PNAS Plus Significance Statements

Broad and efficient control of major foodborne pathogenic strains of *Escherichia coli* by mixtures of plant-produced colicins

Steve Schulz, Anett Stephan, Simone Hahn, Luisa Bortesi, Franziska Jarczowski, Ulrike Bettmann, Anne-Katrin Paschke, Daniel Tusé, Chad H. Stahl, Anatoli Giritch, and Yuri Gleba

Enterohemorrhagic *Escherichia coli*-contaminated food products are among the leading causes of bacterial enteric infections in the United States and worldwide. Currently, other than thermal inactivation, there are no effective methods to control pathogenic bacteria in food. We investigated colicins, nonantibiotic antimicrobial proteins produced by certain *E. coli* strains and active against other strains of the species, as potential pathogen control agents. We demonstrate that most colicins can be expressed at high yields in plants and are fully functional. We show that mixtures of colicins applied at low concentrations are highly and broadly active against all major pathogenic *E. coli* strains of concern for foodborne illness. We propose plant-produced colicins as an inexpensive food treatment for the broad control of pathogenic *E. coli* strains. (See pp. E5454–E5460.)

Nucleosome competition reveals processive acetylation by the SAGA HAT module

Alison E. Ringel, Anne M. Cieniewicz, Sean D. Taverna, and Cynthia Wolberger

Crosstalk between histone modifications regulates transcription by establishing spatial and temporal relationships between histone marks. Despite discoveries of reader domains that physically associate with chromatin-modifying enzymes, the mechanisms by which recognition of one modification triggers other kinds of modifications have remained elusive. Gcn5 is the catalytic subunit of the Spt-Ada-Gcn5 acetyltransferase (SAGA) histone acetyltransferase (HAT) module, which also recognizes histone 3 lysine 4 trimethylation (H3K4me3) through the tandem Tudor domain-containing protein Sgf29. Although previous studies could not connect H3K4me3 recognition to differences in acetylation by Gcn5, we report enhanced processivity by the HAT module on methylated substrates using a previously unpublished histone color-coding assay. Our work defines a mechanism for histone crosstalk that may account for genome-wide patterns of Gcn5-mediated acetylation. (See pp. E5461–E5470.)

Topological patterns in two-dimensional gel electrophoresis of DNA knots

Davide Michieletto, Davide Marenduzzo, and Enzo Orlandini

Gel electrophoresis is a ubiquitous biophysical technique. It consists of dragging charged biopolymers through a porous gel, by applying an electric field. Because the migration speed depends on topology, this method can be used to classify DNA knots. Currently, electrophoresis relies on empirical observations, and its theoretical understanding is limited. No theory can explain why knot mobility under strong fields depends nonmonotonically on complexity. Our study reveals a possible reason: Although complex knots have a smaller size, and hence move faster through the gel, they can become severely entangled with the gel, causing longer pauses. Our results can improve the design of future electrophoresis experiments. (See pp. E5471–E5477.)

Control over overall shape and size in de novo designed proteins

Yu-Ru Lin, Nobuyasu Koga, Rie Tatsumi-Koga, Gaohua Liu, Amanda F. Clouser, Gaetano T. Montelione, and David Baker

We describe how protein size and shape can be sculpted by de novo protein design. Precise control over protein shape will be critical for completely de novo design of high-affinity binding proteins, enzymes, and protein-based nanomaterials. The systematic procedure for design of αβ-protein structures from scratch described in this paper should be broadly useful. (See pp. E5478–E5485.)

Comprehensive assessment of cancer missense mutation clustering in protein structures

Atanas Kamburov, Michael S. Lawrence, Paz Polak, Ignaty Leshchiner, Kasper Lage, Todd R. Golub, Eric S. Lander, and Gad Getz

Tumor sequencing efforts have enabled the identification of cancer genes based on an excess of mutations in the gene or clustering of mutations along the (one-dimensional) DNA sequence of the gene. Here, we show that this approach can be extended to identify cancer genes based on clustering of mutations relative to the 3D structure of the protein product. By analyzing the PanCancer compendium of somatic mutations in nearly 5,000 tumors, we identified known cancer genes and previously unidentified candidates based on clustering of missense mutations in protein structures or at interfaces with binding partners. In addition, we found that 3D clustering is present in both oncoproteins and tumor suppressors—contrary to the view that such clustering is a hallmark of oncoproteins. (See pp. E5486–E5495.)

Beta cells transfer vesicles containing insulin to phagocytes for presentation to T cells

Anthony N. Vomund, Bernd H. Zinselmeyer, Jing Hughes, Boris Calderon, Carolina Valderrama, Stephen T. Ferris, Xiaoxiao Wan, Kohsuke Kanekura, Javier A. Carrero, Fumihiko Urano, and Emil R. Unanue

This report documents that beta cells from islets of Langerhans normally transfer some of their secretory granules to resident phagocytes. The transfer involves a close contact interaction between live beta cells and phagocytes, increases upon glucose stimulation, and requires mobilization of intracellular Ca^{++}. In autoimmune diabetes, the CD4 T cells to various peptides of the insulin B chain recognize the

transferred antigens in the phagocytes represented in islets by macrophages and a subset of dendritic cells. We have identified a process whereby antigens become available for recognition by autoreactive T cells in type 1 diabetes. (See pp. E5496–E5502.)

Plasma DNA tissue mapping by genome-wide methylation sequencing for noninvasive prenatal, cancer, and transplantation assessments

Kun Sun, Peiyong Jiang, K. C. Allen Chan, John Wong, Yvonne K. Y. Cheng, Raymond H. S. Liang, Wai-kong Chan, Edmond S. K. Ma, Stephen L. Chan, Suk Hang Cheng, Rebecca W. Y. Chan, Yu K. Tong, Simon S. M. Ng, Raymond S. M. Wong, David S. C. Hui, Tse Ngong Leung, Tak Y. Leung, Paul B. S. Lai, Rossa W. K. Chiu, and Yuk Ming Dennis Lo

Plasma consists of DNA released from multiple tissues within the body. Using genome-wide bisulfite sequencing of plasma DNA, we obtained a bird's eye view of the identities and contributions of these tissues to the circulating DNA pool. The tissue contributors and their relative proportions are identified by a bioinformatics deconvolution process that draws reference from DNA methylation signatures representative of each tissue type. We validated this approach in pregnant women, cancer patients, and transplant recipients. This method also allows one to identify the tissue of origin of genomic aberrations observed in plasma DNA. This approach has numerous research and diagnostic applications in prenatal testing, oncology, transplantation monitoring, and other fields. (See pp. E5503–E5512.)

Nascent chain-monitored remodeling of the Sec machinery for salinity adaptation of marine bacteria

Eiji Ishii, Shinobu Chiba, Narimasa Hashimoto, Seiji Kojima, Michio Homma, Koreaki Ito, Yoshinori Akiyama, and Hiroyuki Mori

Bacteria living in seawater must cope with low-sodium environments that they may encounter. Here we show an unexpected finding that remodeling of the Sec protein export machinery plays a pivotal role in this adaptation. *Vibrio alginolyticus* possesses alternative SecDF1 and SecDF2 homologs that use the transmembrane gradient of Na^+ and that of H^+, respectively, to enhance protein export by cooperating with the SecYEG translocon. The synthesis of SecDF2 is induced in low-Na^+ environments, and this induction is essential for the bacterium to survive low salinity. Remarkably, the *Vibrio* species use a nascent polypeptide, VemP, to monitor the functional state of the Sec pathway and to up-regulate translation of SecDF2 when activity of the SecDF1-containing Sec machinery declines. (See pp. E5513–E5522.)

Functional divisions for visual processing in the central brain of flying *Drosophila*

Peter T. Weir and Michael H. Dickinson

Neuroanatomical methods are limited in their ability to identify functions of neurons in living brains, and recordings in restrained animals cannot be used to study pathways that are only active during naturalistic behaviors. In this study, we investigated a central brain region thought to be involved in both sensory processing and motor output in insects. To examine its role in the sensory-motor transformation, we imaged neuronal activity in tethered flying flies responding to visual stimuli. While the animals were flying, we observed separate functional subunits defined by their responses to visual stimulation. During quiescence, however, these subunits were inactive and indistinguishable from one another. This context-dependent processing suggests that this brain region is involved in visual navigation during flight. (See pp. E5523–E5532.)

Nanoscale patterning of STIM1 and Orai1 during store-operated Ca^{2+} entry

Stefano Perni, Joseph L. Dynes, Andriy V. Yeromin, Michael D. Cahalan, and Clara Franzini-Armstrong

Almost all cell types rely on calcium signals to maintain homeostasis and trigger specific cell responses. In eukaryotic cells, store-operated Ca^{2+} entry (SOCE) is one of the mechanisms used to ensure control over cytosolic Ca^{2+} signaling and internal Ca^{2+} stores. Mutations in either Orai1 or stromal interaction molecule 1 (STIM1) lead to lethal severe combined immune deficiencies. Using a morphological approach with transmission and freeze–fracture electron microscopy, we describe STIM1-Orai1 interactions and visualize the distribution of individual Orai1 channels on the cell surface. This approach confirms STIM1-Orai1 interaction at specialized endoplasmic reticulum (ER)–plasma membrane junctions following ER depletion and provides new insight on STIM1-Orai1 stoichiometry. (See pp. E5533–E5542.)

Arabidopsis ALIX is required for the endosomal localization of the deubiquitinating enzyme AMSH3

Kamila Kalinowska, Marie-Kristin Nagel, Kaija Goodman, Laura Cuyas, Franziska Anzenberger, Angela Alkofer, Javier Paz-Ares, Pascal Braun, Vicente Rubio, Marisa S. Otegui, and Erika Isono

The regulation of protein abundance of receptors and transporters at the plasma membrane is important for proper signaling in many biological pathways. The removal of plasma membrane proteins can occur via the endocytic protein degradation pathway, in which posttranslational modification by ubiquitin plays a key role. The activity of ubiquitinating and deubiquitinating enzymes can determine the ubiquitination status of a given target protein, and it has been shown that both classes of enzymes have important physiological roles. However, how these enzymes themselves are regulated at the molecular level has not yet been completely understood. In this study, we report a possible mechanism by which the deubiquitinating enzyme AMSH3 is regulated by its interacting protein, apoptosis-linked gene-2 interacting protein X (ALIX), in *Arabidopsis*. (See pp. E5543–E5551.)