PNAS Plus Significance Statements

Cross-resistance to dicamba, 2,4-D, and fluroxypyr in *Kochia scoparia* is endowed by a mutation in an *AUX/IAA* gene

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Because auxin herbicides selectively control broad-leaf weeds, their use is extremely valuable in crops, such as wheat and corn. Although auxin-resistant weeds have appeared rarely over the past 60 years of herbicide use, they pose a major challenge in these cropping systems. Several groups have investigated the mechanisms of resistance for several of these weed species; this paper reports the identification of the underlying genetic mechanism of auxin resistance in a field-derived weed species. This mutation sits within a highly conserved region previously identified in *Arabidopsis* studies as vital for auxin signaling and points to the importance of studies in model systems to predict resistance mechanisms. (See pp. E2911–E2920.)

Noncatalytic aspartate at the exonuclease domain of proofreading DNA polymerases regulates both degradative and synthetic activities

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Replicative DNA polymerases (DNAPs) combine two processes to ensure the extremely faithful synthesis of DNA, nucleotide selectivity, and editing of mis-inserted nucleotides by a 3′-5′ exonuclease activity. The 3′-5′ exonuclease activity is governed by four universally conserved aspartate residues that coordinate the two metal ions responsible for the hydrolysis of the last phosphodiester bond of the primer strand. In this work, we have identified an additional conserved aspartate residue that establishes multiple interactions with residues responsible for the exonucleolysis and for the forward movement of the DNAP along the DNA after inserting a nucleotide, acting as a master organizer for the processive intramolecular proofreading of polymerization errors. (See pp. E2921–E2929.)

Reconstructing a metazoan genetic pathway with transcriptome-wide epistasis measurements

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Transcriptome profiling quantitatively measures gene expression genome-wide. There is widespread interest in using transcriptomic profiles as phenotypes for epistasis analysis. Though epistasis measurements can be performed using individual transcripts, this results in many scores that must be interpreted independently. We developed a statistic that summarizes these measurements, simplifying analysis. Moreover, epistasis analysis has previously only been performed on cDNA extracted from single cells. We show that wholeorganism RNA-sequencing (RNA-seq) can be used to characterize interactions between genes. With the advent of genome engineering, mutants can be created easily in many organisms. Thus, phenotyping is now the rate-limiting step toward reconstructing interaction networks. Our work potentially represents a solution to this problem because RNA-seq is sensitive to a variety of genetic perturbations. (See pp. E2930–E2939.)

CD96 expression determines the inflammatory potential of IL-9–producing Th9 cells

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T helper type (Th) 9 cells demonstrate both pro- and antiinflammatory properties, pointing to a functional heterogeneity not examined so far. Applying single cell gene expression analysis of alloreactive Th9 cells, we revealed the existence of two major subsets, CD96^{high} and CD96^{low} Th9 cells, with strongly opposing inflammatory and, especially, colitis-inducing potential. Mechanistically, we found that CD96 controls cytokine and colitis-inducing potential of Th9 cells, providing strong evidence for an inhibitory role of CD96 in controlling CD4$^+$ T-cell effector functions. Thus, interfering with CD96-mediated immune inhibition would be a promising approach in preventing Th9-mediated diseases, such as ulcerative colitis, or reinforcement of Th9-mediated immune control of tumors and infections. (See pp. E2940–E2949.)

Cyclin-dependent kinase activity is required for type I interferon production

Oya Cingöz and Stephen P. Goff

Innate immune responses are the first line of defense against pathogens. Upon sensing a pathogen, cells produce IFNs: signaling molecules that activate a diverse array of genes involved in antiviral immunity. Cyclin-dependent kinases (CDKs) are enzymes involved in cell cycle progression and transcription. We discovered that CDKs are essential for IFN production

following nucleic acid sensing and virus infection. Depletion of CDK activity results in a block to the translation of IFN mRNA into protein, even though the mRNA levels remain unchanged. This block is specific for IFN, as the translation of other mRNAs proceeds normally. Our results establish a previously unknown and critical requirement for CDKs for IFN production in the response against pathogens. (See pp. E2950–E2959.)

Chronic stress promotes colitis by disturbing the gut microbiota and triggering immune system response

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Stress cannot be avoided in the present competitive world, and chronic stress is associated with detrimental effects on physical health, including the progression of inflammatory bowel disease (IBD). However, the mechanisms behind it are less clearly understood. This study showed that chronic stress disturbed gut microbiota, thereby triggering immune system response and facilitating dextran sulfate sodium-induced colitis. Results also showed stress-deficient expression of mucin-2 and lysozyme, which may contribute to the disorder of gut microbiota. This study adds to our understanding of interactions between microbiota and host and provides the basis for future clinical studies of microbiota manipulation and transplantation and the development of new therapeutic strategies for depression or IBD. (See pp. E2960–E2969.)

Predicting cancer outcomes from histology and genomics using convolutional networks

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Predicting the expected outcome of patients diagnosed with cancer is a critical step in treatment. Advances in genomic and imaging technologies provide physicians with vast amounts of data, yet prognostication remains largely subjective, leading to suboptimal clinical management. We developed a computational approach based on deep learning to predict the overall survival of patients diagnosed with brain tumors from microscopic images of tissue biopsies and genomic biomarkers. This method uses adaptive feedback to simultaneously learn the visual patterns and molecular biomarkers associated with patient outcomes. Our approach surpasses the prognostic accuracy of human experts using the current clinical standard for classifying brain tumors and presents an innovative approach for objective, accurate, and integrated prediction of patient outcomes. (See pp. E2970–E2979.)

Linking imaging to omics utilizing image-guided tissue extraction

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Imaging provides an insight into biological patho-mechanisms of diseases. However, the link between the imaging phenotype and the underlying molecular processes is often not well understood. Methods such as metabolomics and proteomics reveal detailed information about these processes. Unfortunately, they provide no spatial information and thus cannot be easily correlated with functional imaging. We have developed an image-guided milling machine and unique workflows to precisely isolate tissue samples based on imaging data. The tissue samples remain cooled during the entire procedure, preventing sample degradation. This enables us to correlate, at an unprecedented spatial precision, comprehensive imaging information with metabolomics and proteomics data, leading to a better understanding of diseases. (See pp. E2980–E2987.)

Occurrence, evolution, and functions of DNA phosphorothioate epigenetics in bacteria

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Phosphorothioate (PT) modification of the DNA sugar-phosphate backbone is an important microbial epigenetic modification governed by DndABCDE, which together with DndFGH, constitutes a restriction-modification system. We show that up to 45% of 1,349 identified bacterial *dnd* systems exhibit the form of solitary *dndABCDE* without the restriction counterparts of *dndFGH*. The combination of epigenomics, transcriptome analysis, and metabolomics suggests that in addition to providing a genetic barrier against invasive DNA, PT modification is a versatile player involved in the epigenetic control of gene expression and the maintenance of cellular redox homeostasis. This finding provides evolutionary and functional insights into this unusual epigenetic modification. Our results imply that PT systems might evolve similar to other epigenetic modification systems with multiple cellular functions. (See pp. E2988–E2996.)

Combination of cGMP analogue and drug delivery system provides functional protection in hereditary retinal degeneration

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Development of treatments for hereditary degeneration of the retina (RD) is hampered by the vast genetic heterogeneity of this group of diseases and by the delivery of the drug to an organ protected by the blood–retina barrier. Here, we present an approach for the treatment of different types of RD, combining an innovative drug therapy with a liposomal system that facilitates drug delivery into the retina. Using different animal models of RD we show that this pharmacological treatment preserved both the viability of cells in the retina as well as retinal function. Thus, our study provides an avenue for the development of therapies for hereditary diseases which cause blindness, an unmet medical need. (See pp. E2997–E3006.)

Disinhibition of CA1 pyramidal cells by low-dose ketamine and other antagonists with rapid antidepressant efficacy

Allie J. Widman and Lori L. McMahon

Ketamine is an NMDA receptor (NMDAR) antagonist that alleviates depressive symptoms in patients with treatment-resistant depression within hours of administering a single dose. However, psychoactive side effects limit its use. Understanding the mechanism underlying ketamine's antidepressant effect is crucial in identifying targets for novel therapeutics with fewer side effects. Here, we report ketamine enhances excitability of pyramidal cells indirectly by reducing synaptic GABAergic inhibition, thus causing disinhibition. Additionally, we investigated whether other NMDAR

antagonists and the muscarinic acetylcholine receptor antagonist scopolamine shared this disinhibition mechanism. Our results show that only those antagonists with antidepressant efficacy in humans disinhibit pyramidal cells at a clinically relevant concentration, supporting the concept that disinhibition is likely involved in the antidepressant effect of these antagonists. (See pp. E3007–E3016.)

Resonance with subthreshold oscillatory drive organizes activity and optimizes learning in neural networks

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Networks of neurons need to reliably encode and replay patterns and sequences of activity. In the brain, sequences of spatially coding neurons are replayed in both the forward and reverse direction in time with respect to their order in recent experience. As of yet there is no network-level or biophysical mechanism known that can produce both modes of replay within the same network. Here we propose that resonance, a property of neurons, paired with subthreshold oscillations in neural input facilitate network-level learning of fixed and sequential activity patterns and lead to both forward and reverse replay. (See pp. E3017–E3025.)

Bilobal architecture is a requirement for calmodulin signaling to Ca$_V$1.3 channels

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Calmodulin (CaM) regulation of voltage-gated calcium (Ca$_V$) channels constitutes a prototypic biological feedback mechanism that contributes prominently toward Ca^{2+} homeostasis in neurons and cardiac myocytes. Here, by partitioning CaM molecularly into its two elemental domains or lobes, we uncover a distinctive nonlinearity in CaM signaling to Ca$_V$ channels. Ca$_V$ channels detect the coincident binding of two CaM lobes to up-regulate channel activity. This mechanism elaborates a molecular logic operation that enables channels to detect combinations of spatiotemporal Ca^{2+} signals and perform higher-order computations on Ca^{2+} signals. These findings uncover the unified mechanistic basis for Ca$_V$ channel feedback and, in so doing, shed light on the versatility of CaM in decoding cellular Ca^{2+} signals. (See pp. E3026–E3035.)

Complex electrophysiological remodeling in postinfarction ischemic heart failure

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Cardiac arrhythmias often occur in heart failure (HF) patients, but drug therapies using selective ion channel blockers have failed clinical trials and effective drug therapies remain elusive. Here we systematically study the major ionic currents during the cardiac action potential (AP) and arrhythmogenic Ca^{2+} release in post-infarction HF. We found that changes in any individual current are relatively small, and alone could mislead as to consequences. However, differential changes in multiple currents integrate to shorten AP in the infarct border zone but prolong AP in the remote zone, increasing AP repolarization inhomogeneity. Our findings help explain why single channel-blocker therapy may fail, and highlight the need to understand the integrated changes of ionic currents in treating arrhythmias in HF. (See pp. E3036–E3044.)

Arabidopsis TSO1 and MYB3R1 form a regulatory module to coordinate cell proliferation with differentiation in shoot and root

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Plant postembryonic development relies on a small pool of stem cells at the shoot and root tip. The question of how the cell cycle regulatory activities are integrated into the specific stem cell context is not well understood. This study identifies a previously unknown regulatory module in the flowering plant consisting of two regulatory genes, *TSO1* and *MYB3R1*. TSO1 negatively regulates *MYB3R1* to control cell division activity, maintain proper stem cell pool size, and balance cell proliferation with differentiation in shoot and root. Significantly, animal homologs of TSO1 and MYB3R1 are members of a cell cycle regulatory complex, suggesting that this conserved module operates in both plants and animals. (See pp. E3045–E3054.)

Transcriptome landscape of a bacterial pathogen under plant immunity

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Plants have evolved a powerful innate immune system to defend against microbial pathogens. Despite extensive studies, how plant immunity ultimately inhibits bacterial pathogen growth is largely unknown, due to difficulties in profiling bacterial responses *in planta*. In this study, we established two methods for *in planta* bacterial transcriptome analysis using RNA sequencing. By analyzing 27 combinations of plant immunity mutants and *Pseudomonas syringae* strains, we succeeded in the identification of specific bacterial transcriptomic signatures that are influenced by plant immune activation. In addition, we found that over-expression of an immune-responsive *P. syringae* sigma factor gene involved in iron regulation could partially counter bacterial growth restriction during plant immunity. This study illuminates the enigmatic mechanisms of bacterial growth inhibition by plant immunity. (See pp. E3055–E3064.)