM tethers mediated by the extended synaptotagmins (E-Syts) and show that E-Syts regulate ER–PM distance in a cytosolic Ca^{2+}-dependent manner. These findings provide an important foundation towards elucidating E-Syt function and more generally the mechanisms of cross-talk between the ER and the PM.

Virus decomposition provides an important contribution to benthic deep-sea ecosystem functioning

Antonio Dell'Anno, Cinzia Corinaldesi, and Roberto Danovaro

Viruses proliferate at the expense of their hosts. After cell death the released viruses can infect other hosts or undergo decomposition processes. Here we show, for the first time to our knowledge, that in deep-sea ecosystems, the largest biome of the biosphere, approximately 25% of viruses released by lysed prokaryotic cells are decomposed at fast rates. We show that, given the huge viral biomass of the ocean seafloor and the high rates of this process, virus decomposition provides a major source of labile organic compounds able to sustain the microbial food webs and nutrient cycling at a global scale. These findings (pp. E2014–E2019) provide new

insights that will enable a better understanding of the functioning of the global oceans.

Maternal and zygotic *Zfp57* modulate NOTCH signaling in cardiac development

Yulia Shamis, Dana E. Cullen, Lizhi Liu, Guan Yang, Sheau-Fang Ng, Lijuan Xiao, Fong T. Bell, Chelsea Ray, Sachiko Takikawa, Ivan P. Moskowitz, Chen-Leng Cai, Xiao Yang, and Xiajun Li

Abnormal heart development is a common birth defect. Genomic imprinting is absolutely essential for mammalian embryonic development. We found (pp. E2020–E2029) that loss of ZFP57, a master regulator of genomic imprinting, causes a number of heart morphogenetic defects. These cardiac defects are reminiscent of mutant phenotypes observed in the NOTCH signaling pathway, one of the most important pathways in development. Indeed, we demonstrate that NOTCH signaling is diminished without ZFP57. Furthermore, the maternal function of *Zfp57* contributes to NOTCH signaling and embryonic heart development. Maternal and zygotic *Zfp57* play redundant roles in genomic imprinting, NOTCH signaling, and heart development. Thus, our results provide mechanistic links among maternal effect, genomic imprinting, NOTCH signaling, and cardiac development.

Sterile inflammation in the spleen during atherosclerosis provides oxidation-specific epitopes that induce a protective B-cell response

Emilie K. Grasset, Amanda Duhlin, Hanna E. Agardh, Olga Ovchinnikova, Thomas Hägglöf, Mattias N. Forsell, Gabrielle Paulsson-Berne, Göran K. Hansson, Daniel F. J. Ketelhuth, and Mikael C. I. Karlsson

In this study we investigate the origin of the protective B-cell response in the spleen in atherosclerosis. We find an ongoing B-cell activation with production of antibodies against oxidation-specific epitopes. In addition, this response can be accelerated using apoptotic cells alone that reduce lesion development and serum cholesterol in a B-cell–dependent manner. This study (pp. E2030–E2038) pinpoints the spleen as an important organ for atherosclerosis-associated immunity and provides novel pathways to use for treatment.

In vivo characterization of chronic traumatic encephalopathy using [F-18]FDDNP PET brain imaging

Jorge R. Barrio, Gary W. Small, Koon-Pong Wong, Sung-Cheng Huang, Jie Liu (刘捷), David A. Merrill, Christopher C. Giza, Robert P. Fitzsimmons, Bennet Omalu, Julian Bailes, and Vladimir Kepe

Mild traumatic brain injuries are frequent events in the general population and are associated with a severe neurodegenerative disease, chronic traumatic encephalopathy (CTE). This disease is characterized by abnormal accumulation of protein aggregates, primarily tau proteins, which accumulate in brain areas responsible for mood, fear, stress, and cognition. There is no definitive clinical diagnosis of CTE at the present time, and this new work shows how a tau-sensitive brain imaging agent, [F-18]FDDNP, may be able to detect the disease in living people with varying degrees of symptoms (pp. E2039–E2047). Early detection would facilitate the most effective management strategies and provide a baseline to measure the effectiveness of treatments.

Compromised peroxisomes in idiopathic pulmonary fibrosis, a vicious cycle inducing a higher fibrotic response via TGF-β signaling

Gani Oruqaj, Srikanth Karnati, Vijith Vijayan, Lakshmi Kanth Kotarkonda, Eistine Boateng, Wenming Zhang, Clemens Ruppert, Andreas Günther, Wei Shi, and Eveline Baumgart-Vogt

This study enhances the knowledge on the molecular pathogenesis of idiopathic pulmonary fibrosis (IPF). To date, there is no information available on the role of peroxisomes in lung fibrosis. In our study (pp. E2048–E2057) we demonstrate that peroxisomal biogenesis and metabolism is compromised in tissue samples as well as in fibroblasts of IPF patients and in bleomycin-induced fibrosis mouse model. Moreover, RNAi-mediated knockdown of peroxisomal biogenesis leads to a profibrotic response in control and IPF fibroblasts suggesting that the reduction of peroxisomal function in IPF would contribute to the profibrotic phenotype of this devastating disease. Our work opens a new field of research in the area of lung fibrosis and might lead to novel treatment strategies against IPF by modulating the peroxisomal compartment.

Visualized macrophage dynamics and significance of S100A8 in obese fat

Ryohei Sekimoto, Shiro Fukuda, Norikazu Maeda, Yu Tsushima, Keisuke Matsuda, Takuya Mori, Hideaki Nakatsuji, Hitoshi Nishizawa, Ken Kishida, Junichi Kikuta, Yumiko Maijima, Tohru Funahashi, Masaru Ishii, and Iichiro Shimomura

Infiltration of immune cells into adipose tissue has been observed in obesity, but, until now, these data were based on immunohistological microscopic analysis. Here (pp. E2058–E2066), to our knowledge for the first time, we applied an intravital multiphoton imaging technique to adipose tissue with lysozyme M-EGFP transgenic ($LysM^{EGFP}$) mice whose EGFP was expressed in the myelomonocytic lineage. Mobility of $LysM^{EGFP}$-positive macrophages was activated just 5 d after high-fat and high-sucrose (HF/HS) diet before the development of obesity. Furthermore, a significant increase of S100A8, one of the alarmins, was detected in fat tissue just 5 d after HF/HS diet. S100A8 stimulated chemotactic migration, and neutralization of S100A8 suppressed the HF/HS diet-induced activation of $LysM^{EGFP}$-positive cells. Time-lapse intravital imaging first identified the very early event exhibiting increased mobility of adipose macrophages.

Motor role of parietal cortex in a monkey model of hemispatial neglect

Jan Kubanek, Jingfeng M. Li, and Lawrence H. Snyder

Humans and animals often choose between targets in space. Parietal cortex is known to be critical to this function. However, the exact role of parietal cortex in spatial choice remains controversial. Does parietal cortex implement choices at the general cognitive level, or are the choices made in the context of specific upcoming actions? We placed focused reversible lesions into specific parietal circuits of monkeys making choices between two targets (pp. E2067–E2072).

Lesions of specific circuits only affected choices made using specific actions: lesions of lateral intraparietal area (LIP) affected eye movements but not reaches, while lesions of the parietal reach region had converse effects. This illuminates the nature of spatial choice in parietal cortex, and suggests that choice is implemented in dedicated parietal circuits, each responsible for a specific class of actions.

Asphyxia-activated corticocardiac signaling accelerates onset of cardiac arrest

Duan Li, Omar S. Mabrouk, Tiecheng Liu, Fangyun Tian, Gang Xu, Santiago Rengifo, Sarah J. Choi, Abhay Mathur, Charles P. Crooks, Robert T. Kennedy, Michael M. Wang, Hamid Ghanbari, and Jimo Borjigin

How does the heart of a healthy individual cease to function within just a few minutes in the absence of oxygen? We addressed this issue by simultaneously examining the heart and the brain in animal models during asphyxiation and found that asphyxia markedly stimulates neurophysiological and neurochemical activities of the brain. Furthermore, previously unidentified corticocardiac coupling showed increased intensity as the heart deteriorated. Blocking efferent input to the heart markedly increased survival time of both the heart and the brain. The results (pp. E2073–E2082) show that targeting the brain's outflow may be an effective strategy to delay the death of the heart and the brain from asphyxia.

Cortical activity is more stable when sensory stimuli are consciously perceived

Aaron Schurger, Ioannis Sarigiannidis, Lionel Naccache, Jacobo D. Sitt, and Stanislas Dehaene

Recently, a reduction in the variability of neural activity across trials has been proposed as a general property of sensory perception. However, in order for this increased reliability in the neuronal response to be relevant for perception it must be present at the single-trial level. Here (pp. E2083–E2092) we show that the within-trial stability of the activity evoked by a threshold-level visual stimulus is a reliable and specific indicator of whether or not the stimulus was reported as "seen." This within-trial difference in stability coincides in time with a difference in variability across trials. We also show that the same neural stability can be used to discriminate the conscious state of brain-injured patients. These findings validate the relevance of transient neural stability for conscious perception.

Architecture of the cerebral cortical association connectome underlying cognition

Mihail Bota, Olaf Sporns, and Larry W. Swanson

Connections between cerebral cortex regions are known as association connections, and neural activity in the network formed by these connections is thought to generate cognition. Network analysis of microscopic association connection data produced over the last 40 years in a small, easily studied mammal suggests a new way to describe the organization of the cortical association network. Basically, it consists of four modules with an anatomical shell–core arrangement and asymmetric connections within and between modules, implying at least partly "hardwired," genetically determined biases of information flow through the cortical association network. The results (pp. E2093–E2101) advance the goal of achieving a global nervous system wiring diagram of connections and provide another step toward understanding the cellular architecture and mechanisms underpinning cognition.

The human sex ratio from conception to birth

Steven Hecht Orzack, J. William Stubblefield, Viatcheslav R. Akmaev, Pere Colls, Santiago Munné, Thomas Scholl, David Steinsaltz, and James E. Zuckerman

The human sex ratio has long interested cell biologists, developmental biologists, demographers, epidemiologists, evolutionary biologists, gynecologists, and statisticians. Nonetheless, the trajectory of the human sex ratio from conception to birth has been poorly characterized. We present the most comprehensive analysis of this trajectory ever done. Our dataset is the largest ever assembled to estimate the sex ratio at conception and is the first, to our knowledge, to include data from 3- to- 6-d-old embryos, induced abortions, chorionic villus sampling, amniocentesis, and fetal deaths and live births. Our results (pp. E2102–E2111) indicate that the sex ratio at conception is unbiased, the proportion of males increases during the first trimester, and total female mortality during pregnancy exceeds total male mortality; these are fundamental insights into early human development.