PNAS Plus Significance Statements

Quantification of labile heme in live malaria parasites using a genetically encoded biosensor

James R. Abshire, Christopher J. Rowlands, Suresh M. Ganesan, Peter T. C. So, and Jacquin C. Niles

Malaria parasites degrade substantial quantities of hemoglobin to release heme within a specialized digestive vacuole. Most of this heme is sequestered in an inert crystal. However, the concentration of bioavailable, labile heme in the parasite's cytosol was unknown. We developed a biosensor to provide the first quantitative insights into labile heme concentrations in malaria parasites. We find that ~1.6 μM labile cytosolic heme is maintained, including during a period coincident with intense hemoglobin degradation. The heme-binding antimalarial drug, chloroquine, which interferes with heme crystallization, specifically induces an increase in labile heme. The ability to quantify labile heme in malaria parasites opens opportunities for better understanding heme homeostasis, signaling, and metabolism, and its association with antimalarial potency. (See pp. E2068–E2076.)

Bioinformatic analysis of riboswitch structures uncovers variant classes with altered ligand specificity

Zasha Weinberg, James W. Nelson, Christina E. Lünse, Madeline E. Sherlock, and Ronald R. Breaker

In the 15 y since metabolite-binding riboswitches were first experimentally validated, only 4 examples of riboswitch classes with altered specificity have been confirmed by experiments out of ~30 distinct structural architectures. In contrast, evolutionary changes in ligand specificity of proteins are routinely reported. To further investigate the propensity for natural adaptation of riboswitch specificity, we developed a structural bioinformatics method to systematically search for variant riboswitches with altered ligand recognition. This search method yielded evidence for altered specificity within five riboswitch classes, including validation of a second riboswitch class that senses 2′-deoxyguanosine. (See pp. E2077–E2085.)

Synergy of cAMP and calcium signaling pathways in CFTR regulation

Zoltan Bozoky, Saumel Ahmadi, Tal Milman, Tae Hun Kim, Kai Du, Michelle Di Paola, Stan Pasyk, Roman Pekhletski, Jacob P. Keller, Christine E. Bear, and Julie D. Forman-Kay

Cystic fibrosis is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that encodes a chloride channel located in the apical membrane of epithelia cells. The cAMP signaling pathway and protein phosphorylation are known to be primary controlling mechanisms for channel function. In this study, we present an alternative activation pathway that involves calcium-activated calmodulin binding of the intrinsically disordered regulatory (R) region of CFTR. Beyond their potential therapeutic value, these data provide insights into the intersection of calcium signaling with control of ion homeostasis and the ways in which the local CFTR microdomain organizes itself. (See pp. E2086–E2095.)

Structural basis of autoregulatory scaffolding by apoptosis signal-regulating kinase 1

Johannes F. Weijman, Abhishek Kumar, Sam A. Jamieson, Chontelle M. King, Tom T. Caradoc-Davies, Elizabeth C. Ledgerwood, James M. Murphy, and Peter D. Mace

Phosphorylation catalyzed by protein kinases governs many aspects of cellular behavior. Apoptosis signal-regulating kinases (ASK1–3) trigger responses to stress, but the structural basis of their regulation remains unclear. Here, we show that a domain directly adjacent to the ASK1 kinase domain promotes activity of ASK1 on a key substrate and also orients an additional ASK1 domain nearby to suppress kinase activity. The structure of this regulatory domain appears to be shared by all ASK kinases and provides a versatile mechanism to control ASK activity in response to various stress stimuli. (See pp. E2096–E2105.)

Structure of human IFIT1 with capped RNA reveals adaptable mRNA binding and mechanisms for sensing N1 and N2 ribose 2′-O methylations

Yazan M. Abbas, Beatrice Theres Laudenbach, Saúl Martínez-Montero, Regina Cencic, Matthias Habjan, Andreas Pichlmair, Masad J. Damha, Jerry Pelletier, and Bhushan Nagar

IFIT1 is an antiviral effector of host innate-immunity that selectively recognizes the 5′-end of viral mRNAs, which are often capped to mimic host mRNA, and blocks their translation. Our X-ray structural analysis reveals that the cap and four additional nucleotides are encircled by IFIT1 through a central tunnel in an adaptable manner, which gives it the flexibility required to defend against many different viruses, and to deter their ability to rapidly evolve. Host mRNA, normally ribose methylated at the first and second nucleotides following the cap, avoids IFIT1 recognition through tight complementary interfaces at these positions. This study uncovers the molecular basis for how IFIT1 selectively recognizes viral mRNAs and will

help guide development of viral vaccines and mRNA therapeutics. (See pp. E2106–E2115.)

Genome-wide transcription-coupled repair in *Escherichia coli* is mediated by the Mfd translocase

Ogun Adebali, Yi-Ying Chiou, Jinchuan Hu, Aziz Sancar, and Christopher P. Selby

In transcription-coupled repair (TCR), nucleotide excision repair occurs most rapidly in the template strand of actively transcribed genes. TCR has been observed in a limited set of genes directly assayed in *Escherichia coli* cells. In vitro, Mfd translocase performs reactions necessary to mediate TCR: It removes RNA polymerase blocked by a template strand lesion and rapidly delivers repair enzymes to the lesion. This study applied excision repair sequencing methodology to map the location of repair sites in different *E. coli* strains. Results showed that Mfd-dependent TCR is widespread in the *E. coli* genome. Results with UvrD helicase demonstrated its role in basal repair, but no overall role in TCR. (See pp. E2116–E2125.)

Rps3/uS3 promotes mRNA binding at the 40S ribosome entry channel and stabilizes preinitiation complexes at start codons

Jinsheng Dong, Colin Echeverría Aitken, Anil Thakur, Byung-Sik Shin, Jon R. Lorsch, and Alan G. Hinnebusch

In the initiation of protein synthesis, a preinitiation complex (PIC) of the 40S ribosomal subunit, initiation factors, and initiator $tRNA_i$ scans the mRNA leader for an AUG codon in favorable context; and AUG recognition evokes a closed conformation of the PIC with more tightly bound $tRNA_i$. uS3 (Rps3 in yeast) is a protein in the 40S mRNA entry channel, whose function during initiation was unknown. Substituting uS3 arginine residues in contact with mRNA reduces initiation at suboptimal start codons (UUG and AUG in poor context), weakens ribosome–mRNA interaction specifically at the entry channel, and destabilizes $tRNA_i$ binding selectively at UUG codons. Thus, uS3 promotes mRNA:40S interaction at the entry channel to enhance initiation accuracy. (See pp. E2126–E2135.)

Ligand binding to telomeric G-quadruplex DNA investigated by funnel-metadynamics simulations

Federica Moraca, Jussara Amato, Francesco Ortuso, Anna Artese, Bruno Pagano, Ettore Novellino, Stefano Alcaro, Michele Parrinello, and Vittorio Limongelli

A thorough characterization of the binding interaction between a drug and its molecular target is fundamental to successfully lead drug design. We demonstrate that this characterization is also possible using the recently developed method of funnel-metadynamics (FM), here applied to investigate the binding of berberine to DNA G-quadruplex. We computed a quantitatively well-characterized free-energy landscape that allows identifying two low-energy ligand binding modes and the presence of higher energy prebinding states. We validated the accuracy of our calculations by steady-state fluorescence experiments. The good agreement between the theoretical and experimental binding free-energy value demonstrates that FM is a most reliable method to study ligand/DNA interaction. (See pp. E2136–E2145.)

Mechanism and bottlenecks in strand photodissociation of split green fluorescent proteins (GFPs)

Chi-Yun Lin (林其勻), Johan Both, Keunbong Do, and Steven G. Boxer

Split GFPs have been used exclusively as imaging tools to study protein–protein interactions, but the irreversible nature of split GFP complementation can be highly perturbative. Dissociating the split GFP into constituent parts with light irradiation not only remedies this problem but also invites the opportunity for noninvasive optical control of biological processes. By elucidating an energetics–function model for split GFP strand photodissociation, we show that photodissociation is achieved by light-activated *cis-trans* isomerization of the chromophore, a mechanism pervasive among reversibly photoswitchable fluorescent proteins. This observation suggests that these proteins can be engineered to provide active control of in vivo processes as optogenetic tools in addition to their conventional roles as passive imaging reporters. (See pp. E2146–E2155.)

Localization of the gate and selectivity filter of the full-length P2X7 receptor

Anja Pippel, Michaela Stolz, Ronja Woltersdorf, Achim Kless, Günther Schmalzing, and Fritz Markwardt

The P2X7 receptor (P2X7R) was the first ion channel that was suggested to transform from cation selective into nonselective by undergoing a dilatation in the diameter of its transmembrane pathway following sustained activation. This change requires that the selectivity filter behave as a dynamic structure. Here, we used a single-channel analysis of cysteine substitution mutants to find that the gate and selectivity filter of P2X7R are colocalized and primarily determined by one single residue, S342. We found the agonist-opened selectivity filter to be completely stable over time, indicating that use-dependent dilatation of the channel diameter does not occur. Instead, P2X7R exhibits striking susceptibility to remain in the open state for longer when the channel pore contains slowly or nonpermeating cations. (See pp. E2156–E2165.)

LEM2 recruits CHMP7 for ESCRT-mediated nuclear envelope closure in fission yeast and human cells

Mingyu Gu, Dollie LaJoie, Opal S. Chen, Alexander von Appen, Mark S. Ladinsky, Michael J. Redd, Linda Nikolova, Pamela J. Bjorkman, Wesley I. Sundquist, Katharine S. Ullman, and Adam Frost

The molecular mechanism for sealing newly formed nuclear envelopes was unclear until the recent discovery that endosomal sorting complexes required for transport III (ESCRT-III) proteins mediate this process. Cmp7p (CHMP7), in particular, was identified as an early acting factor that recruits other ESCRT-III proteins to the nuclear envelope. A fundamental aspect of the varied roles of ESCRT factors is their recruitment by site-specific adaptors, yet the central question of how the ESCRT machinery is targeted to nuclear membranes has remained outstanding. Our study identifies the inner nuclear membrane protein LEM2 as a key, conserved factor that recruits CHMP7 and downstream ESCRT-III proteins to breaches in the nuclear envelope. (See pp. E2166–E2175.)

SM protein Munc18-2 facilitates transition of Syntaxin 11-mediated lipid mixing to complete fusion for T-lymphocyte cytotoxicity

Waldo A. Spessott, Maria L. Sanmillan, Margaret E. McCormick, Vineet V. Kulkarni, and Claudio G. Giraudo

Vital physiological processes—such as the cytotoxic immune response—require the coordinated action of the atypical fusion protein Syntaxin 11 (STX11) and the Sec/Munc protein Munc18-2 for releasing effector proteins housed in membrane-enclosed secretory granules. Human mutations in STX11 and Munc18-2 genes lead to severe immunodeficiency and hemostasis disorders. However it is still unclear how STX11, a

lipid-anchored SNARE, and Munc18-2 mediate membrane fusion. By using an in vitro fusion assay, we found that STX11 mainly mediates lipid mixing when combined with human lymphocyte-interacting SNAREs. Remarkably, Munc18-2 induces association among these SNAREs and facilitates the transition from a hemifusion-like state to complete fusion. Our findings support a model in which SM proteins play a direct role in membrane merging. (See pp. E2176–E2185.)

Vitamin D receptor regulates autophagy in the normal mammary gland and in luminal breast cancer cells

Luz E. Tavera-Mendoza, Thomas Westerling, Eric Libby, Andriy Marusyk, Laura Cato, Raymundo Cassani, Lisa A. Cameron, Scott B. Ficarro, Jarrod A. Marto, Jelena Klawitter, and Myles Brown

Epidemiological evidence suggests that vitamin D can protect women from developing breast cancer (BC). This study reveals that vitamin D and its receptor regulate autophagy in both normal mammary epithelial cells and luminal BCs, and suggests a potential mechanism underlying the link between vitamin D levels and BC risk. In addition, this work suggests that vitamin D receptor ligands could be exploited therapeutically for the treatment of a significant subset of BCs. (See pp. E2186–E2194.)

Aberrant splicing in maize *rough endosperm3* reveals a conserved role for U12 splicing in eukaryotic multicellular development

Christine M. Gault, Federico Martin, Wenbin Mei, Fang Bai, Joseph B. Black, W. Brad Barbazuk, and A. Mark Settles

The last eukaryotic common ancestor had two spliceosomes. The major spliceosome acts on nearly all introns, whereas the minor spliceosome removes rare, U12-type introns. Based on in vitro RNA-splicing assays, the RGH3/ZRSR2 RNA-splicing factor has functions in both spliceosomes. Here, we show that the maize *rgh3* mutant allele primarily disrupts U12 splicing, similar to human *ZRSR2* mutants, indicating a conserved in vivo function in the minor spliceosome. These mutant alleles block cell differentiation leading to overaccumulation of stem cells in endosperm and blood, respectively. We found extensive conservation between maize and human U12-type intron-containing genes, demonstrating that a common genetic architecture controls at least a subset of cell differentiation pathways in both plants and animals. (See pp. E2195–E2204.)

Monoubiquitylation of histone H2B contributes to the bypass of DNA damage during and after DNA replication

Shih-Hsun Hung, Ronald P. Wong, Helle D. Ulrich, and Cheng-Fu Kao

DNA damage-bypass mechanisms that facilitate the resolution of replication blocks in proliferating cells during and after S phase are important for the defense against damage-induced mutations, genome instability, and cancer. Lesion bypass, mediated either by the ubiquitylation of the replication factor proliferating cell nuclear antigen or by homologous recombination, takes place in the context of chromatin. However, the implications of nucleosome dynamics and chromosome packaging in the efficiency of replication-associated damage processing are still largely unknown. Our physical, genetic, and cytological studies suggest that ubiquitylation of histone H2B facilitates the replicative bypass of fork-stalling DNA lesions by contributing to both DNA damage tolerance and homologous recombination during and after replication, thus revealing a direct link between chromatin architecture and lesion bypass. (See pp. E2205–E2214.)

Transposon insertional mutagenesis in mice identifies human breast cancer susceptibility genes and signatures for stratification

Liming Chen, Piroon Jenjaroenpun, Andrea Mun Ching Pillai, Anna V. Ivshina, Ghim Siong Ow, Motakis Efthimios, Tang Zhiqun, Tuan Zea Tan, Song-Choon Lee, Keith Rogers, Jerrold M. Ward, Seiichi Mori, David J. Adams, Nancy A. Jenkins, Neal G. Copeland, Kenneth Hon-Kim Ban, Vladimir A. Kuznetsov, and Jean Paul Thiery

Despite concerted efforts to identify causal genes that drive breast cancer (BC) initiation and progression, we have yet to establish robust signatures to stratify patient risk. Here we used in vivo transposon-based forward genetic screening to identify potentially relevant BC driver genes. Integrating this approach with survival prediction analysis, we identified six gene pairs that could prognose human BC subtypes into high-, intermediate-, and low-risk groups with high confidence and reproducibility. Furthermore, we identified susceptibility gene sets for basal and claudin-low subtypes (21 and 16 genes, respectively) that stratify patients into three relative risk subgroups. These signatures offer valuable prognostic insight into the genetic basis of BC and allow further exploration of the interconnectedness of BC driver genes during disease progression. (See pp. E2215–E2224.)

Glyoxylate detoxification is an essential function of malate synthase required for carbon assimilation in *Mycobacterium tuberculosis*

Susan Puckett, Carolina Trujillo, Zhe Wang, Hyungjin Eoh, Thomas R. Ioerger, Inna Krieger, James Sacchettini, Dirk Schnappinger, Kyu Y. Rhee, and Sabine Ehrt

A better understanding of essential processes in *Mycobacterium tuberculosis* (*Mtb*) is required for the development of new chemotherapeutics. Isocitrate lyase (ICL) and malate synthase (MS) function in the glyoxylate shunt, a pathway required by *Mtb* to metabolize fatty acids (FAs). Here, we demonstrate that *Mtb* MS enables growth and survival on fatty acids through its ability to simultaneously detoxify a metabolic byproduct arising from the initial assimilation of acetyl coenzyme A (acetyl-CoA), glyoxylate, while assimilating a second molecule of acetyl-CoA. We further show that MS depletion during acute and chronic mouse infections kills *Mtb*. These studies expand our fundamental understanding of the glyoxylate shunt and biologically validate MS as an attractive drug target in *Mtb*. (See pp. E2225–E2232.)

Manganese scavenging and oxidative stress response mediated by type VI secretion system in *Burkholderia thailandensis*

Meiru Si, Chao Zhao, Brianne Burkinshaw, Bing Zhang, Dawei Wei, Yao Wang, Tao G. Dong, and Xihui Shen

As an essential micronutrient, Gram-negative bacteria must concentrate Mn^{2+} into the cytosol via active transport systems to meet cellular demands. Whereas inner membrane Mn^{2+} transporters have been characterized, an active transporter for translocation of Mn^{2+} across the outer membrane has not been described. Here we report a Mn^{2+}-scavenging pathway consisting of a newly identified TonB-dependent outer membrane manganese transporter, MnoT, and a type VI secretion system (T6SS)-secreted Mn^{2+}-binding protein, TseM. Traditionally, T6SS is recognized as a contact-dependent nanomachine to inject effectors into eukaryotic or prokaryotic cells for virulence or for interbacterial competition. The contact-independent functions of T6SS for metal acquisition and bacteria–bacteria competition,

reported here, suggest that T6SS may have been retrofitted by some bacteria to gain additional adaptive functions during evolution. (See pp. E2233–E2242.)

Rapid and efficient generation of oligodendrocytes from human induced pluripotent stem cells using transcription factors

Marc Ehrlich, Sabah Mozafari, Michael Glatza, Laura Starost, Sergiy Velychko, Anna-Lena Hallmann, Qiao-Ling Cui, Axel Schambach, Kee-Pyo Kim, Corinne Bachelin, Antoine Marteyn, Gunnar Hargus, Radia Marie Johnson, Jack Antel, Jared Sterneckert, Holm Zaehres, Hans R. Schöler, Anne Baron-Van Evercooren, and Tanja Kuhlmann

Understanding of myelin diseases and development of new treatment options are at least partly hampered by the limited availability of human oligodendrocytes. Induced pluripotent stem cells (iPSC) may be an ideal tool to circumvent this problem; however, rapid and efficient protocols to generate oligodendrocytes from human iPSC are currently lacking. The induction of the transcription factors SOX10, OLIG2, and NKX6.2 in iPSC-derived neural progenitors accelerates oligodendroglial differentiation significantly resulting in up to 70% of $O4^+$ oligodendrocytes within 28 d. These oligodendrocytes myelinate the CNS during development and after demyelination, and are suitable for pharmacological screens and disease modeling. The strategy presented herein will markedly facilitate the studying of human myelin diseases and the development of screening platforms for drug discovery. (See pp. E2243–E2252.)

Synaptic vesicle glycoprotein 2C (SV2C) modulates dopamine release and is disrupted in Parkinson disease

Amy R. Dunn, Kristen A. Stout, Minagi Ozawa, Kelly M. Lohr, Carlie A. Hoffman, Alison I. Bernstein, Yingjie Li, Minzheng Wang, Carmelo Sgobio, Namratha Sastry, Huaibin Cai, W. Michael Caudle, and Gary W. Miller

Here we describe a role for the synaptic vesicle glycoprotein 2C (SV2C) in dopamine neurotransmission and Parkinson disease (PD). SV2C is expressed on the vesicles of dopamine-producing neurons, and genetic deletion of SV2C causes a reduction in synaptic release of dopamine. The reduced dopamine release is associated with a decrease in motor activity. SV2C is suspected of mediating the neuroprotective effects of nicotine, and we show an ablated neurochemical response to nicotine in SV2C-knockout mice. Last, we demonstrate that SV2C expression is specifically disrupted in mice that express mutated α-synuclein and in humans with PD. Together, these data establish SV2C as an important mediator of dopamine homeostasis and a potential contributor to PD pathogenesis. (See pp. E2253–E2262.)