PNAS Plus Significance Statements

Direct observation of grain rotations during coarsening of a semisolid Al–Cu alloy

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Computational modeling of materials phenomena promises to reduce the time and cost of developing new materials and processing techniques—a goal made feasible by rapid advances in computer speed and capacity. Validation of such simulations, however, has been hindered by a lack of 3D experimental data of simultaneously high temporal and spatial resolution. In this study, we exploit 3D X-ray diffraction microscopy to capture the evolution of crystallographic orientations during particle coarsening in a semisolid Al–Cu alloy. The data confirm a long-standing hypothesis that particle rotation is driven (in part) by the dependence of grain boundary energy on misorientation. In addition, the results constitute an experimental foundation for testing the predictive power of next-generation computational models for sintering. (See pp. E5998–E6006.)

Shape-programmable magnetic soft matter

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At small scales, shape-programmable magnetic materials have significant potential to achieve mechanical functionalities that are unattainable by traditional miniature machines. Unfortunately, these materials have only been programmed for a small number of specific applications, as previous work can only rely on human intuition to approximate the required magnetization profile and actuating magnetic fields for such materials. Here, we propose a universal programming methodology that can automatically generate the desired magnetization profile and actuating fields for soft materials to achieve new time-varying shapes. The proposed method can enable other researchers to fully capitalize the potential of shape-programming technologies, allowing them to create a wide range of novel soft active surfaces and devices that are critical in robotics, material science, and medicine. (See pp. E6007–E6015.)

Statecraft and expansionary dynamics: A Virú outpost at Huaca Prieta, Chicama Valley, Peru

Jean-François Millaire, Gabriel Prieto, Flannery Surette, Elsa M. Redmond, and Charles S. Spencer

Cross-cultural analyses of early statecraft suggest that territorial expansion was an integral part of the process of primary state formation, closely associated with the delegation of authority to subordinate administrators and the construction of core outposts of the state in foreign territories. Understood as instruments of territorial expansion that were closely tied to historical processes, such outposts offer important viewpoints on the evolutionary trajectories of specific early states and also on the nature and extent of the foreign policy of archaic states in general. (See pp. E6016–E6025.)

Integrated analysis of phenome, genome, and transcriptome of hybrid rice uncovered multiple heterosis-related loci for yield increase

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Because of its practical importance and scientific significance, heterosis (hybrid vigor) is an interesting topic for both breeders and biologists. However, although heterosis has been applied successfully to increase crop yields, the molecular mechanisms involved remain obscure. In this study, using an integrative approach, we found that multiple quantitative trait loci (QTLs) cumulatively drive yield heterosis in hybrid rice by regulating two grain-yield component traits in which the *RH8* (rice heterosis 8) gene plays a major role. Our research highlights the importance of integrative methods to uncover the molecular mechanism of heterosis and thus pave a way toward revealing the molecular mechanisms in rice heterosis in detail. (See pp. E6026–E6035.)

Intracellular mechanisms of molecular recognition and sorting for transport of large extracellular matrix molecules

Yoshihiro Ishikawa, Shinya Ito, Kazuhiro Nagata, Lynn Y. Sakai, and Hans Peter Bächinger

The discovery of Transmembrane Protein Transport and Golgi Organization 1 (TANGO1) has given insight into how large extracellular molecules like collagens are loaded into special coat protein complex II (COPII) vesicles for transport. However, because there are more than 20 different collagens in humans, it is not clear how TANGO1 recognizes all collagens. In this manuscript, we show that TANGO1 does not directly interact with collagens. We show instead that the Src homology 3 (SH3) domain of TANGO1 interacts with heat shock protein (Hsp) 47, a chaperone that binds to all different types of collagens. We propose a model in which Hsp47 serves as

a guide molecule directing collagens to special COPII vesicles. (See pp. E6036–E6044.)

Tyrosine phosphorylation stimulates activity of human RAD51 recombinase through altered nucleoprotein filament dynamics

Shyamal Subramanyam, Mohammed Ismail, Ipshita Bhattacharya, and Maria Spies

Homologous recombination provides the most accurate means to repair genotoxic DNA lesions. It depends on the assembly of the RAD51 DNA strand exchange protein into a dynamic nucleoprotein filament. The c-Abl tyrosine kinase and its oncogenic counterpart BCR-ABL control RAD51 by phosphorylating tyrosine residues 54 and 315. A nonnatural phosphotyrosine mimetic was used to represent phosphorylated RAD51 and to parse out the importance of Y54 and Y315 phosphorylation. By combining biochemical and single-molecule analyses, we found that Y54 phosphorylation enhances the RAD51 DNA strand exchange activity by altering the nucleoprotein filament properties. In contrast, Y315 phosphorylation has little effect on the RAD51 activities. (See pp. E6045–E6054.)

Redox-assisted regulation of Ca^{2+} homeostasis in the endoplasmic reticulum by disulfide reductase ERdj5

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Ca^{2+} is one of the most important second messengers regulating numerous cellular functions; therefore, the regulation of Ca^{2+} release from and its uptake into the endoplasmic reticulum (ER) are both critical for calcium signaling. The activity of sarco/endoplasmic reticulum Ca^{2+}-ATPase isoform 2b (SERCA2b), a calcium pump on the ER membrane, was reported to be negatively regulated by the oxidation of two cysteines in its ER-luminal portion, and it is expected to be activated by its reduction. However, no molecules responsible for this reduction have been identified. Here, we showed for the first time that ERdj5, the reductase in the ER of mammalian cells, activates SERCA2b by reducing its disulfide bonds in a $[Ca^{2+}]_{ER}$-dependent manner. (See pp. E6055–E6063.)

Structure-guided enzymology of the lipid A acyltransferase LpxM reveals a dual activity mechanism

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Lysophospholipid acyltransferase (LPLAT) proteins are required for many essential biological activities involving the transfer of acyl chains. One LPLAT, LpxM, is necessary for the biosynthesis of lipid A, which comprises the outer leaflet of the outer membrane in Gram-negative bacteria. Lipid A is important because it is a potent activator of the innate immune system and because of its role in preventing xenobiotics from permeating Gram-negative bacteria. In this work, we structurally and mechanistically characterize LpxM, providing insights that may enable the targeted discovery of inhibitors that prevent lipid A maturation; these might potentiate the uptake of extant antibiotics whose clinical efficacy is hitherto limited by poor permeability. Our insights into the mechanism of LpxM may facilitate the study of diverse LPLATs. (See pp. E6064–E6071.)

Mammalian Period represses and de-represses transcription by displacing CLOCK–BMAL1 from promoters in a Cryptochrome-dependent manner

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The mammalian circadian clock is controlled by a transcription-translation feedback loop consisting of transcriptional activators circadian locomotor output cycles kaput (CLOCK)–brain and muscle Arnt-like protein-1 (BMAL1), which function as a complex at E/E′-box elements, and repressors Cryptochrome 1 (CRY1)/CRY2 and PER1/PER2. CRYs repress upon binding as CRY–CLOCK–BMAL1–E-box complexes. Period proteins (PERs) repress by removing the heterotrimeric complexes from the E-box. We report here that in the *Cry1* promoter, the CRY1–CLOCK–BMAL1–E-box complex represses a transcriptional activator acting in cis, and removal of the heterotrimeric complex by PER2 de-represses the transcriptional activator. ChIP-seq and RNA-seq experiments identified other genes also de-repressed by PER2. These data clarify the role of PER2 and reveal the level of complexity in regulation of *Cry1* and other circadian-controlled genes. (See pp. E6072–E6079.)

Tunable allosteric library of caspase-3 identifies coupling between conserved water molecules and conformational selection

Joseph J. Maciag, Sarah H. Mackenzie, Matthew B. Tucker, Joshua L. Schipper, Paul Swartz, and A. Clay Clark

The interconversion of states in the caspase-3 native ensemble is affected by binding of ligands that either stabilize or destabilize active-site loops. It is not clear how the ensemble is regulated in cells, aside from modulating levels of endogenous caspase inhibitors. We describe a library of caspase-3 variants with activities that vary by more than four orders of magnitude and show that removal of conserved water molecules may provide a strategy to design novel allosteric inhibitors that globally destabilize the active conformation within the ensemble. Our results suggest that posttranslational modifications fine-tune caspase activity by disrupting conserved water networks, and our database provides an approach to examine caspase signaling in cells by modifying caspase-3 activity while simultaneously maintaining endogenous enzyme levels. (See pp. E6080–E6088.)

C-terminal domain of the RNA chaperone Hfq drives sRNA competition and release of target RNA

Andrew Santiago-Frangos, Kumari Kavita, Daniel J. Schu, Susan Gottesman, and Sarah A. Woodson

The RNA chaperone Hfq binds hundreds of small noncoding RNAs (sRNAs) and facilitates their interactions with mRNAs, regulating bacterial stress responses and virulence. Hfq is limiting in the cell and must release RNAs after they base pair. Most bacterial Hfqs contain an intrinsically disordered C-terminal domain (CTD), with unknown function. Time-resolved assays now show that CTDs are needed to displace base-paired RNA, recycling Hfq. The CTDs also enable kinetic competition between different sRNAs in *Escherichia coli*, allowing some sRNAs to bind Hfq and accumulate, whereas other sRNAs are degraded. We propose that the CTDs sweep sRNAs from the surface of Hfq. This displacement allows Hfq to search among potential RNA partners and establishes a hierarchy of sRNA regulation. (See pp. E6089–E6096.)

Miro phosphorylation sites regulate Parkin recruitment and mitochondrial motility

Evgeny Shlevkov, Tal Kramer, Jason Schapansky, Matthew J. LaVoie, and Thomas L. Schwarz

In mitophagy, damaged mitochondria stabilize PTEN-induced putative kinase 1 (PINK1) and recruit Parkin, an E3-ligase that ubiquitinates proteins on the outer membrane and targets mitochondria for degradation. The crucial roles of PINK1 phosphorylation of Parkin and ubiquitin in mitophagy are well-established. Other substrates of PINK1, however, have also been reported but the significance of those phosphorylations is less clear. We now show that Miro phosphorylations can regulate Parkin recruitment to Miro and trigger Miro degradation. The consequence of this branch of the PINK1/Parkin pathway is the disruption of mitochondrial motility, an event that may spatially restrict the deleterious effects of mitochondrial damage prior to the mitophagic removal of the organelle. (See pp. E6097–E6106.)

Repression of p63 and induction of EMT by mutant Ras in mammary epithelial cells

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The oncogenes Harvey Rat Sarcoma Virus GTPase (*H-RAS*) and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) are well known for altering cell growth and morphology. We show here that they are also able to modify the differentiation state of mammary epithelial cells by inducing an epithelial-to-mesenchymal transition (EMT). This transition leads to greater invasiveness, a hallmark of the progression of tumors toward metastasis. Expression of p63, a protein required for the development of mammary epithelial cells, is strongly repressed by these oncogenes. In turn, loss of p63, which occurs at the transcriptional level, causes a shift in microRNAs and transcription factors that control EMT. Targeting specific genes within this Ras-to-EMT axis may be useful as a therapy to block breast cancer progression. (See pp. E6107–E6116.)

Codon usage is an important determinant of gene expression levels largely through its effects on transcription

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Codon usage bias is an essential feature of all genomes. The effects of codon usage biases on gene expression were previously thought to be mainly due to its impacts on translation. Here, we show that codon usage bias strongly correlates with protein and mRNA levels genome-wide in the filamentous fungus *Neurospora*, and codon usage is an important determinant of gene expression. Surprisingly, we found that the impacts of codon usage on gene expression are mainly due to effects on transcription and are largely independent of translation. Together, these results uncovered an unexpected role of codon biases in determining transcription levels by affecting chromatin structures and suggest that codon biases are results of genome adaptation to both transcription and translation machineries. (See pp. E6117–E6125.)

An evolutionarily conserved element in initiator tRNAs prompts ultimate steps in ribosome maturation

Sunil Shetty and Umesh Varshney

Ribosomes are complex molecular machines requiring an intricate pathway for their biogenesis. Deficiencies in ribosome biogenesis result in inefficient and inaccurate translation, causing cellular toxicities and ribosomopathies. As ribosomes have multiple functions during the translation process, how cells ensure fidelity of the newly synthesized ribosomes for their functions has remained unclear. The immature ribosomes possess rRNAs whose ends have not been fully processed. What licenses final trimming of immature rRNAs is also unclear. Here, using *Escherichia coli*, we show that participation of initiator tRNA via its universally conserved three consecutive GC base pairs, in the first round of the initiation complex formation licenses the final steps of ribosome biogenesis by signaling RNases to trim the immature 16S rRNAs. (See pp. E6126–E6134.)

Dual chromatin recognition by the histone deacetylase complex HCHC is required for proper DNA methylation in *Neurospora crassa*

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Modifications of chromatin proteins (e.g. histones) and DNA play vital roles in genome function. Both hypo- and hypermethylation of DNA are associated with human diseases, including cancers, but the underlying mechanisms are not well understood. Using the filamentous fungus *Neurospora crassa*, one of the simplest eukaryotes with DNA methylation, we report a DNA methylation pathway that depends partially on the histone deacetylase complex HCHC [heterochromatin protein 1 (HP1)–chromodomain protein 2 (CDP-2)–histone deacetylase 1 (HDA-1)– CDP-2/HDA-1–associated protein (CHAP)]. Genome-wide DNA methylation analyses revealed both hypo- and hyper-DNA methylation in strains with defective HCHC components. We show the interrelationship of HCHC components and genetically dissect the proteins to define domains critical for proper DNA methylation and centromeric silencing. This work provides insights into the crosstalk between DNA methylation and histone modifications. (See pp. E6135–E6144.)

Laquinimod arrests experimental autoimmune encephalomyelitis by activating the aryl hydrocarbon receptor

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Laquinimod is an oral drug currently being evaluated for the treatment of relapsing, remitting, and primary progressive multiple sclerosis as well as Huntington's disease. It is thought that laquinimod has a primary effect on the peripheral innate immune system and also acts directly on resident cells within the CNS. However, the exact mechanism of action of laquinimod has not been fully elucidated. We investigated gene expression in laquinimod-treated mice and show induction of genes downstream to activation of the aryl hydrocarbon receptor (AhR). In this paper, we examine the role of the AhR in laquinimod treatment of experimental autoimmune encephalomyelitis and demonstrate that AhR is the molecular target of laquinimod in this model. (See pp. E6145–E6152.)

Exogenous remodeling of lung resident macrophages protects against infectious consequences of bone marrow-suppressive chemotherapy

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Infectious complications can be lethal in patients with cancer when chemotherapy depletes white blood cells (WBCs) needed

to clear microbes. Prevention of infection by vaccination also requires WBCs, and thus has not been effective in saving patients with low WBC counts during chemotherapy. Using a mouse model, we discovered a kind of lung WBC that survives chemotherapy. This cell is found in the lung and can engulf and remove bacteria when activated by a vaccine. This vaccination strategy results in excellent survival in a mouse model of lethal bacterial pneumonia in the setting of chemotherapy. These findings suggest that a protective, chemotherapy-stable lung WBC could be exogenously induced to protect patients with cancer who are at high risk of life-threatening infections. (See pp. E6153–E6161.)

Keratinocytes contribute intrinsically to psoriasis upon loss of *Tnip1* function

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Psoriasis is a complex inflammatory disease with clear genetic contribution that affects roughly 2% of the population in Europe and North America. Inflammation of the skin, and in many cases the joints, leads to severe clinical symptoms, including disfiguration and disability. Immune cells and their inflammatory effector functions have been identified as critical factors for disease development; however, how genetic susceptibility contributes to disease remains largely unclear. Here we developed mouse models based on the gene *TNIP1*, whose loss-of-function in humans is linked to psoriasis. Based on these models, we provide evidence that non-immune cells, specifically skin-resident keratinocytes, contribute causally to disease. This work shifts attention to keratinocytes as causal contributors and therapeutic targets in psoriasis. (See pp. E6162–E6171.)

MiR-155–regulated molecular network orchestrates cell fate in the innate and adaptive immune response to *Mycobacterium tuberculosis*

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The mechanism by which *Mycobacterium tuberculosis* (Mtb) modulates the host immune response is not fully understood. We have used a systems biology approach to generate a microRNA regulatory network composed of 77 microRNAs that are associated with Mtb–macrophage interactions. We have determined a unique and dual role for one of these regulators, miR-155, as a rheostat regulating the survival of both innate and adaptive immune cells. On the one hand, miR-155 maintains the survival of Mtb-infected macrophages, providing a niche favoring bacterial replication. On the other hand, miR-155 maintains the survival of Mtb-specific T cells, enabling an effective adaptive response. Our work underscores the value of systems-based prediction of pathogen-specific microRNA networks as a tool to define host–pathogen interactions. (See pp. E6172–E6181.)

B7-H1 shapes T-cell–mediated brain endothelial cell dysfunction and regional encephalitogenicity in spontaneous CNS autoimmunity

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A crucial step in the pathogenesis of autoimmune diseases, such as multiple sclerosis (MS), is transmigration of pathogenic T cells across the blood–brain barrier. These T cells mediate inflammation and subsequent lesion formation in the CNS. However, molecular mechanisms underlying lesion distribution and formation are not well understood. We here show that genetic ablation of a single immunoregulatory molecule on T cells, B7-homolog 1 (B7-H1), causes local endothelial dysfunction and determines lesion topography in a spontaneous mouse model of CNS autoimmunity. These findings can lead to new therapeutic approaches in targeting pathogenic T cell responses in MS. (See pp. E6182–E6191.)

Antigen exposure shapes the ratio between antigen-specific Tregs and conventional T cells in human peripheral blood

Laura F. Su, Daniel del Alcazar, Erietta Stelekati, E. John Wherry, and Mark M. Davis

Herein we used peptide–MHC tetramer staining and enrichment to identify antigen-specific Tregs directly ex vivo and found that most $CD4^+$ T-cell specificities, self or foreign, contain 5–15% Tregs. A notable exception is that two dominant influenza specificities contain very small proportions of Tregs. We demonstrated using human cord blood cells and murine lymphocytic choriomeningitis virus infection that antigen exposure can alter the balance between Tregs and conventional T cells in an antigen-specific and context-dependent manner. The ratio between specific Tregs and cognate effectors correlates with protective immunity in mice and T-cell dominance hierarchy in humans. Our finding provides insight into Treg specificity and highlights the importance of Treg and effector balance in an antigen-specific context. (See pp. E6192–E6198.)

Suppression of NF-κB activity via nanoparticle-based siRNA delivery alters early cartilage responses to injury

Huimin Yan, Xin Duan, Hua Pan, Nilsson Holguin, Muhammad Farooq Rai, Antonina Akk, Luke E. Springer, Samuel A. Wickline, Linda J. Sandell, and Christine T. N. Pham

Osteoarthritis is a common debilitating joint disease that affects millions in the United States and for which there are few therapeutic options. Critical barriers to the successful development of osteoarthritis treatment include limited understanding of the pathways governing early cartilage degradation and ineffective delivery of therapeutic agents to the resident chondrocytes in the avascular cartilage. Using a peptidic nanoparticle carrying siRNA that specifically suppresses NF-κB, we show that early antiinflammatory intervention reduces chondrocyte death caused by joint injury, a known predisposing factor for osteoarthritis. The peptidic nanoparticle deeply penetrates human cartilage to deliver its therapeutic cargo to the chondrocytes, demonstrating its ability to permeate the dense cartilage matrix. This approach promises to overcome the barriers to effectively treat osteoarthritis. (See pp. E6199–E6208.)

Mutant PFN1 causes ALS phenotypes and progressive motor neuron degeneration in mice by a gain of toxicity

Chunxing Yang, Eric W. Danielson, Tao Qiao, Jake Metterville, Robert H. Brown Jr., John E. Landers, and Zuoshang Xu

ALS is an incurable neurodegenerative disease caused by loss of motor neurons leading to paralysis and death. To understand the disease mechanism and develop therapeutics, mammalian models that phenocopy human disease are crucial. For more than two decades, transgenic animals expressing mutant copper zinc superoxide dismutase (SOD1) gene represented the only model that faithfully reproduced the human disease. Despite recent identification of new causal genes, construction of another mammalian

model with progressive loss of motor neurons and concomitant clinical phenotypes has proven difficult. In this study, we have generated a transgenic mouse model by expressing mutant profilin 1. These mice replicate key features of human ALS and thus provide an in vivo system for study of disease mechanisms and development of therapeutics. (See pp. E6209–E6218.)

24-Hydroxycholesterol participates in pancreatic neuroendocrine tumor development

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Oxysterols promote tumor growth directly or through the dampening of tumor-infiltrating immune cells. Whether oxysterols contribute to pancreatic neuroendocrine tumor (pNET) development and how they are generated within the pNET microenvironment are currently unknown. Here, we show that the 24S-hydroxycholesterol (24S-HC) oxysterol-generating enzyme Cyp46a1 is overexpressed during the angiogenic switch in rat insulin promoter 1–T-antigen 2 (RIP1-Tag2) pNET formation. Moreover, we report that Cyp46a1 overexpression requires hypoxia inducible factor-1a (HIF-1α). Importantly, we show that pharmacologic blockade and genetic inactivation of 24S-HC delays angiogenic switch and therefore tumor formation in RIP1-Tag2. Overexpression of Cyp46a1 transcripts in some human pNET samples suggests that targeting this axis in patients affected by pancreatic neuroendocrine tumors may be an effective therapeutic strategy. (See pp. E6219–E6227.)

A penicillin-binding protein inhibits selection of colistin-resistant, lipooligosaccharide-deficient *Acinetobacter baumannii*

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Antimicrobial drug resistance is a major threat to public health. Gram-negative bacteria are exceptionally resistant to antibiotics because of their outer-membrane barrier. Glycolipids called lipopolysaccharide (LPS) or lipooligosaccharide (LOS) fortify the outer membrane from many antimicrobials and biocides and were thought to be essential for Gram-negative bacterial survival. The last-resort treatment for multidrug-resistant Gram-negative infections is colistin, which targets the lipid A domain of LPS/LOS to disrupt the membrane, but the emerging pathogen *Acinetobacter baumannii* can develop colistin resistance by inactivating lipid A biosynthesis. This analysis advances our understanding of lipid A/LOS essentiality in *A. baumannii* and identifies antimicrobial targets. (See pp. E6228–E6237.)

Follicular dendritic cell disruption as a novel mechanism of virus-induced immunosuppression

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Arboviruses cause increasingly important human and veterinary diseases. Currently, there is a critical lack of understanding about the nature of arbovirus–host interactions in the lymph nodes (LNs), where the adaptive immune response initiates. We used a hemorrhagic arbovirus of sheep, bluetongue virus (BTV), to unveil the early phases of infection in the natural host. We discovered that BTV modulates the humoral immune response by rapidly infecting and destroying follicular dendritic cells (FDCs) in the host LNs. FDC destruction impairs B-cell activation and antibody production, inducing an immunosuppressive phase associated with virus spread in the animal's tissues. The novel virus evasion strategy described here provides key insights on the initiation of the immune response and the pathogenesis of arboviral diseases. (See pp. E6238–E6247.)

Functional receptor molecules CD300lf and CD300ld within the CD300 family enable murine noroviruses to infect cells

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Norovirus is the leading cause of acute gastroenteritis worldwide. Since the discovery of norovirus, a receptor for norovirus internalization into cells has not been identified. Murine norovirus (MNV) binding to cells that were originally not susceptible to the virus can be mediated by ectopically expressed CD300 molecule like family members f or d (CD300lf or CD300ld). The expression of CD300lf or CD300ld is sufficient to render cells permissive to infection by the virus. We conclude that CD300lf and CD300ld are essential for MNV infection and that each molecule can function independently as the viral receptor. (See pp. E6248–E6255.)

Neural correlate of the construction of sentence meaning

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How do circuits of neurons in your brain extract and hold the meaning of a sentence? To start to address this unanswered question, we measured neural activity from the surface of the human brain in patients being mapped out before neurosurgery, as they read sentences. In many electrodes, neural activity increased steadily over the course of the sentence, but the same was not found when participants read lists of words or pronounceable nonwords, or grammatical nonword strings ("Jabberwocky"). This build-up of neural activity appears to reflect neither word meaning nor syntax alone, but the representation of complex meanings. (See pp. E6256–E6262.)

Two subdivisions of macaque LIP process visual-oculomotor information differently

Mo Chen, Bing Li, Jing Guang, Linyu Wei, Si Wu, Yu Liu, and Mingsha Zhang

The lateral intraparietal cortex (LIP) in primates links circuits for vision and saccadic eye movements. Neurons in LIP discharge during visually evoked saccades, but it is not certain whether LIP activity is primarily related to vision or to eye movement. From neuroanatomical evidence, we propose that LIP has two distinct subdivisions, dorsal and ventral LIP, with the dorsal division more concerned with visual processing and the ventral division more concerned with saccades. We provide evidence from both electrophysiological recordings and local inactivation to support our hypothesis. We therefore demonstrate that the two subdivisions of LIP play distinct roles in linking vision with visually guided action. (See pp. E6263–E6270.)

SARM1-specific motifs in the TIR domain enable NAD^+ loss and regulate injury-induced SARM1 activation

Daniel W. Summers, Daniel A. Gibson, Aaron DiAntonio, and Jeffrey Milbrandt

Axon degeneration is an important pathological event in multiple neurodegenerative disorders. Axon injury stimulates the prodestructive

factor SARM1, leading to the precipitous loss in the metabolite NAD^+. Remarkably, enforcing dimerization of the Toll/interleukin receptor (TIR) domain from SARM1 is sufficient to promote NAD^+ loss and axon degeneration. In this study, we uncover fundamental elements within the SARM1 TIR domain responsible for this activity, including a unique motif that is highly specific to SARM1. In addition, we discover a role for the SARM1 TIR domain in injury-induced activation of the SARM1 protein, suggesting that this domain contributes to SARM1 regulation in addition to the execution of axon degeneration. These studies identify potential avenues for therapeutic intervention in SARM1-dependent axon destruction pathways. (See pp. E6271–E6280.)

Pallidal spiking activity reflects learning dynamics and predicts performance

Eitan Schechtman, Maria Imelda Noblejas, Aviv D. Mizrahi, Omer Dauber, and Hagai Bergman

The basal ganglia (BG) are a set of interconnected nuclei deeply buried within the brain that are involved in action selection and habit formation. Classically considered motor nuclei, their role in cognitive performance has become widely appreciated over time. Current models of learning in the BG focus on striatal neurons and the neurotransmitter dopamine, but these do not fully account for observed behaviors. In this paper, we considered the learning-related activity of the external globus pallidus (GPe), a downstream BG nucleus. We show that GPe spiking activity predicts future performance, corresponds with learning dynamics, and decreases as performance becomes more automatic. Taken together, our data reveal the role of GPe in learning and open new avenues for research. (See pp. E6281–E6289.)

Elevated ERK/p90 ribosomal S6 kinase activity underlies audiogenic seizure susceptibility in fragile X mice

Kirsty Sawicka, Alexander Pyronneau, Miranda Chao, Michael V. L. Bennett, and R. Suzanne Zukin

Fragile X syndrome is the most common heritable cause of intellectual disability and autism. Elevated protein synthesis is considered a major contributor to the pathophysiology of fragile X. ERK (extracellular signal-regulated kinase) and mTOR (mechanistic target of rapamycin) are key signaling molecules in two prominent pathways that regulate protein synthesis. A major finding of this study is that ERK (but not mTOR) signaling is elevated in the neocortex of fragile X mice. Elevated ERK activity causes overactivation of p90-ribosomal S6 kinase (RSK) and hyperphosphorylation of ribosomal protein S6. Audiogenic seizures in fragile X mice, which mimic sensory hypersensitivity in fragile X humans, are prevented by RSK inhibition. Thus, RSK is a potential therapeutic target for treatment of fragile X. (See pp. E6290–E6297.)

Threshold-dependent transcriptional discrimination underlies stem cell homeostasis

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Various mechanisms have been proposed to explain dose-dependent transcriptional regulation mediated by morphogen gradients in animal development. In plant development, on the other hand, transcriptional mechanisms that underlie dose-dependent modulation of gene expression have not been discovered, despite the well-documented importance of positional signals in cell-fate specification. Here we show that the stem cell-promoting transcription factor WUSCHEL (WUS) regulates transcription in a concentration-dependent manner, activating transcription at a lower level and repressing transcription at a higher level, thus leading to the transcriptional control of its own negative regulator. Our work also shows that WUS binds a group of tightly clustered *cis* elements, each with different affinities; this binding suggests a buffering mechanism in maintaining a stable *CLAVATA3* (*CLV3*) expression. (See pp. E6298–E6306.)

DNA-dependent homodimerization, sub-cellular partitioning, and protein destabilization control WUSCHEL levels and spatial patterning

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The growing tips of plants maintain a constant number of stem cells in shoot apical meristems despite a continuous differentiation of stem cell descendants. The levels of homeodomain transcription factor WUSCHEL (WUS) determine the number of stem cells. Although WUSCHEL has been demonstrated to move across cells, the protein signatures that control nuclear levels and spatial patterning have remained elusive. We show that transcriptional regulatory domains also influence nuclear levels of WUS through nuclear-cytoplasmic partitioning and determine the spatial distribution of WUS through DNA binding and homodimerization. Our data also suggest that maintenance of WUSCHEL levels involves protein destabilization. Utilization of the same protein domains to regulate transcription and protein concentration may provide robustness to the spatiotemporal regulation of gene expression. (See pp. E6307–E6315.)