PNAS Plus Significance Statements

Longitudinal multiparameter assay of lymphocyte interactions from onset by microfluidic cell pairing and culture

Burak Dura, Mariah M. Servos, Rachel M. Barry, Hidde L. Ploegh, Stephanie K. Dougan, and Joel Voldman

Many immune responses are mediated by direct cell–cell interactions and develop over multiple timescales. A mechanistic understanding of how diverse outcomes arise during these interactions entails identifying the relationships between various responses occurring at different stages by correlated measurements. Typical approaches that rely on population-wide correlations, however, reveal these connections broadly and mask the fine details that might be discernible only at the single-cell level. Here, we present a microfluidics-based cell–cell interaction assay that allows defined generation, real-time imaging, and longitudinal assay of lymphocyte interactions, thereby permitting direct correlative studies within each single cell. Our studies using this platform indicate a possible role for the strength of calcium signaling in selective regulation of cytotoxicity and interferon-gamma production of natural killer cells. (See pp. E3599–E3608.)

Outer membrane vesicles displaying engineered glycotopes elicit protective antibodies

Linxiao Chen, Jenny L. Valentine, Chung-Jr Huang, Christine E. Endicott, Tyler D. Moeller, Jed A. Rasmussen, Joshua R. Fletcher, Joseph M. Boll, Joseph A. Rosenthal, Justyna Dobruchowska, Zhirui Wang, Christian Heiss, Parastoo Azadi, David Putnam, M. Stephen Trent, Bradley D. Jones, and Matthew P. DeLisa

Conjugate vaccines have proven to be an effective and safe strategy for reducing the incidence of disease caused by bacterial pathogens. However, the manufacture of these vaccines is technically demanding, inefficient, and expensive, thereby limiting their widespread use. Here, we describe an alternative methodology for generating glycoconjugate vaccines whereby recombinant polysaccharide biosynthesis is coordinated with vesicle formation in nonpathogenic *Escherichia coli*, resulting in glycosylated outer membrane vesicles (glycOMVs) that can effectively deliver pathogen-mimetic glycotopes to the immune system. An attractive feature of our approach is the fact that different plasmid-encoded polysaccharide biosynthetic pathways can be readily transformed into *E. coli*, enabling a "plug-and-play" platform for the on-demand creation of glycOMV vaccine candidates that carry heterologous glycotopes from numerous pathogenic bacteria. (See pp. E3609–E3618.)

Fragile X Mental Retardation Protein (FMRP) controls diacylglycerol kinase activity in neurons

Ricardos Tabet, Enora Moutin, Jérôme A. J. Becker, Dimitri Heintz, Laetitia Fouillen, Eric Flatter, Wojciech Krężel, Violaine Alunni, Pascale Koebel, Doulaye Dembélé, Flora Tassone, Barbara Bardoni, Jean-Louis Mandel, Nicolas Vitale, Dominique Muller, Julie Le Merrer, and Hervé Moine

Fragile X syndrome (FXS), the most frequent form of inherited intellectual disability, is caused by the absence of the protein Fragile X Mental Retardation Protein (FMRP) in neurons. In the absence of FMRP, the translation of a high number of mRNAs is increased in glutamatergic synapses, leading to abnormal synaptic function. It is unclear whether FMRP individually controls each of these mRNAs and whether some mRNAs are more important for the pathology. This study shows that FMRP mostly associates with and controls one main mRNA target in neurons, diacylglycerol kinase kappa (Dgkκ), a master regulator that controls two key signaling pathways activating protein synthesis. The deregulation of Dgkκ could account for many of the symptoms associated with FXS and could represent a novel therapeutic target. (See pp. E3619–E3628.)

Conformational dynamics of a G-protein α subunit is tightly regulated by nucleotide binding

David Goricanec, Ralf Stehle, Pascal Egloff, Simina Grigoriu, Andreas Plückthun, Gerhard Wagner, and Franz Hagn

G proteins are molecular switches for cellular signaling induced by G-protein–coupled receptor activation. The Gα subunit is the central timer of signal transduction regulated by GTP hydrolysis, which returns the system to its inactive state. Although previous work has characterized the structural states of Gα during the GTPase cycle, we show here that Gα is highly dynamic in the apo and GDP-bound states but in complex with GTP is completely rigid and is locked in a defined domain orientation. These insights help demonstrate that the conformational plasticity of G proteins is a central feature of their switching functionality. (See pp. E3629–E3638.)

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Phosphorylation of CMG helicase and Tof1 is required for programmed fork arrest

Deepak Bastia, Pankaj Srivastava, Shamsu Zaman, Malay Choudhury, Bidyut K. Mohanty, Julien Bacal, Lance D. Langston, Philippe Pasero, and Michael E. O'Donnell

Programmed replication fork arrest (PFA) at specific terminator sites and the proteins that bind to these sites functionally interconnect replication, transcription, and recombination. PFA prevents collision between replication and transcription that can cause genome instability, promotes intrachromatid recombination at ribosomal (r)DNA that controls replicative life span, and maintains rDNA homeostasis. This work reveals the mechanism of PFA by showing that the Tof1 protein of budding yeast remains associated with the replication fork only when it is phosphorylated, and uses the CMG helicase that drives the replication fork as a landing pad. Tof1–Csm3 promotes PFA by preventing the terminator protein Fob1 from getting displaced by other factors such as the Rrm3 helicase by counteracting the latter. This important mechanism appears to be evolutionarily conserved. (See pp. E3639–E3648.)

Defining chromosomal translocation risks in cancer

Marc A. Hogenbirk, Marinus R. Heideman, Iris de Rink, Arno Velds, Ron M. Kerkhoven, Lodewyk F. A. Wessels, and Heinz Jacobs

Applying innovative integrative analyses of multifactorial genome-wide data, we now demonstrate that an open chromatin configuration, which is generically enriched promoter-proximal but not promoter-specific, is the common denominator and key translocation risk-determinant of active chromatin. The finding that gene size directly correlated with its translocation risk, in both mice and cancer patients, independently emphasized the generic irrelevance of any promoter-specific activity. These data exclude activation-induced cytidine deaminase, Spt5, transcription, and promoter-proximal regions as critical risk-determinants and specific targets for genome-wide chromosomal translocations. Our insights are fundamental in understanding the origin of chromosome translocations and, consequently, cancer. (See pp. E3649–E3656.)

Rational design and validation of a vanilloid-sensitive TRPV2 ion channel

Fan Yang (杨帆), Simon Vu, Vladimir Yarov-Yarovoy, and Jie Zheng (郑劼)

Understanding how small molecules regulate activities of ion channels, which form the second largest family of drug targets, is important for both basic and translational research. By learning from how capsaicin activates TRPV1, we have introduced high-affinity binding of both capsaicin and resiniferatoxin to TRPV2 with only four point mutations. This successful outcome not only validated the working model for capsaicin–TRPV1 interactions but also suggested that vanilloids such as resiniferatoxin use a similar structural mechanism. Our findings further revealed previously unrecognized structural requirements for capsaicin-induced TRPV1 activation, which will guide future efforts to design drug candidates targeting this important pain sensor. In addition, the modified TRPV2–resiniferatoxin pair has the potential to be further engineered as a tool for chemogenetic studies. (See pp. E3657–E3666.)

Profiling DNA damage-induced phosphorylation in budding yeast reveals diverse signaling networks

Chunshui Zhou, Andrew E. H. Elia, Maria L. Naylor, Noah Dephoure, Bryan A. Ballif, Gautam Goel, Qikai Xu, Aylwin Ng, Danny M. Chou, Ramnik J. Xavier, Steven P. Gygi, and Stephen J. Elledge

The DNA damage response (DDR) promotes survival and genome maintenance. It involves a network of kinases that phosphorylate a multitude of effector proteins. Although the protein kinases involved have been studied extensively, many targets remain to be discovered. We have used an unbiased approach to profile DDR phosphorylation in budding yeast. We reveal a link between DDR signaling and the metabolic pathways of inositol phosphate and phosphatidyl inositol synthesis, which are required for resistance to DNA damage. Taken together, these data shed new light on the organization of DDR signaling in budding yeast. (See pp. E3667–E3675.)

Identification of S-phase DNA damage-response targets in fission yeast reveals conservation of damage-response networks

Nicholas A. Willis, Chunshui Zhou, Andrew E. H. Elia, Johanne M. Murray, Antony M. Carr, Stephen J. Elledge, and Nicholas Rhind

The cellular response to DNA damage during DNA replication promotes survival and genome maintenance. It involves a network of kinases that phosphorylate a variety of target proteins. Although the protein kinases involved have been studied extensively in fission yeast, only a handful of their targets have been identified. We used an unbiased approach to profile protein phosphorylation in response to DNA damage during DNA replication. We found target proteins involved in gene expression, stress response, regulation of mitosis and cytokinesis, and DNA replication and repair, many of which are required for resistance to DNA damage. These protein targets are conserved in budding yeast and human cells, demonstrating the deep conservation of the response. (See pp. E3676–E3685.)

Phosphoinositide 5- and 3-phosphatase activities of a voltage-sensing phosphatase in living cells show identical voltage dependence

Dongil Keum, Martin Kruse, Dong-Il Kim, Bertil Hille, and Byung-Chang Suh

Phosphatase and tensin homolog (PTEN) is a phosphatidylinositol 3,4-bisphosphate [$PI(3,4)P_2$] and phosphatidylinositol 3,4,5-trisphosphate [$PI(3,4,5)P_3$] 3-phosphatase that plays important roles in cell polarization, division, and development as well as in tumor suppression. Voltage-sensing phosphatases (VSPs) are PTEN family members but have catalytic activity in response to membrane depolarization with a wider range of substrates: They cleave the 5-phosphate of $PI(3,4,5)P_3$ and phosphatidylinositol 4,5-bisphosphate as well as the 3-phosphate of $PI(3,4)P_2$ and perhaps $PI(3,4,5)P_3$. Using in-cell observations accompanied by quantitative analysis, we demonstrated that VSPs clearly cleave 3-phosphate from $PI(3,4,5)P_3$ as PTEN does. Our results suggest that VSPs might act as surrogates for PTEN in several biological events related to membrane depolarization in addition to their inherent role. (See pp. E3686–E3695.)

Biogenesis of sperm acrosome is regulated by pre-mRNA alternative splicing of *Acrbp* in the mouse

Yoshinori Kanemori, Yoshitaka Koga, Mai Sudo, Woojin Kang, Shin-ichi Kashiwabara, Masahito Ikawa, Hidetoshi Hasuwa, Kiyoshi Nagashima, Yu Ishikawa, Narumi Ogonuki, Atsuo Ogura, and Tadashi Baba

Mammalian sperm possess a Golgi-derived exocytotic organelle, the acrosome, located on the apical region of the head. Proper biogenesis of the acrosome is essential for the fertilization process because the aberrant acrosome formation results in the sterility or subfertility of males. Here, we show that the acrosome formation is governed by two forms of proacrosin-binding protein ACRBP, wild-type ACRBP-W and variant ACRBP-V5, which are generated by pre-mRNA alternative splicing of *Acrbp*. ACRBP-V5 is involved in the formation and configuration of the acrosomal granule during early spermiogenesis, whereas the inactive status of proacrosin in the acrosome is maintained by ACRBP-W until acrosomal exocytosis. (See pp. E3696–E3705.)

Mutation of *Fnip1* is associated with B-cell deficiency, cardiomyopathy, and elevated AMPK activity

Owen M. Siggs, Alexander Stockenhuber, Mukta Deobagkar-Lele, Katherine R. Bull, Tanya L. Crockford, Bethany L. Kingston, Greg Crawford, Consuelo Anzilotti, Violetta Steeples, Sahar Ghaffari, Gabor Czibik, Mohamed Bellahcene, Hugh Watkins, Houman Ashrafian, Benjamin Davies, Angela Woods, David Carling, Arash Yavari, Bruce Beutler, and Richard J. Cornall

Cellular metabolism is tightly regulated by AMP-activated protein kinase (AMPK): the function of which is influenced by folliculin (FLCN), folliculin-interacting protein (FNIP)1, and FNIP2. FLCN is a known tumor-suppressor protein that is mutated in Birt–Hogg–Dubé syndrome, whereas FNIP1 and FNIP2 are binding partners of FLCN. Previous reports have suggested that the FLCN/FNIP1/FNIP2 complex acts a positive regulator of AMPK, whereas other reports suggest the opposite. Using a new mouse model of FNIP1 deficiency, our findings support the latter: we found that mutation of *Fnip1* leads to B-cell deficiency and the development of a cardiomyopathy similar to mice and humans with gain-of-function mutations in AMPK. (See pp. E3706–E3715.)

Conserved 33-kb haplotype in the MHC class III region regulates chronic arthritis

Anthony C. Y. Yau, Jonatan Tuncel, Sabrina Haag, Ulrika Norin, Miranda Houtman, Leonid Padyukov, and Rikard Holmdahl

The role of the MHC region has been a long-standing issue in chronic inflammatory diseases, such as rheumatoid arthritis, and it has not been possible to identify the underlying specific polymorphism. Here, we provide evidence that some of the MHC association must be explained by how closely linked genes operate together as haplotype blocks. We identified a conserved haplotype, *Ltab-Ncr3*, comprising five genes (*lymphotoxin α* and *β*, *Tnf*, *leukocyte-specific transcript 1*, and *natural cytotoxicity-triggering receptor 3*) within MHC class III, regulating arthritis. We found significant coexpression of the *Ltab-Ncr3* genes, indicating how these genes may work together as a haplotype. Furthermore, haplotype-specific differences in *Ltab-Ncr3* gene expression and alternative splicing correlate remarkably to susceptibility to arthritis. Our data show that a conserved haplotype within MHC class III regulates arthritis development. (See pp. E3716–E3724.)

Connectivity mapping (ssCMap) to predict A20-inducing drugs and their antiinflammatory action in cystic fibrosis

Beth Malcomson, Hollie Wilson, Eleonora Veglia, Gayathri Thillaiyampalam, Ruth Barsden, Shauna Donegan, Amal El Banna, Joseph S. Elborn, Madeleine Ennis, Catriona Kelly, Shu-Dong Zhang, and Bettina C. Schock

This study reports that publicly available gene array expression data together with statistically significant connections' map successfully predicts licensed drugs able to modify genes of interest. We used this method to predict drugs able to induce A20 [TNFα-induced protein 3 (TNFAIP3)], which is reduced in cystic fibrosis (CF) airway cells, and thus normalize the inflammatory response. Using CF and non-CF airway epithelial cells, ikarugamycin and quercetin had antiinflammatory effects mediated by induction of A20. We confirmed that this was mainly due to A20 induction, because no antiinflammatory effects were seen in bronchial epithelial cells with A20 knockdown. We have identified a process whereby already licensed drugs can be successfully repositioned for chronic inflammatory airway diseases. (See pp. E3725–E3734.)

HIFI-α activation underlies a functional switch in the paradoxical role of Ezh2/PRC2 in breast cancer

Sylvia Mahara, Puay Leng Lee, Min Feng, Vinay Tergaonkar, Wee Joo Chng, and Qiang Yu

The plasticity of Polycomb repressive complex 2 (PRC2) in the context of tumorigenesis has remained a subject of contention. Here we demonstrate that the equilibrium between the oncogenic and tumor-suppressive activity of PRC2 in promoting breast cancer invasion is tightly regulated by hypoxia-inducible factor 1-α. PRC2 acts as a tumor-suppressor barrier to the hypoxia-driven invasion pathway, and the impaired PRC2 activity upon hypoxia promotes a chromatin switch at proinvasion matrix metalloproteinase gene loci. The study fundamentally changed our understanding of the role of PRC2 in breast cancer and also identified a previously unidentified function of enhancer of zeste 2 to complex with Forkhead box M1 to promote cancer invasion. (See pp. E3735–E3744.)

Cross-class metallo-β-lactamase inhibition by bisthiazolidines reveals multiple binding modes

Philip Hinchliffe, Mariano M. González, Maria F. Mojica, Javier M. González, Valerie Castillo, Cecilia Saiz, Magda Kosmopoulou, Catherine L. Tooke, Leticia I. Llarrull, Graciela Mahler, Robert A. Bonomo, Alejandro J. Vila, and James Spencer

Bacterial diseases remain a huge burden on healthcare worldwide, with the emergence and re-emergence of strains resistant to currently used antibiotics posing an increasing clinical threat. Metallo-β-lactamases (MBLs) are key determinants of antibiotic resistance because they hydrolyze almost all β-lactam antibiotics and are unaffected by currently available β-lactamase inhibitors (βLIs). The structural diversity between MBLs has proved problematic when designing βLIs effective against all MBL targets. Here we show a series of small compounds, bisthiazolidines, which act as inhibitors of all MBL types, restoring the efficacy of currently used antibiotics against resistant bacterial strains producing different MBLs. High-resolution crystal structures reveal how diverse MBLs are inhibited by the unexpected versatility of bisthiazolidine binding, raising implications for future βLI design. (See pp. E3745–E3754.)

Tau protein is essential for stress-induced brain pathology

Sofia Lopes, João Vaz-Silva, Vitor Pinto, Christina Dalla, Nikolaos Kokras, Benedikt Bedenk, Natalie Mack, Michael Czisch, Osborne F. X. Almeida, Nuno Sousa, and Ioannis Sotiropoulos

Exposure to stressful events is a well-known inducer of neuronal atrophy implicated in the development of neuropsychiatric and neurological pathologies (e.g., depression and Alzheimer's disease), although the underlying molecular mechanisms remain elusive. The current study demonstrates that absence of the cytoskeletal protein Tau blocks stress-evoked hippocampal synaptic signaling and morphofunctional damages related to both neuronal structure and connectivity as well as subsequent behavioral deficits. These findings suggest, for the first time to our knowledge, that Tau protein is a key regulator of neuronal malfunction found in stress-driven hippocampal pathology. (See pp. E3755–E3763.)

Activation of the molecular chaperone, sigma 1 receptor, preserves cone function in a murine model of inherited retinal degeneration

Jing Wang, Alan Saul, Penny Roon, and Sylvia B. Smith

The role of sigma 1 receptor (Sig1R) in rescuing cone photoreceptor function was investigated in *Pde6β*^{rd10} (*rd10*) mice, a model of severe retinal degeneration. Sig1R, a putative molecular chaperone, is implicated in several human neurodegenerative diseases. We administered (+)-pentazocine, a high-affinity Sig1R ligand, to *rd10* mice, which lose rod and subsequently cone photoreceptor cells (PRC) within the first few weeks of life, rendering them completely blind. Regular administration of (+)-pentazocine rescued cone PRC responses, which were markedly preserved and were similar to those in wild-type mice. To our knowledge, this is the first demonstration of significant preservation of retinal function as a consequence of Sig1R activation. The data are highly relevant to potential therapeutic interventions in human retinal disease. (See pp. E3764–E3772.)

Tau accumulation induces synaptic impairment and memory deficit by calcineurin-mediated inactivation of nuclear CaMKIV/CREB signaling

Yaling Yin, Di Gao, Yali Wang, Zhi-Hao Wang, Xin Wang, Jinwang Ye, Dongqin Wu, Lin Fang, Guilin Pi, Ying Yang, Xiao-Chuan Wang, Chengbiao Lu, Keqiang Ye, and Jian-Zhi Wang

Memory deterioration is a characteristic clinical symptom in patients with Alzheimer's disease (AD); however, the mechanisms underlying the memory loss are poorly understood. Here, we found that intraneuronal tau accumulation, the hallmark pathology seen in AD brains, induced a remarkable dephosphorylation/inactivation of nuclear cAMP response element binding protein (CREB), an important memory-associated protein. Further studies demonstrated that the abnormal tau accumulation could activate calcineurin, a calcium/calmodulin-dependent protein phosphatase and cause CREB dephosphorylation. Importantly, simultaneous inhibition of calcineurin remarkably attenuated tau-induced CREB inactivation and memory deficits. These findings not only reveal new mechanisms underlying AD memory deficits, but also provide a potential drug target for arresting tauopathies. (See pp. E3773–E3781.)

Brain aerobic glycolysis and motor adaptation learning

Benjamin J. Shannon, Sanjeev Neil Vaishnavi, Andrei G. Vlassenko, Joshua S. Shimony, Jerrel Rutlin, and Marcus E. Raichle

A substantial fraction of glucose used by the brain does not enter the oxidative phosphorylation pathway despite the presence of adequate oxygen, a phenomenon known as aerobic glycolysis. Among its several functions, aerobic glycolysis makes substantial contributions to biosynthesis, thus becoming a marker of synaptic plasticity. Combining PET and MRI brain-imaging techniques, we characterized the role of aerobic glycolysis in plasticity during the performance of a motor adaptation learning task. Our findings support a link between aerobic glycolysis and learning as well as providing unexpected evidence of a potential role of microglia in long-term depression and synaptic pruning. (See pp. E3782–E3791.)

Singlet oxygen- and EXECUTER1-mediated signaling is initiated in grana margins and depends on the protease FtsH2

Liangsheng Wang (王良省), Chanhong Kim, Xia Xu, Urszula Piskurewicz, Vivek Dogra, Somesh Singh, Hanno Mahler, and Klaus Apel

Singlet oxygen ($^1O_2$)- and EXECUTER1 (EX1)-dependent signaling triggers programmed cell death in seedlings and inhibits growth of mature plants of the *fluorescent* (*flu*) mutant of *Arabidopsis*. The EX1 protein has been located in chloroplasts to the grana margins close to where chlorophyll is synthesized and the disassembly of damaged photosystem II (PSII) and reassembly of active PSII take place. With the onset of $^1O_2$-mediated signaling there is a rapid decline of EX1 that depends on the ATP-dependent zinc metalloprotease FtsH. Generation of $^1O_2$ without the decline of EX1 is not sufficient to activate $^1O_2$ signaling. As FtsH cleaves also the D1 reaction center protein of damaged PSII, EX1-dependent signaling seems not only spatially but also functionally linked to the repair of PSII. (See pp. E3792–E3800.)

Comparative genome-scale modelling of *Staphylococcus aureus* strains identifies strain-specific metabolic capabilities linked to pathogenicity

Emanuele Bosi, Jonathan M. Monk, Ramy K. Aziz, Marco Fondi, Victor Nizet, and Bernhard Ø. Palsson

Comparative analysis of multiple strains within a species is a powerful way to uncover pathoadaptive genetic acquisitions. Hundreds of genome sequences are now available for the human pathogen *Staphylococcus aureus*, mostly known for its antibiotic-resistant variants that threaten the emergence of panresistant superbugs. In this study, genome-scale models of metabolism are used to analyze the shared and unique metabolic capabilities of this pathogen and its strain-specific variants. The models are used to distinguish *S. aureus* strains responsible for severe infections based solely on growth capabilities and presence of different virulence factors. The results identify metabolic similarities and differences between *S. aureus* strains that provide insights into the epidemiology of *S. aureus* and may help to combat its spread. (See pp. E3801–E3809.)