PNAS Plus Significance Statements

Intrinsic cellular chirality regulates left–right symmetry breaking during cardiac looping

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Cell chirality, or handedness of the cell, is a newly discovered, fundamental property of the cell, so far studied in cell culture only with micropatterning or graded biomaterial-based approaches. The relevance of intrinsic cell chirality on organ laterality is yet to be established. Cardiac looping is the first organ-specific left–right asymmetry evident during embryogenesis. Despite extensive insights into the molecular signals regulating cardiac left–right asymmetry, the biophysical mechanism is still unknown. Our findings establish intrinsic cell chirality as a regulator of cardiac laterality. This study combines an in vitro chirality assay with embryonic left–right asymmetry in vivo and will significantly impact the understanding and future studies of embryonic left–right asymmetry and congenital heart diseases. (See pp. E11568–E11577.)

Visualizing atomic sizes and molecular shapes with the classical turning surface of the Kohn–Sham potential

Egor Ospadov, Jianmin Tao, Viktor N. Staroverov, and John P. Perdew

Can quantum mechanics predict a well-defined and chemically intuitive size and shape for an atom or a molecule? We show that the bounding surface of a chemical species can be naturally defined as the classical turning surface of the Kohn–Sham potential—an effective potential that, acting on noninteracting electrons, yields the ground-state density of the real system. The atomic and ionic radii defined in this manner display all expected periodic trends, while the ratio of a bond length to the sum of atomic or ionic radii identifies the type of the bond (covalent, ionic, hydrogen, or van der Waals). The proposed approach permits a visual representation of chemical species that is intuitive and quantum-mechanically rigorous at the same time. (See pp. E11578–E11585.)

Eurasian river spring flood observations support net Arctic Ocean mercury export to the atmosphere and Atlantic Ocean

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Elevated levels of mercury in Arctic marine wildlife have been linked to midlatitude anthropogenic mercury emissions which are transported to the Arctic Ocean by air. Modeling studies, however, suggest that Arctic rivers contribute equal amounts of mercury to the Arctic Ocean. In this study, we provide comprehensive mercury data on large Eurasian rivers. We find that the spring flood mercury flux from Eurasian rivers is indeed large, which confirms a new Arctic mercury cycling paradigm: Mid-latitude anthropogenic emissions reach the terrestrial Arctic by air, whereby vegetation uptake transfers atmospheric mercury to tundra and boreal peat soils. Springtime snowmelt subsequently mobilizes peat soil mercury to the Arctic Ocean, where photochemistry drives net export of mercury back to the atmosphere. (See pp. E11586–E11594.)

Strong impact of wildfires on the abundance and aging of black carbon in the lowermost stratosphere

Jeannine Ditas, Nan Ma, Yuxuan Zhang, Denise Assmann, Marco Neumaier, Hella Riede, Einar Karu, Jonathan Williams, Dieter Scharffe, Qiaoqiao Wang, Jorge Saturno, Joshua P. Schwarz, Joseph M. Katich, Gavin R. McMeeking, Andreas Zahn, Markus Hermann, Carl A. M. Brenninkmeijer, Meinrat O. Andreae, Ulrich Pöschl, Hang Su, and Yafang Cheng

Unique information about the abundance and evolution of wildfire-emitted black carbon (BC) in the lowermost part of the stratosphere (LMS) was obtained from long-term airborne measurements made in cooperation with Lufthansa through the Civil Aircraft for the Regular Investigation of the atmosphere Based on an Instrument Container (CARIBIC) project, part of the In-service Aircraft for a Global Observing System (IAGOS) framework. Our results demonstrate that wildfires can dramatically increase BC mass concentration in the LMS, substantially enhance regional climate forcing, and are a challenge for model simulations. Climate change is expected to increase the frequency and spread of wildfires. Thus, recording a present-day baseline with extensive and long-term measurements should help to constrain model estimations of the climate impact of BC and foster our fundamental understanding of future climate change. (See pp. E11595–E11603.)

The calculation of transcript flux ratios reveals single regulatory mechanisms capable of activation and repression

Eric A. Galburt

Cells adapt to their environment by modulating the amount of RNA transcripts produced in a gene-specific manner. While some transcription factors are recruited via DNA sequence motifs, other factors bind directly to

RNA polymerase to modulate the rate constants underlying the initiation process. Here, I describe a freely available web-based tool to quantitatively evaluate how factor-induced changes in the rate constants connecting initiation intermediates combine to regulate the amount of RNA produced per time. I use these calculations to show that multifaceted kinetic mechanisms may lead to differential and promoter-specific regulation. Finally, I explore the mechanisms of two factors essential for the response of bacteria to stress. The insights provided help to understand the logic of bacterial gene regulation. (See pp. E11604–E11613.)

Phage Mu Gam protein promotes NHEJ in concert with *Escherichia coli* ligase

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Prophages constitute a large portion of the abundant phage genomes on our planet. They live in symbiosis with their host, replicating together, and potentially conferring increased fitness. Many fitness-enhancing prophage functions are known, famously the cholera toxin, but the majority are unknown. Prophage Mu encodes numerous genes of undetermined function. The product of one of these, Gam, protects linear DNA ends from exonucleases. We report two distinct roles for MuGam, both involved in promoting survival of the phage and of its host under conditions where the bacterial genome suffers double-stranded DNA breaks. The novel finding is that MuGam, in concert with *Escherichia coli* ligase, endows the cell with a nonhomologous end-joining repair pathway. (See pp. E11614–E11622.)

Structural snapshots of OxyR reveal the peroxidatic mechanism of H_2O_2 sensing

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The bacterial transcription factor OxyR is a model example of a highly sensitive and specific hydrogen peroxide (H_2O_2) sensor. H_2O_2 reduction by its active-site cysteine triggers protein structural changes leading to an increased transcription of antioxidant genes. By solving the crystal structures of full-length OxyR in both reduced and oxidized states, we provide molecular insight into these structural changes. We also present a H_2O_2-bound structure with a threonine activating the peroxide, and argue that this H_2O_2-bound structure may be catalytically more relevant than that seen previously in the study of a sulfinic acid-mimic mutant of the active-site cysteine. Finally, we discuss the commonalities and differences between the peroxidatic mechanisms of peroxiredoxins and OxyR. (See pp. E11623–E11632.)

NETSeq reveals heterogeneous nucleotide incorporation by RNA polymerase I

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It is well known that ribosomal RNA processing is directly impacted by the rate of transcription elongation by RNA polymerase I (Pol I). To understand how these processes are orchestrated, we must carefully define transcription elongation properties in vitro and in living cells. Here, we characterize DNA sequence elements that pause and terminate Pol I transcription in vitro. We also establish methods for analyzing Pol I transcription elongation properties in vivo using native elongating transcript sequencing (NETSeq). Our NETSeq data revealed frequent pausing by Pol I and decreased Pol I occupancy at G residues, suggesting unequal rates of nucleotide incorporation by the enzyme. These findings redefine our understanding of Pol I transcription elongation and its heterogeneity in vivo. (See pp. E11633–E11641.)

UBL domain of Usp14 and other proteins stimulates proteasome activities and protein degradation in cells

Hyoung Tae Kim and Alfred L. Goldberg

26S proteasomes catalyze most of the protein degradation in eukaryotic cells, and their activity is precisely regulated. Upon binding ubiquitinated proteins, proteasomes become activated. This activation is triggered by binding of the ubiquitin chain to the proteasome-associated deubiquitinating enzyme Usp14/Ubp6. In studying this activation mechanism, we discovered that Usp14's ubiquitin-like (UBL) domain by itself stimulates multiple proteasome activities and thus appears to mediate the activation by ubiquitinated proteins. Many other proteins contain UBL domains, and we show that the UBL domains of other proteins also stimulate the proteasomes' degradative capacity. Thus, activation of proteasomes may represent a general new role for UBL-containing proteins. Furthermore, overexpressing the UBL domain in cells increases overall protein breakdown, which may have therapeutic applications. (See pp. E11642–E11650.)

Mechanistic insights into the interactions of NAP1 with the SKICH domains of NDP52 and TAX1BP1

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Selective autophagy is critical for the regulation of cellular homeostasis and physiology in mammals. Selective autophagy of invading pathogens and damaged mitochondria requires the TBK1 kinase and the autophagy receptors NDP52 and TAX1BP1, but with poorly understood mechanisms. In this study, we present the crystal structures of the SKIP carboxyl homology (SKICH) domains of NDP52 and TAX1BP1 in complex with NAP1, which not only uncover how NDP52 interacts with NAP1 for the recruitment of TBK1 but also provide an atomic picture showing how a SKICH domain functions as a protein–protein interaction module to interact with its binding partners. Furthermore, the determined complex structures also help to evaluate the currently known TBK1-mediated phosphorylation sites in the SKICH domains of NDP52 and TAX1BP1. (See pp. E11651–E11660.)

Dual functions for OVAAL in initiation of RAF/MEK/ERK prosurvival signals and evasion of p27-mediated cellular senescence

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Here, we report that the long noncoding RNA (lncRNA) ovarian adenocarcinoma-amplified lncRNA (OVAAL) is a mediator of cancer cell resistance, counteracting the effects of apoptosis-inducing agents acting through both the extrinsic and intrinsic pathways. Building upon previous reports associating OVAAL amplification with ovarian and endometrial cancers, we now show that OVAAL overexpression occurs during the pathogenesis of colorectal cancer and melanoma. Mechanistically, our findings also establish that OVAAL expression more generally contributes a prosurvival role to cancer cells under steady-state conditions. OVAAL accomplishes these actions utilizing distinct functional modalities: one promoting activation of RAF/MEK/ERK

signaling and the other blocking cell entry into senescence. Our study demonstrates that expression of a single OVAAL in cancer cells drives two distinct but coordinated actions contributing to cancer pathology. (See pp. E11661–E11670.)

Cancer-associated fibroblasts suppress SOX2-induced dysplasia in a lung squamous cancer coculture

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Tumor–stroma interactions play a critical role in regulating tumorigenesis. However, how these interactions contribute to changes in tissue architecture and cell polarity observed during tumor development is unclear. Here we report a 3D coculture system that recapitulates key phenotypic changes during the progression of lung squamous carcinoma (LUSC) as well as the dynamic interactions between LUSC cells and components of the tumor microenvironment (TME). Our data suggest that two major components of TME, including the extracellular matrix and cancer-associated fibroblasts, could override cell intrinsic oncogenic changes in determining the disease phenotype in the context of LUSC. These findings may have broad implications for LUSC biology as well as the design of future therapies. (See pp. E11671–E11680.)

Coordinated histone modifications and chromatin reorganization in a single cell revealed by FRET biosensors

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We have developed a fluorescence resonance energy transfer (FRET) biosensor to visualize in single live cells the histone H3 Lys-9 trimethylation (H3K9me3) dynamics. Together with another FRET biosensor for simultaneous monitoring of the neighboring histone H3 Ser-10 phosphorylation (H3S10p) in the same cell, we found an anticorrelation between the dynamics of H3K9me3 and H3S10p during cell cycles, with H3S10p facilitating the decrease of H3K9me3 at onset of mitosis. This decrease of H3K9me3 is accompanied by dissolution of heterochromatin structures before chromatin condensation and nuclear envelope dissolution. The coordinated regulation of H3S10p and H3K9me3 may enhance access of remodeling complexes to the chromatin. Our results hence provide insights on how histone modifications and chromatin structures are coordinated to regulate mitosis. (See pp. E11681–E11690.)

Evolution of host support for two ancient bacterial symbionts with differentially degraded genomes in a leafhopper host

Meng Mao, Xiushuai Yang, and Gordon M. Bennett

Nutritional symbionts in sap-feeding insects are characterized by highly degenerate genomes. It is poorly understood how hosts evolve to maintain these symbionts, particularly when hosts rely on more than one symbiont that requires distinct support for basic cell functions. We show that the aster leafhopper (*Macrosteles quadrilineatus*), which depends on two symbionts with tiny genomes (*Sulcia* and *Nasuia*), has differentially reprogramed gene-expression patterns in symbiont-associated cells. The host has acquired novel genetic traits and likely recruited preexisting mitochondrial support mechanisms to meet the specific needs of each symbiont. Broad comparisons across anciently diverged sap-feeding hosts reveal that the evolution of symbiont support mechanisms is largely unique to each host lineage. Important parallels are further observed with organelle evolution. (See pp. E11691–E11700.)

Germline genetic polymorphisms influence tumor gene expression and immune cell infiltration

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Our DNA contains a blueprint for phenotypic traits, which include the immune response to tumors. As cancer immunotherapies continue to show clinical promise, it is important to understand how inherited genetic variation may account for variable immune responses, as reflected by gene expression within the tumor. We systematically identified the germline genetic polymorphisms associated with variable tumor tissue gene expression across 24 human cancer types. We showed that expression of major regulators of immunity, such as ICOSLG and ERAP2, was under strong genetic control. Additionally, we defined germline variants associated with the abundance of immune cells that infiltrated the tumor. These data demonstrate that germline genetics is a major player in shaping the tumor environment and immune response. (See pp. E11701–E11710.)

Transcriptional landscape of B cell precursor acute lymphoblastic leukemia based on an international study of 1,223 cases

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In BCP ALL, molecular classification is used for risk stratification and influences treatment strategies. We reanalyzed the transcriptomic landscape of 1,223 BCP ALLs and identified 14 subgroups based on their transcriptional profiles. Eight of these (G1 to G8) are previously well-known subgroups, harboring specific genetic abnormalities. The sample size allowed the identification of six previously undescribed subgroups, consisting of cases harboring *PAX5* or *CRLF2* fusions (G9), *PAX5* (p.P80R) mutations (G10), *IKZF1* (p.N159Y) mutations (G11), either *ZEB2* (p.H1038R) mutations or *IGH–CEBPE* fusions (G12), *HLF* rearrangements (G13), or *NUTM* rearrangements (G14). In addition, this study allowed us to determine the prognostic impact of several recently defined subgroups. This study suggests that RNA sequencing should be a valuable tool in the routine diagnostic workup for ALL. (See pp. E11711–E11720.)

Molecular mechanisms of biogenesis of apoptotic exosome-like vesicles and their roles as damage-associated molecular patterns

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Exosomes, present in most of body fluids, play essential roles in intercellular communication. Here, we found extracellular vesicles, termed "apoptotic exosome-like vesicles" (AEVs), released in the apoptotic cells. Their biogenesis was dependent on the sphingosine-1-phosphate/sphingosine-1-phopshate receptors

(S1P/S1PRs) signaling. The S1P/S1PRs complex in the AEVs induced inflammatory mediators such as IL-1α/β, Cox2, Cxcl1, and Ccl2 in macrophages and mice. Therefore, the AEVs in the dying cells are implicated in the pathogenesis of various inflammatory diseases including cancer, autoimmune diseases, chronic allergy, and neurodegenerative diseases. In the future AEVs could be utilized as carriers for delivery of genetic materials or drugs. (See pp. E11721–E11730.)

CD226 regulates natural killer cell antitumor responses via phosphorylation-mediated inactivation of transcription factor FOXO1

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CD226 is an important activating receptor involved in mediating natural killer (NK) cell responses against tumors, but how CD226 exerts control over NK cell function is not fully understood. CD226 belongs to the poliovirus receptor (PVR)-nectin family that includes TIGIT and CD96, with TIGIT garnering much attention as a key checkpoint in T cell and NK cell antitumor responses and as an immunotherapy target. Thus, it is imperative to determine how CD226 counteracts the actions of TIGIT and CD96 with which it competes for binding to its ligands such as CD155 (PVR). We demonstrate that CD226 engagement of CD155 is required for phosphorylation of transcription factor FOXO1, resulting in inactivation of its negative regulatory control over NK cell effector function. (See pp. E11731–E11740.)

Citrate-based materials fuel human stem cells by metabonegenic regulation

Chuying Ma, Xinggui Tian, Jimin P. Kim, Denghui Xie, Xiang Ao, Dingying Shan, Qiaoling Lin, Maria R. Hudock, Xiaochun Bai, and Jian Yang

Differentiation of mesenchymal stem cells (MSCs) to bone-forming cells is central to bone regeneration, the extent of which is largely regulated by microenvironment factors. Here, we find that citrate as a metabolic factor that is abundant in bone can be consumed by MSCs to fuel osteogenesis by regulating metabolic pathways. We explored the mechanism of citrate benefit and designed a biomimetic citrate-based material that could provide a citrate- and phosphoserine-rich environment during degradation based on the previously unexplored concerted action between the two bioactive factors in accelerating bone regeneration. Together, these studies open up avenues for the study of stem cell biology and design of bone biomaterials to treat critically sized defects and bone disorders of metabolic origin. (See pp. E11741–E11750.)

Structure and architecture of immature and mature murine leukemia virus capsids

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Immature retroviruses are built by the Gag polyprotein; Gag is then cut into domains, and the resulting CA capsid proteins form the mature capsid, which can mediate infection of a new cell. Murine leukemia virus (MLV) is a model retrovirus and the basis for gene-delivery vectors. We determined the capsid structures and architectures for immature and mature MLV. The mature MLV core does not enclose the genome in a closed capsid by using only part of the available proteins, as is the case for HIV-1. Instead, it wraps the genome in curved sheets incorporating most CA proteins. Retroviruses therefore have fundamentally different modes of core assembly and genome protection, which may relate to differences in their early replication. (See pp. E11751–E11760.)

Comparative genomics reveals the molecular determinants of rapid growth of the cyanobacterium *Synechococcus elongatus* UTEX 2973

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There has been recent interest in fast-growing microbes to accelerate discoveries in medicine, biology, and biotechnology. Additionally, microbes with rapid-growth properties are of significant industrial interest as a chassis for bioproduction. While such microbes have been identified, the determinants of their rapid growth remain poorly understood. In this study, we have elucidated the molecular basis for the rapid growth of *Synechococcus* 2973, a fast-growing oxygenic photosynthetic organism, and applied these insights to significantly enhance the growth rate of a related model cyanobacterium, *Synechococcus* 7942. Such engineered photosynthetic microbes are expected to open the doors for significantly higher productivity under sustainable conditions. (See pp. E11761–E11770.)

Staphylococcus aureus coagulases are exploitable yet stable public goods in clinically relevant conditions

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Clotting of blood is not exclusive to host physiology; pathogens are also able to generate clots as part of their life cycle. Here, we show that coagulases, enzymes responsible for bacteria-mediated clotting, can act as public goods in clinical conditions. Coagulases, secreted by producers, generate protective layers of fibrin around the bacteria, shielding them from antimicrobials and host immune factors. Remarkably, we find that this protection is also conferred onto strains that do not produce coagulases but still benefit from those made by others. Although this is a social trait, overexploitation of coagulases does not occur due to spatial segregation and population viscosity. Our study provides a social evolution perspective on the critical role of coagulases. (See pp. E11771–E11779.)

DksA–DnaJ redox interactions provide a signal for the activation of bacterial RNA polymerase

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Transcription in Gram-negative bacteria is greatly influenced by the synergistic interactions of DksA and the nucleotide alarmone guanosine tetraphosphate. Our investigations reveal a unique, previously unknown layer of transcriptional regulation that depends on redox-based protein–protein interactions between DksA and the molecular chaperone DnaJ. Tripartite connections between DksA, DnaJ, and guanosine tetraphosphate afford a dynamic range of transcriptional responses to H_2O_2 concentrations associated with redox signaling or oxidative stress. The redox-based gene regulation dependent on the combined actions of DksA, DnaJ, and guanosine tetraphosphate confers resistance of

Salmonella to the antimicrobial activity of the NADPH phagocyte oxidase. (See pp. E11780–E11789.)

Integrative approach using *Yersinia pestis* genomes to revisit the historical landscape of plague during the Medieval Period

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While our knowledge of modern plague reservoirs and their hosts is extensive, we have little to no knowledge about the origin of the Medieval plague pandemics or the routes of transmission involved in their spread. Prior genomic data provide a patchy low-resolution picture of the transmission dynamics involved during the Second Plague Pandemic, with only five distinct genomes. We have reevaluated all Medieval strains under the light of archaeological and historical evidence to carefully discuss the involvement of different transmission routes during the Second Plague Pandemic. Our interpretation showcases the importance of trade routes and human movements and further supports the identification of *Yersinia pestis* as the pathogenic agent of the so-called *pestis secunda* (1357–1366). (See pp. E11790–E11797.)

Emergent elasticity in the neural code for space

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We develop a theoretical model, grounded in known properties of neural dynamics and synaptic plasticity, that can fuse information gathered from the past history of velocity and sequence of encountered landmarks during exploratory behavior, to construct a self-consistent internal representation of space. Moreover, through model reduction techniques, we obtain conceptual insights into how consistent internal spatial representations naturally emerge through an elastic relaxation process in an effective spring–particle system. We verify several experimentally testable predictions of our model involving the spatial behavior of grid cells in the medial entorhinal cortex, as well as suggest additional experiments. (See pp. E11798–E11806.)

The adult oligodendrocyte can participate in remyelination

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Remyelination of the CNS is a critical process in restoring function and protecting nerve fibers from degeneration in multiple sclerosis and other demyelinating diseases. It is currently thought that myelin can only be repaired by the generation of new oligodendrocytes from progenitor cells and that remaining mature cells are unable to participate. Here we show, using unique large animal models, including a nonhuman primate, that oligodendrocytes that are partially injured can participate in myelin repair. The capacity of mature oligodendrocytes to remyelinate in demyelinating disease remains unknown, yet it provides an additional cell source for recruitment for myelin repair. (See pp. E11807–E11816.)

Photoreceptive retinal ganglion cells control the information rate of the optic nerve

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Noise in the visual signal falls as ambient light increases, allowing the retina to extract more information from the scene. We show here that a measure of ambient light produced by the small number of inner retinal photoreceptors [intrinsically photosensitive retinal ganglion cells (ipRGCs)] regulates intrinsic rates of spike firing across the population of retinal ganglion cells that form the optic nerve. Increased firing at higher irradiance allows the ganglion cells to convey more information. Our findings reveal a potential mechanism for increasing visual performance at high ambient light and show that changes in maintained activity can be used to provide proactive control over rates of information flow in the CNS. (See pp. E11817–E11826.)

Interplay of the Norrin and Wnt7a/Wnt7b signaling systems in blood–brain barrier and blood–retina barrier development and maintenance

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The blood–brain barrier (BBB) and the blood–retina barrier (BRB) play essential roles in maintaining the health of the central nervous system. Two partially redundant ligand–receptor systems—the Norrin and Wnt7a/Wnt7b systems—activate β-catenin signaling in vascular endothelial cells to control BBB and BRB development and maintenance. The present study explores the partially overlapping roles of these two systems in the postnatal brain and retinal vasculatures. In the cerebellum, the two signaling systems are substantially redundant in maintaining the BBB, with isolated loss of some components, such as the ligand Wnt7a or the coactivator Tspan12, producing little or no barrier defect but combined loss of the two components producing a large defect in barrier integrity. (See pp. E11827–E11836.)

TRPV1 pore turret dictates distinct DkTx and capsaicin gating

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The TRPV1 channel integrates noxious stimuli from multiple sources to evoke an appropriate pain response. However, while different stimuli evoke distinct channel activation, the underlying molecular mechanisms remain unclear. Here, we aim at elucidating the structural determinants that regulate the different TRPV1 responses to two toxins: capsaicin and double-knot toxin (DkTx). We found that the channel pore turret domain imposes two opposite effects on the responses to these toxins. While it restricts the ion conductance of the DkTx-evoked current, it stabilizes the open channel state evoked by capsaicin. Together, our results indicate that TRPV1 pore turret dictates different gating mechanisms in response to agonists acting through different binding domains. (See pp. E11837–E11846.)

Role of human Hv1 channels in sperm capacitation and white blood cell respiratory burst established by a designed peptide inhibitor

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Some peptide toxins act by stabilizing the voltage sensor domains (VSDs) of voltage-gated channels in open or closed conformations. hHv1 is a human voltage-gated proton channel and has lacked a specific inhibitor to assess its roles in physiology. We designed a phage-display library of 1 million novel peptides sharing an inhibitor cysteine knot (ICK) scaffold by combining elements of natural toxins; phagemids expressing Corza6 (C6) were selected by their capacity to bind to hHv1 protein. Two C6 peptides bind to the two VSDs in hHv1 and thereby inhibit

channel operation. C6 was employed to confirm hypothesized roles for hHv1 in human sperm and white blood cells. The method should prove amenable to designer toxin identification for other voltage-gated channels. (See pp. E11847–E11856.)

PYL8 mediates ABA perception in the root through non-cell-autonomous and ligand-stabilization–based mechanisms

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The phytohormone abscisic acid (ABA) controls root responses to environmental signals such as abiotic stress. ABA signaling in roots depends on the nonredundant role of the PYL8 receptor. This study reveals special features of this ABA receptor. ABA binding triggers hormone-dependent stabilization of PYL8 through reduced ubiquitination and induces nuclear localization of the receptor. ABA-induced stabilization also allows movement of the PYL8 receptor from the root epidermis and stele to adjacent tissues. Hence, like mobile transcription factors that regulate plant development, the PYL8 protein can move between cells. In summary, our study reports a novel non-cell-autonomous mechanism to regulate hormone perception and root growth. (See pp. E11857–E11863.)

Hinge region of *Arabidopsis* phyA plays an important role in regulating phyA function

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The plant phytochrome molecule consists of an N-terminal photosensory domain and a C-terminal dimerization domain, connected by a flexible hinge region. It was previously shown that a serine residue in the hinge region of oat phytochrome A (phyA) could be phosphorylated in vivo and plays an important role in regulating phyA function. However, little is known regarding the function of the hinge region of *Arabidopsis* phyA. Here, we show that three residues in the hinge region of *Arabidopsis* phyA are essential in regulating phyA phosphorylation and function. Our study thus provides new insights into the regulatory role of the phytochrome hinge region, suggesting that phosphorylation and function of the hinge region may differ between oat and *Arabidopsis* phyA photoreceptors. (See pp. E11864–E11873.)

Metabolic network-based stratification of hepatocellular carcinoma reveals three distinct tumor subtypes

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Hepatocellular carcinoma (HCC) is a heterogeneous and deadly form of liver cancer. Here, we stratified and characterized HCC tumors by applying graph and control theory concepts to the topology of genome-scale metabolic networks. We identified three HCC subtypes with distinct differences in metabolic and signaling pathways and clinical survival and validated our results by performing additional experiments. We further identified HCC subtype-specific genes pivotal in controlling the entire metabolism and discovered genes that can be targeted for development of efficient treatment strategies for specific HCC subtypes. Our systems-level analyses provided a systematic way for characterization of HCC subtypes and identification of drug targets for effective treatment of HCC patients. (See pp. E11874–E11883.)