PNAS Plus Significance Statements

Numerosity representation is encoded in human subcortex

Elliot Collins, Joonkoo Park, and Marlene Behrmann

Despite major neuroanatomical differences, adults, infants, nonhuman primates, and invertebrates possess the ability to evaluate relative quantities. Humans' ability starts with coarse granularity (distinguishing ratios of numerical quantities of 3:1 or larger), but this becomes increasingly precise over development. This series of experiments demonstrates a role of the subcortex in discriminating numerosities in larger (4:1 or 3:1), but not in smaller ratios. These findings map onto the precision with which newborns evaluate number. Combined with evidence from the development of numerical skills, this study implicates the human subcortex as a possible source of core number knowledge that is both related to phylogenetic numerical competence and serves as the foundation on which more complex ontogenetic numerical skills may be built. (See pp. E2806–E2815.)

Recurrent rewiring and emergence of RNA regulatory networks

Daniel Wilinski, Natascha Buter, Andrew D. Klocko, Christopher P. Lapointe, Eric U. Selker, Audrey P. Gasch, and Marvin Wickens

Regulatory networks change during evolution. A protein that controls many genes in one species may control a different set of genes in another. We examined how mRNA networks evolve, focusing on the PUF (Pumilio and FBF) family of RNA-binding proteins. These govern stability and translation of hundreds of mRNAs and enable coordinate regulation of discrete biological outcomes. To understand how RNA networks evolve, we used knowledge of the RNA specificity of each PUF protein to predict its mRNA targets and directly identified mRNAs bound to each protein in divergent fungi via biochemical methods. We find networks controlled by one protein switch during evolution to be controlled by another and that proteins with different specificities can share, gain, or lose batteries of mRNAs. (See pp. E2816–E2825.)

Inositol phosphates and phosphoinositides activate insulin-degrading enzyme, while phosphoinositides also mediate binding to endosomes

Eun Suk Song, Hyeln Jang, Hou-Fu Guo, Maria A. Juliano, Luiz Juliano, Andrew J. Morris, Emilia Galperin, David W. Rodgers, and Louis B. Hersh

A diverse collection of peptides mediates cell–cell communication. Enzymes that cleave these peptides modulate their signals and thus play an important role in the physiology of multicellular organisms. Insulin-degrading enzyme (IDE) is one such enzyme that cleaves a number of bioactive peptides. IDE is activated by polyanions, but physiological activators remain unidentified. Here we show that inositol-containing molecules, known to modulate various cellular functions, activate IDE, identifying them as potential physiological regulators. Inositol phosphates are potent soluble activators of IDE. Phosphatidylinositol phosphates, lipid components of cell membranes, also activate but in addition facilitate the localization of IDE to intracellular compartments, where the enzyme gains access to substrates, such as insulin, internalized by cells. (See pp. E2826–E2835.)

EGF and NRG induce phosphorylation of HER3/ERBB3 by EGFR using distinct oligomeric mechanisms

Bettina van Lengerich, Christopher Agnew, Elias M. Puchner, Bo Huang, and Natalia Jura

Signaling by human epidermal growth factor receptor (HER) receptors relies on heteromeric interactions between four members of the family: EGF receptor, HER2, HER3, and HER4. These interactions remain remarkably poorly understood. Using super-resolution microscopy imaging and signaling assays, we demonstrate a rich scope of HER receptor organization patterns that are differentially influenced by ligands and coreceptor expression, resulting in unique phosphorylation signatures of HER receptors. We also show that there are fundamental differences in molecular mechanisms that govern HER receptor cross-activation, which do not always follow the canonical kinase dimerization mechanism. Our data underscore an emerging concept in the field that HER receptor signaling needs to be interpreted in the context of higher-order receptor oligomers, redefining the basic signaling unit relevant for receptor function. (See pp. E2836–E2845.)

Ion and inhibitor binding of the double-ring ion selectivity filter of the mitochondrial calcium uniporter

Chan Cao, Shuqing Wang, Tanxing Cui, Xun-Cheng Su, and James J. Chou

Mitochondrial calcium homeostasis is vital to cellular activities such as aerobic ATP production and cell death. Calcium uptake by mitochondria is achieved using an intricately regulated calcium channel, known as mitochondrial calcium uniporter (MCU). MCU demonstrates

high calcium selectivity and conductance but little is known about its ion selectivity filter. We used paramagnetic NMR to show that the double carboxylate rings formed by the "DXXE" signature sequence of MCU is the ion selectivity filter and that the two ion binding sites can afford high ion affinity via positive cooperativity. We also showed that the channel inhibitor Ru360 directly blocks at the selectivity filter. Our approach demonstrates the promising application of NMR in investigating ion channels interacting with ions and blockers. (See pp. E2846–E2851.)

Three-dimensional culture system identifies a new mode of cetuximab resistance and disease-relevant genes in colorectal cancer

Cunxi Li, Bhuminder Singh, Ramona Graves-Deal, Haiting Ma, Alina Starchenko, William H. Fry, Yuanyuan Lu, Yang Wang, Galina Bogatcheva, Mohseen P. Khan, Ginger L. Milne, Shilin Zhao, Gregory Daniel Ayers, Nenggan Li, Huaying Hu, Mary Kay Washington, Timothy J. Yeatman, Oliver G. McDonald, Qi Liu, and Robert J. Coffey

By culturing a human colorectal cancer (CRC) cell line (HCA-7) in 3D, we have generated two cell lines (CC and SC) with distinct morphological, genetic, biochemical, and functional properties. Using this 3D system, we have discovered that increased tyrosine phosphorylation of MET and RON results in cetuximab resistance in the SC cell line that can be overcome by addition of the dual MET/RON tyrosine kinase inhibitor, crizotinib. We have also identified that increased epithelial, but not stromal, versican staining correlates with reduced survival in a clinically annotated CRC tissue microarray. (See pp. E2852–E2861.)

Hepatic FcRn regulates albumin homeostasis and susceptibility to liver injury

Michal Pyzik, Timo Rath, Timothy T. Kuo, Sanda Win, Kristi Baker, Jonathan J. Hubbard, Rosa Grenha, Amit Gandhi, Thomas D. Krämer, Adam R. Mezo, Zachary S. Taylor, Kevin McDonnell, Vicki Nienaber, Jan Terje Andersen, Atsushi Mizoguchi, Laurence Blumberg, Shalaka Purohit, Susan D. Jones, Greg Christianson, Wayne I. Lencer, Inger Sandlie, Neil Kaplowitz, Derry C. Roopenian, and Richard S. Blumberg

Neonatal crystallizable fragment receptor (FcRn) regulates immunity and homeostasis of the two most abundant circulating proteins, IgG and albumin. FcRn is expressed in hepatocytes, but hepatic FcRn function is unknown. We show that hepatic FcRn regulates albumin biodistribution. Absence of FcRn in the liver leads to hypoalbuminemia by preventing efficient albumin delivery into the circulation, causing albumin retention within hepatocytes and increasing biliary albumin excretion. Blockade of albumin–FcRn interactions protects liver from damage induced by acetaminophen, a hepatotoxin. This protection results from hepatocyte accumulation of albumin, which scavenges superoxide radicals, and from the redirection of albumin-bound acetaminophen into the bile. Therefore, FcRn-mediated homeostatic distribution of albumin into the bloodstream renders hepatocytes susceptible to acute hepatotoxin exposure, and inhibition of FcRn in the hepatocyte is protective. (See pp. E2862–E2871.)

Dual-utility NLS drives RNF169-dependent DNA damage responses

Liwei An, Yiyang Jiang, Howin H. W. Ng, Ellen P. S. Man, Jie Chen, Ui-Soon Khoo, Qingguo Gong, and Michael S. Y. Huen

The work describes the first nuclear localization signal (NLS) peptide that not only promotes nuclear shuttling of a DNA damage response (DDR) protein but mediates a direct interaction with a deubiquitylase for enhanced stability. Its identification suggests that NLS peptides, aside from their canonical function in nuclear import, may have acquired additional properties. The study also reports on an important role of the ubiquitin-specific protease 7 (USP7)–ring finger protein 169 (RNF169) axis in driving DNA repair and poly (ADP-ribose) polymerase inhibition resistance. Several lines of evidence indicate that USP7 deubiquitylates and enforces RNF169-dependent DDRs. Together, these data highlight a critically important role of the USP7–RNF169 axis in genome stability maintenance. (See pp. E2872–E2881.)

Allele-specific non-CG DNA methylation marks domains of active chromatin in female mouse brain

Christopher L. Keown, Joel B. Berletch, Rosa Castanon, Joseph R. Nery, Christine M. Disteche, Joseph R. Ecker, and Eran A. Mukamel

Mammalian cells contain two copies of the genome inherited from the two parents. Although most genes are expressed using both, a small but critical part of the genome has different levels of expression from each copy. These parts include the X chromosome in females and imprinted genes in both genders, which play key roles in brain development and cognition. We measured gene expression and DNA methylation, an epigenetic modification of the genome, in the brains of mice using a technique that allowed us to analyze the maternal and paternal copies of the genome separately. Our findings show that a brain-specific form of DNA methylation called non-CG methylation marks regions of active transcription within the inactive X chromosome. (See pp. E2882–E2890.)

Activin-A co-opts IRF4 and AhR signaling to induce human regulatory T cells that restrain asthmatic responses

Sofia Tousa, Maria Semitekolou, Ioannis Morianos, Aggelos Banos, Aikaterini I. Trochoutsou, Tess M. Brodie, Nikolaos Poulos, Konstantinos Samitas, Maria Kapasa, Dimitris Konstantopoulos, Giannis Paraskevopoulos, Mina Gaga, Catherine M. Hawrylowicz, Federica Sallusto, and Georgina Xanthou

Here, we demonstrate that the cytokine activin-A instructs the differentiation of human IL-10–producing type 1 regulatory T (Tr1)-like cells that exhibit strongly suppressive functions against allergen-induced naive and effector $CD4^+$ T-cell responses. In addition, we show that activin-A induces the activation of interferon regulatory factor (IRF4), which, along with aryl hydrocarbon receptor (AhR) and its binding partner, AhR nuclear translocator, forms a tripartite transcription factor complex that is essential for the differentiation and effector functions of human Tr1 cells. Importantly, administration of human activin-A–induced Tr1 cells in a humanized model of asthma confers protection against cardinal disease manifestations in preventive and therapeutic regimes. Collectively, our studies unravel a biological function for activin-A in the generation of suppressive human Tr1 cells that may be exploited for the control of allergic diseases. (See pp. E2891–E2900.)

Ena/VASP proteins regulate activated T-cell trafficking by promoting diapedesis during transendothelial migration

Miriam L. Estin, Scott B. Thompson, Brianna Traxinger, Marlie H. Fisher, Rachel S. Friedman, and Jordan Jacobelli

T-cell trafficking is essential for the function of the adaptive immune system, and regulation of T-cell entry into tissues can be an effective therapy in diseases such as autoimmunity. However, the mechanisms regulating T-cell migration and trafficking are poorly understood. We have identified a key role for Ena/VASP (vasodilator-stimulated phosphoprotein) family cytoskeletal effectors selectively in activated T-cell trafficking to secondary lymphoid organs and to

peripheral sites of inflammation. Ena/VASP deficiency in T cells causes a defect in α4 integrin function, which impairs transendothelial migration. Our work suggests that further studies of the Ena/VASP pathway in T cells could identify therapeutically useful ways to more selectively modulate α4 integrin activity and activated T-cell trafficking. (See pp. E2901–E2910.)

Common nonmutational *NOTCH1* activation in chronic lymphocytic leukemia

Giulia Fabbri, Antony B. Holmes, Mara Viganotti, Claudio Scuoppo, Laura Belver, Daniel Herranz, Xiao-Jie Yan, Yasmine Kieso, Davide Rossi, Gianluca Gaidano, Nicholas Chiorazzi, Adolfo A. Ferrando, and Riccardo Dalla-Favera

A pathogenetic role of *NOTCH1* in chronic lymphocytic leukemia (CLL) has been implied by the presence of deregulating mutations in a relatively small fraction of cases. Our results now indicate that ∼50% of CLL cases devoid of mutations express the active form of NOTCH1 ICN1 (intracellular portion of NOTCH1), thus implicating a much broader role of this transcription factor in the disease. ICN1$^+$ CLL cases display equivalent NOTCH1-dependent transcriptional responses regardless of the gene mutation status, indicating that the detection of ICN1 represents a reliable biomarker of *NOTCH1* activation for diagnostic and therapeutic targeting. Finally, our results identify the NOTCH1-dependent transcriptional program in CLL cells, thus providing direct insights into the pathogenesis of a large fraction of CLL cases. (See pp. E2911–E2919.)

Discovery of *scmR* as a global regulator of secondary metabolism and virulence in *Burkholderia thailandensis* E264

Dainan Mao, Leah B. Bushin, Kyuho Moon, Yihan Wu, and Mohammad R. Seyedsayamdost

Silent biosynthetic gene clusters represent fertile grounds for the discovery of new small molecules, and a number of approaches have been developed for activating them. Their regulation, however, has received far less attention, especially in Gram-negative bacteria. Here, we show that a LysR-type transcriptional regulator, which we name *scmR*, acts as a gatekeeper of secondary metabolism and virulence in the model organism *Burkholderia thailandensis* E264. Specifically, genetic, chemical, and "omics"-based approaches unveil ScmR as a master regulator that interacts with pathway-specific transcriptional regulators to control, and primarily suppress, secondary metabolism. Because this gene is conserved in other *Burkholderia* species, our results suggest that *scmR*-mediated control of secondary metabolism and virulence may be common within this genus and the *Pseudomallei*-group pathogens. (See pp. E2920–E2928.)

Structure of the infectious salmon anemia virus receptor complex illustrates a unique binding strategy for attachment

Jonathan D. Cook, Azmiri Sultana, and Jeffrey E. Lee

The infectious salmon anemia virus (ISAV), an aquatic pathogen with lethal hemorrhagic potential, decimates farmed and freshwater fish populations globally. Here, we determined the crystallographic structures of the hemagglutinin-esterase (HE) viral glycoprotein responsible for the dynamic attachment of the virus to its receptor in Atlantic salmon. We identified surface features of ISAV HE that are conserved across isolates known to cause significant economic burden to fisheries worldwide. This provides a molecular blueprint for the design of a broadly protective vaccine. Furthermore, we showed that ISAV HE has a distinct receptor recognition strategy from those of other influenza-like viruses and coronaviral HE proteins, contributing to our understanding of the diversity of viral entry mechanisms. (See pp. E2929–E2936.)

In vivo optophysiology reveals that G-protein activation triggers osmotic swelling and increased light scattering of rod photoreceptors

Pengfei Zhang, Robert J. Zawadzki, Mayank Goswami, Phuong T. Nguyen, Vladimir Yarov-Yarovoy, Marie E. Burns, and Edward N. Pugh Jr.

Complete activation of the phototransduction G-protein cascade of dark-adapted rod photoreceptors causes outer segments to undergo 10% elongation and large local increases in backscattering, as measured in vivo with noninvasive, high-resolution optical coherence tomography. Maximal elongation is caused by a potentially harmful 20% increase in internal osmotic pressure generated by excess osmolytes arising from phototransduction. The light-stimulated elongation and backscattering responses can be explained by an osmo-elastic model of cytoplasmic swelling, combined with changes in refractive index consequent to the swelling and translocation of the G-protein subunits into the cytosol. Disease conditions that affect the structural integrity of rods may cause them to be especially vulnerable to osmotic stress caused by bright light. (See pp. E2937–E2946.)

Deficiency of a sulfotransferase for sialic acid-modified glycans mitigates Alzheimer's pathology

Zui Zhang, Yoshiko Takeda-Uchimura, Tahmina Foyez, Shiori Ohtake-Niimi, Narentuya, Hiroyasu Akatsu, Kazuchika Nishitsuji, Makoto Michikawa, Tony Wyss-Coray, Kenji Kadomatsu, and Kenji Uchimura

Keratan sulfate (KS) is an extracellular sulfated glycan covalently attached to core proteins in the brain. Here, we show that a type of KS with a certain molecular mass in microglia and its synthetic enzyme GlcNAc6ST1, previously known as a sulfotransferase for ligands of L-selectin, are upregulated in model mice and patients with Alzheimer's disease. GlcNAc6ST1 deficiency resulted in increased amyloid-β phagocytosis and hyperresponsiveness to an antiinflammatory cytokine in primary microglia. Moreover, amyloid-β pathology was mitigated in GlcNAc6ST1-deficient Alzheimer's model mice. These data support a model in which GlcNAc6ST1 regulates microglial functions via synthesizing sialic acid-modified KS, a potential ligand for microglial carbohydrate-recognizing receptors, in Alzheimer's pathology. (See pp. E2947–E2954.)

BORC/kinesin-1 ensemble drives polarized transport of lysosomes into the axon

Ginny G. Farías, Carlos M. Guardia, Raffaella De Pace, Dylan J. Britt, and Juan S. Bonifacino

Lysosomes are found in all neuronal domains, including the soma, axon, and dendrites. How neurons are transported in these domains, however, remains poorly understood. In the present study, we show that a protein ensemble comprising BORC, Arl8, SKIP, and kinesin-1 specifically drives lysosome transport into the axon and not the dendrites. We also show that this mechanism of axonal lysosome transport is essential for maintenance of growth-cone dynamics and turnover of autophagosomes in the distal axon. These findings imply that transport of lysosomes into the axon and the dendrites occurs by different mechanisms, and

demonstrate that BORC-regulated lysosome transport is critical for axonal functions. (See pp. E2955–E2964.)

Small RNA-mediated repair of UV-induced DNA lesions by the DNA DAMAGE-BINDING PROTEIN 2 and ARGONAUTE 1

Catherine Schalk, Valérie Cognat, Stéfanie Graindorge, Timothée Vincent, Olivier Voinnet, and Jean Molinier

As obligate photosynthetic and sessile organisms, plants are particularly exposed to the damaging effects of excess light and UV wavelengths, which can impact genome integrity by inducing DNA sequence alterations. As a response, plants have evolved efficient genome surveillance processes, some of which appear to also overlap with mechanisms of gene expression control. Our study extends this emerging notion by uncovering complex interconnections linking DNA repair and RNA silencing in *Arabidopsis*, illustrating the ever-expanding array of biological functions mediated by silencing small RNAs in plants. (See pp. E2965–E2974.)

Sensing relative signal in the Tgf-β/Smad pathway

Christopher L. Frick, Clare Yarka, Harry Nunns, and Lea Goentoro

It is not fully understood how cells process information in the face of noise. We posed this question in the transforming growth factor-β (Tgf-β) pathway, a major intercellular signaling pathway in animal cells. We found evidence that rather than sensing the signaling state of the Tgf-β pathway, cells sense the signaling state relative to background. Finding that signaling dynamics are interpreted in a relative manner may have implications for how we understand the pathway's context-dependent outcomes and roles in diseases. Our work reinforces an emerging principle that individual cells process signal in a relative manner. (See pp. E2975–E2982.)