PNAS Plus Significance Statements

Structural insights into the mechanism of inhibition of AHAS by herbicides

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Herbicide-resistant weeds are a major threat to the world's food security and result in the loss of billions of dollars of income to crop producers. Penoxsulam, a member of the triazolopyrimidine family of commercial herbicides, has become a center of focus due to an increase in the number of weeds that have developed resistance to this compound. Thus, understanding its mode of action will assist in managing this problem. Here, our crystallographic data capture "in action" the molecular mechanisms that underpin how this herbicide operates. As well as having an effective binding affinity for acetohydroxyacid synthase, it is able to induce and enhance the production of peracetate, a highly reactive oxidant that induces the accumulative inhibition of its target. (See pp. E1945–E1954.)

Lineage-specific gene acquisition or loss is involved in interspecific hybrid sterility in rice

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Hybrid sterility, a major reproductive barrier between species, hinders the transfer of desirable traits from one species to another. We report a forward genetic approach for creating a "neutral" allele of the S_1 locus, a major interspecific hybrid sterility locus in rice. This neutral allele does not induce hybrid sterility in combination with alleles from either Asian or African rice species. The allele carries a deletion in the peptidase-coding gene, *SSP*, in the S_1 locus. This work provides mechanistic and evolutionary insights into hybrid sterility and demonstrates the feasibility of the approach that allows broader access to desirable traits in distantly related species during crop breeding. (See pp. E1955–E1962.)

Digital signaling network drives the assembly of the AIM2-ASC inflammasome

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The complexity of biological signaling pathways parallels the design of modern electronic circuits. The AIM2-ASC inflammasome is a filamentous signaling platform that plays an essential role in defense against foreign dsDNA arising from invading pathogens. Currently, the design principles of the AIM2-ASC "signaling circuit" remain poorly understood. Here, we found that the assembly of the AIM2-ASC inflammasome filters short dsDNA as noise at each signaling step. dsDNA induces hysteresis, and the assembly generates multistage, virtually infinite signal amplification. Moreover, an array of positive feedback loops reinforces the assembly. Together with a quantitative model of the assembly pathway, we demonstrate that an ultrasensitive digital circuit drives the assembly of the AIM2-ASC inflammasome on foreign dsDNA. (See pp. E1963–E1972.)

Structure of a zosuquidar and UIC2-bound human-mouse chimeric ABCB1

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The ATP binding cassette transporter ABCB1 (also termed P-glycoprotein) is a physiologically essential multidrug efflux transporter of key relevance to biomedicine. Here, we report the conformational trapping and structural analysis of ABCB1 in complex with the antigen-binding fragment of UIC2, a human ABCB1-specific inhibitory antibody, and zosuquidar, a third-generation ABCB1 inhibitor. The structures outline key features underlining specific ABCB1 inhibition by antibodies and small molecules, including a dual mode of inhibitor binding in a fully occluded ABCB1 cavity. Finally, our analysis sheds light on the conformational transitions undergone by the transporter to reach the inhibitor-bound state. (See pp. E1973–E1982.)

PI5P4Kγ functions in DTX1-mediated Notch signaling

Li Zheng and Sean D. Conner

The Notch signaling pathway performs a vital role in biological processes ranging from stem cell maintenance to cell viability. This highly conserved pathway must be tightly controlled, since defects in signaling can promote disease. The E3 ubiquitin ligase DTX1 has emerged as a key negative regulator of Notch signaling, where Notch ubiquitination by DTX1 is thought to control intracellular sorting decisions of the receptor. Here we show that DTX1 can regulate Notch activity independent of directly ubiquitinating the receptor, suggesting that DTX1 targets other factors involved in Notch transport. Using an activity-based screen for DTX1 substrates, we identify PI5P4Kγ, a lipid kinase, and discover that PI5P4Kγ and

DTX1 have opposing activities in regulating Notch transit through recycling endosomes. (See pp. E1983–E1990.)

Interacting-heads motif has been conserved as a mechanism of myosin II inhibition since before the origin of animals

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All animals have the ability to move. Myosin II is the motor protein that generates this movement by powering muscular contraction; it also drives motility in nonmuscle cells. In relaxed muscle and in quiescent nonmuscle cells, myosin II is switched off by intramolecular interactions between its heads that inhibit its activity. This interacting-heads motif (IHM) is a fundamental contributor to contractile regulation. Given its importance in cell contractility, we wanted to determine when the IHM first evolved. Using electron microscopy, image averaging, and sequence analysis of myosin II from primitive organisms, we show that the IHM has existed since the earliest animals and before. This ancient origin highlights the central role of the IHM in regulating myosin II function. (See pp. E1991–E2000.)

Death-domain dimerization-mediated activation of RIPK1 controls necroptosis and RIPK1-dependent apoptosis

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While the critical role of RIPK1 kinase activity in mediating necroptosis and RIPK1-dependent apoptosis has been established, we still know little about how the nonkinase domains of RIPK1 regulate its kinase activity. Establishing the role of RIPK1-death domain (DD) in mediating RIPK1 activation and formation of complex II provides an important insight into the molecular mechanism by which RIPK1 is activated during the transition from complex I to complex II. These results suggest that RIPK1-DD self-association may provide an amplification mechanism to promote the activation of RIPK1 kinase activity for mediating signal transduction to lead to cell death. Our results also suggest that the activation of RIPK1 may be regulated by its concentration as increased expression of RIPK1 under pathological conditions may promote its dimerization and activation. (See pp. E2001–E2009.)

Light color acclimation is a key process in the global ocean distribution of *Synechococcus cyanobacteria*

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Understanding the functional diversity of specific microbial groups at the global scale is critical yet poorly developed. By combining the considerable knowledge accumulated through recent years on the molecular bases of photosynthetic pigment diversity in marine *Synechococcus*, a major phytoplanktonic organism, with the wealth of metagenomic data provided by the *Tara* Oceans expedition, we have been able to reliably quantify all known pigment types along its transect and provide a global distribution map. Unexpectedly, cells able to dynamically change their pigment content to match the ambient light color were ubiquitous and predominated in many environments. Altogether, our results unveiled the role of adaptation to light quality on niche partitioning in a key primary producer. (See pp. E2010–E2019.)

Evolutionary stability of antibiotic protection in a defensive symbiosis

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Insights from natural applications of antibiotics are important to gain a deeper understanding of the evolutionary processes that underlie the maintenance of an antibiotic defense and prevent the rise and spread of antibiotic resistance. Using 25 species and subspecies of beewolf digger wasps that engage in a defensive symbiosis with *Streptomyces* bacteria, we tracked evolutionary changes in the antibiotic cocktail that protects the wasps' larval offspring against mold fungi. Our results yield insights into the mechanistic basis as well as the ecological and evolutionary implications of producing a complex cocktail of antimicrobial compounds in a symbiotic setting. (See pp. E2020–E2029.)

Pivotal roles of PCNA loading and unloading in heterochromatin function

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DNA replication poses a unique logistical challenge for cells in that structural features of chromatin and their regulatory functions must be carefully coordinated with the passage of replication machinery so faithful duplication of both the genome and its chromatin structures may be achieved. Nucleosome assembly is fundamental to reestablishment of chromatin in the wake of DNA replication. Here, a mechanism for coordinating nucleosome assembly with DNA replication to maintain silenced chromatin is described. (See pp. E2030–E2039.)

CRISPR/Cas9 cleavages in budding yeast reveal templated insertions and strand-specific insertion/deletion profiles

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Using budding yeast, we address how Cas9 protein and its guide RNA (gRNA) create double-strand chromosome breaks (DSBs), and explore whether binding of Cas9::gRNA influences subsequent DSB repair by nonhomologous end-joining. We created pairs of gRNAs that are complementary to opposite DNA strands but direct cleavage at the same chromosomal location. The resulting repair profiles (insertion/deletions) are different for the two ostensibly identical DSBs. Most notably, there are frequent +1 insertions that are templated after cleavage creates a 1-nt 5′ overhang that is filled in before ends are ligated. DNA polymerase 4 is required for most +1 insertions and for longer (+2 and +3) insertions. We found similar templating of +1 insertions in published studies of mammalian DSBs created by Cas9. (See pp. E2040–E2047.)

The ZBED6–IGF2 axis has a major effect on growth of skeletal muscle and internal organs in placental mammals

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Insulin-like growth factor 2 (IGF2) is an important growth factor with a critical role for fetal growth in mammals. The ZBED6 transcription factor is unique to placental mammals and has evolved from a domesticated DNA transposon. This study

shows that ZBED6 and its interaction with the *Igf2* locus play a prominent role in regulating postnatal growth of skeletal muscle and internal organs (kidney, liver, and heart) in placental mammals. This prominent role in mammalian biology provides a reasonable explanation why ZBED6 is highly conserved among all families of placental mammals and why 16 base pairs encompassing the ZBED6 binding site in an intron of *Igf2* are conserved among the great majority of, if not all, placental mammals. (See pp. E2048–E2057.)

STING-dependent translation inhibition restricts RNA virus replication

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In mammalian cells, a protein called STING (stimulator of IFN genes) is typically viewed as a factor dedicated to defense strategies induced by DNA viruses. The antiviral activities of STING are linked to its ability to induce the expression of genes that combat viral replication cycles. In our study, we have discovered a transcription-independent function for STING in the restriction of viruses that contain RNA genomes. We found that cells lacking STING are sensitive to RNA virus infections and that during these infections, STING inhibits the translation machinery to prevent viral protein synthesis. This study therefore establishes that STING has dual functions in host defense, regulating antiviral gene expression or interfering with translation, to restrict replication of distinct classes of viruses. (See pp. E2058–E2067.)

Chimeric antigen receptor T cells form nonclassical and potent immune synapses driving rapid cytotoxicity

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Davenport et al. discovered that the chimeric antigen receptor (CAR) immune synapse structure is different from the T cell receptor (TCR) synapse. The CAR immune synapse formed a disorganized pattern of Lck and more rapidly recruited lytic granules compared with the TCR. The differing immune synapse correlated with faster killing of tumor target cells and detachment from dying tumor cells by CAR-T cells. These findings provide a mechanism whereby CAR-T cells can effectively reduce large tumor burden in patients. This study will form a basis upon which to compare future receptor design to modulate signaling and programming of cytotoxic CAR-T cells to improve treatment of solid cancers. (See pp. E2068–E2076.)

Targeting the cMET pathway augments radiation response without adverse effect on hearing in NF2 schwannoma models

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In patients with progressive vestibular schwannoma (VS), radiotherapy is associated with the risk of debilitating hearing loss. There is an urgent need to identify an adjunct therapy that, by enhancing the efficacy of radiation, can help lower the radiation dose and preserve hearing. In our newly developed cerebellopontine angle model of schwannomas that faithfully recapitulates the tumor-induced hearing loss, we demonstrate that cMET blockade sensitizes schwannomas to radiation therapy (RT) in neurofibromatosis type II schwannoma animal models without any adverse effects on hearing. Using an organoid brain slice culture model, cMET blockade inhibited the growth of patient-derived schwannomas. Our study provides the rationale and critical data for the clinical translation of combined cMET blockade with RT in patients with VSs. (See pp. E2077–E2084.)

Suppression of RGSz1 function optimizes the actions of opioid analgesics by mechanisms that involve the Wnt/β-catenin pathway

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Opioids are used to alleviate severe pain, but their long-term use leads to analgesic tolerance, dependence, and addiction. Here, we targeted specific intracellular pathways to dissociate the analgesic actions of opioids from addiction-related effects. Using genetically modified male and female mice in models of addiction and analgesia, we revealed a key role of an intracellular modulator of the mu opioid receptor, RGSz1, in opioid actions. We applied next-generation sequencing and biochemical assays to delineate the mechanism of RGSz1 action in the mouse periaqueductal gray. Findings from this work point to novel intracellular pathways that can be targeted to optimize the actions of opioids for the treatment of chronic pain. (See pp. E2085–E2094.)

Compartmentalization of antagonistic Ca^{2+} signals in developing cochlear hair cells

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Cochlear inner hair cells (IHCs) are responsible for transducing sound waves and relaying acoustic information to the brain through its afferent synapse. During development, IHCs exhibit sensory-independent activity, supported by voltage-gated Ca^{2+} influx, which is critical for the normal maturation of the auditory system. This spontaneous activity is modulated by an inhibitory cholinergic input, mediated by nicotinic receptors with unusually high Ca^{2+} permeability, leading to the activation of hyperpolarizing SK channels. Thus, Ca^{2+} should play distinct excitatory and inhibitory roles in small, compact IHCs. This work presents evidence for specialized cellular mechanisms that maintain local compartmentalization of Ca^{2+} signals and prevent synaptic cross-talk. Thus, the cholinergic input preserves its inhibitory signature to ensure normal development of the auditory system. (See pp. E2095–E2104.)

Ridding fMRI data of motion-related influences: Removal of signals with distinct spatial and physical bases in multiecho data

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Spontaneous fMRI signals are used to understand human brain organization throughout the life span and in disease states. Spontaneous fMRI signals contain many artifacts, and removing these artifacts is vital to properly studying neurobiological signals. We report successful removal of a major artifact, spatially focal motion artifact, from resting state fMRI signals via multiecho imaging techniques. By removing motion artifact, we isolate a second kind of motion-associated signal, a respiratory signal, that occurs across the entire brain. We illustrate several techniques that remove this respiratory artifact, yielding fMRI data free of motion-related influences. These two kinds of motion-related

signals have distinct physical and spatial bases, and each can strongly and differentially influence signal patterns in fMRI data. (See pp. E2105–E2114.)

GABAergic inhibition of leg motoneurons is required for normal walking behavior in freely moving *Drosophila*

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Inhibition is an important feature of the neuronal circuit, and in walking, it aids in controlling coordinated movement of legs, leg segments, and joints. Recent studies in *Drosophila* report the role of premotor inhibitory interneurons in regulation of larval locomotion. However, in adult walking, the identity and function of premotor interneurons are poorly understood. Here, we use genetic methods for targeted knockdown of inhibitory neurotransmitter receptors in leg motoneurons, combined with automated video recording methods we have developed for quantitative analysis of fly leg movements and walking parameters, to reveal the resulting slower walking speed and defects in walking parameters. Our results indicate that GABAergic premotor inhibition to leg motoneurons is required to control the normal walking behavior in adult *Drosophila*. (See pp. E2115–E2124.)

Targeted DNA demethylation of the *Arabidopsis* genome using the human TET1 catalytic domain

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DNA methylation is an epigenetic modification involved in gene silencing. Studies of this modification usually rely on the use of mutants or chemicals that affect methylation maintenance. Those approaches cause global changes in methylation and make difficult the study of the impact of methylation on gene expression or chromatin at specific loci. In this study, we develop tools to target DNA demethylation in plants. We report efficient on-target demethylation and minimal effects on global methylation patterns, and show that in one case, targeted demethylation is heritable. These tools can be used to approach basic questions about DNA methylation biology, as well as to develop new biotechnology strategies to modify gene expression and create new plant trait epialleles. (See pp. E2125–E2134.)

Temporal–prefrontal cortical network for discrimination of valuable objects in long-term memory

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Animals, including humans, are surrounded by many objects, only some of which are valuable. To survive, it is critical to efficiently discriminate valuable objects, particularly those that are only occasionally or seasonally available. Here, we use fMRI to show that, in macaques, a network consisting of areas in the temporal and prefrontal cortex and their associated subcortical structures maintained value memories for a large number of objects. This memory representation lasted for many months after the objects were last seen and accordingly the monkeys were able to find valuable objects efficiently. We postulate that this temporal–prefrontal circuit is critical for drawing on learned value memory to guide goal-oriented behavior toward certain objects. (See pp. E2135–E2144.)