PNAS Plus Significance Statements

Enantiomer excesses of rare and common sugar derivatives in carbonaceous meteorites

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The majority of biological sugars and their derivatives contain higher abundances of the "D" mirror-image forms relative to the "L" forms. For example, nucleic acids are composed of only D sugars. Carbonaceous meteorites can potentially assist in understanding the long-sought origin of such phenomena; They preserve a record of the earliest (~4.5 Gy) chemical processes in the Solar System. To date, there have been no systematic studies of D/L (i.e., enantiomer) ratios of meteoritic sugar derivatives. In multiple meteorites, we demonstrate that rare and common sugar acids contain large excesses of the D enantiomer. Such data indicate that early meteoritic compounds may have influenced the enantiomer profile of subsequent biological sugars and their derivatives. (See pp. E3322–E3331.)

Sustained deposition of contaminants from the *Deepwater Horizon* spill

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Despite numerous publications reporting the accumulation of petroleum hydrocarbons associated with the *Deepwater Horizon* spill on the seafloor, the mechanisms of their delivery to the seafloor remain unclear. We demonstrate sedimentation of black carbon derived from the in situ burning of surface oil slicks for about 2 mo following the cessation of burning while other contaminants from the spill, including bioactive barium derived from drilling mud, continued to sediment for at least 5 mo after the well was capped. We also show that the episodic sinking of spill-associated substances was mainly mediated by marine particles, especially diatoms. Together, these data demonstrate delivery mechanisms of contaminants from the spill to benthic ecosystems in the deep Gulf of Mexico. (See pp. E3332–E3340.)

Teaching a lay theory before college narrows achievement gaps at scale

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In the United States, large, persistent gaps exist in the rates at which racial, ethnic, and socioeconomic groups complete postsecondary education, even when groups are equated on prior preparation. We test a method for preventing some of those gaps by providing individuals with a lay theory about the meaning of commonplace difficulties before college matriculation. Across three experiments, lay theory interventions delivered to over 90% of students increased full-time enrollment rates, improved grade point averages, and reduced the overrepresentation of socially disadvantaged students among the bottom 20% of class rank. The interventions helped disadvantaged students become more socially and academically integrated in college. Broader tests can now be conducted to understand in which settings lay theories can help remedy postsecondary inequality at scale. (See pp. E3341–E3348.)

Dynamic recruitment and activation of ALS-associated TBK1 with its target optineurin are required for efficient mitophagy

Andrew S. Moore and Erika L. F. Holzbaur

Mitochondria are key regulators of cellular metabolism and are defective in a number of human disorders. Here, we examine how living cells selectively eliminate damaged mitochondria through the autophagosome-lysosome system. We find that the serine/threonine kinase TANK-binding kinase 1 (TBK1) and its downstream target optineurin (OPTN) are recruited to mitochondria after acute damage, where they coordinate engulfment by autophagosomes. Loss or chemical inhibition of TBK1 stalls mitophagy, resulting in the accumulation of damaged mitochondria. Because mutations in both TBK1 and OPTN are associated with amyotrophic lateral sclerosis and frontotemporal dementia, these results suggest that disordered mitophagy may be associated with selective neurodegeneration. (See pp. E3349–E3358.)

Spatial scale modulates the strength of ecological processes driving disease distributions

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For four decades, ecologists have hypothesized that biotic interactions predominantly control species' distributions at local scales, whereas abiotic factors operate more at regional scales. Here, we demonstrate that the drivers of three emerging diseases (amphibian chytridiomycosis, West Nile virus, and Lyme disease) in the United States support the predictions of this fundamental hypothesis. Humans are contributing to biodiversity loss, changes in dispersal patterns, and global climate

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change at an unprecedented rate. Our results highlight that common single-scale analyses can misestimate the impact that humans are having on biodiversity, disease, and the environment. (See pp. E3359–E3364.)

Delineating ecologically significant taxonomic units from global patterns of marine picocyanobacteria

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Metagenomics has become an accessible approach to study complex microbial communities thanks to the advent of high-throughput sequencing technologies. However, molecular ecology studies often face interpretation issues, notably due to the lack of reliable reference databases for assigning reads to the correct taxa and use of fixed cutoffs to delineate taxonomic groups. Here, we considerably refined the phylogeography of marine picocyanobacteria, responsible for about 25% of global marine productivity, by recruiting reads targeting a high-resolution marker from *Tara* Oceans metagenomes. By clustering lineages based on their distribution patterns, we showed that there is significant diversity at a finer resolution than the currently defined "ecotypes," a diversity that is tightly controlled by environmental cues. (See pp. E3365–E3374.)

Highly variable individual donor cell fates characterize robust horizontal gene transfer of an integrative and conjugative element

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DNA conjugation is a fascinating process that bestows the prokaryotic world with the ability to exchange hundreds of genes in a single event. What has long been puzzling is the low rate of horizontal gene transfer at the population level. Here, we develop a fluorescent tool to study the dynamic fate of a chromosomally located conjugative DNA. We find that donor cells becoming transfer proficient undergo highly variable cell fates, likely as a result of the conjugative element wreaking havoc in the cell. Modeling of the partnership between the cell and integrative and conjugative element (ICE) suggests that ICE fitness is optimal at low activation rates, explaining why gene transfer in bacterial populations occurs at low frequencies despite its ecological importance for adaptation. (See pp. E3375–E3383.)

Transposon mutagenesis identifies genes and cellular processes driving epithelial-mesenchymal transition in hepatocellular carcinoma

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Epithelial-mesenchymal transition (EMT) contributes to metastasis and chemoresistance in patients with hepatocellular carcinoma (HCC), but the genes driving EMT are poorly understood. Here, we describe a transposon mutagenesis screen that made it possible to identify 233 candidate cancer genes (CCGs) that are enriched for genes driving EMT in HCC. Twenty-three CCGs are predicted to function early in tumorigenesis, and alterations in these genes are associated with poor HCC patient survival. Validation studies showed that deregulation of the most highly mutated CCGs activates an EMT program that enhances HCC cell migration and also confers sorafenib resistance. Thus, transposon mutagenesis appears to provide an excellent resource for identifying genes regulating EMT in human HCC and for potentially identifying new drug targets for HCC. (See pp. E3384–E3393.)

Transcriptional regulator Bhlhe40 works as a cofactor of T-bet in the regulation of IFN-γ production in *i*NKT cells

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A unique characteristic of invariant natural killer T (*i*NKT) cells is their ability to immediately produce large amounts of interferon-gamma (IFN-γ) upon activation, which enables these cells to play critical roles in initiating immune responses in various pathological conditions. In this study, we demonstrate a previously unidentified mechanism mediated by basic helix–loop–helix transcription factor family, member e40 (Bhlhe40) for accelerating IFN-γ production in *i*NKT cells. Bhlhe40 is required for normal physiological functions in *i*NKT cells, where it positively regulates IFN-γ production. Bhlhe40 also contributes to acetylating histone H3-lysine 9 of the *Ifng* locus in *i*NKT cells. These findings may help in understanding the molecular mechanisms related to the biology of *i*NKT cells. (See pp. E3394–E3402.)

Structural basis for concerted recruitment and activation of IRF-3 by innate immune adaptor proteins

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Type I IFNs are key cytokines involved in antiviral immunity. A number of innate sensing pathways regulate the induction of type I IFNs. These pathways converge at the activation of the transcription factor IRF-3 (IFN regulatory factor 3). Three different adaptors mediate the recruitment of IRF-3 using a conserved structural motif. In this study, we determined the molecular mechanisms by which these adaptors recruit IRF-3 upon phosphorylation, the mechanism of IRF-3 activation, and how rotavirus subverts these signaling mechanisms to evade innate immune surveillance. These results provide critical insights into the molecular basis of innate immunity against microbial and viral infections. (See pp. E3403–E3412.)

Envelope residue 375 substitutions in simian–human immunodeficiency viruses enhance CD4 binding and replication in rhesus macaques

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Simian–human immunodeficiency viruses (SHIVs) are an invaluable tool for assessing HIV-1 vaccines, developing therapeutic "cure" strategies, and understanding viral immunopathogenesis. However, only limited success has been achieved in creating SHIVs that incorporate HIV-1 envelopes (Envs) that retain the antigenic features of clinically relevant viruses. Here we focus on a critical residue of the CD4-binding region, Env375, which is under strong positive selection across the broad range of primate lentiviruses. We find that genotypic variation of residue 375 allows for the creation of pathogenic SHIVs that retain the antigenicity, tier 2 neutralization sensitivity, and persistence properties characteristic of primary HIV-1 strains. Taken

together, our findings suggest a new paradigm for SHIV design and modeling with important applications to HIV-1 vaccine, cure, and pathogenesis research. (See pp. E3413–E3422.)

Argininosuccinate synthetase regulates hepatic AMPK linking protein catabolism and ureagenesis to hepatic lipid metabolism

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The CDC projects that by the year 2050 one in three adults will be affected by diabetes in the United States alone. A key feature of type 2 diabetes is insulin resistance, which is associated with ectopic lipid deposition in tissues such as the liver. We have discovered a novel regulatory mechanism in the liver linking protein catabolism and ureagenesis to increased lipid oxidation. By increasing urea production, flux through the enzyme argininosuccinate synthetase is enhanced, leading to activation of AMP-activated protein kinase and increased hepatic fat oxidation. These findings may lead to the development of new drugs designed to reduce fat accumulation in the liver and reverse insulin resistance. (See pp. E3423–E3430.)

Engineering control of bacterial cellulose production using a genetic toolkit and a new cellulose-producing strain

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Bacterial cellulose is a remarkable material that is malleable, biocompatible, and over 10-times stronger than plant-based cellulose. It is currently used to create materials for tissue engineering, medicine, defense, electronics, acoustics, and fabrics. We describe here a bacterial strain that is readily amenable to genetic engineering and produces high quantities of bacterial cellulose in low-cost media. To reprogram this organism for biotechnology applications, we created a set of genetic tools that enables biosynthesis of patterned cellulose, functionalization of the cellulose surface with proteins, and tunable control over cellulose production. This greatly expands our ability to control and engineer new cellulose-based biomaterials, offering numerous applications for basic research, materials science, and biotechnology. (See pp. E3431–E3440.)

Morphine paradoxically prolongs neuropathic pain in rats by amplifying spinal NLRP3 inflammasome activation

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Pain after disease/damage of the nervous system is predominantly treated with opioids, but without exploration of the long-term consequences. We demonstrate that a short course of morphine after nerve injury doubles the duration of neuropathic pain. Using genetic and pharmacological interventions, and innovative Designer Receptor Exclusively Activated by Designer Drugs disruption of microglia reactivity, we demonstrate that opioid-prolonged neuropathic pain arises from spinal microglia and NOD-like receptor protein 3 inflammasome formation/activation. Inhibiting these processes permanently resets amplified pain to basal levels, an effect not previously reported. These data support the "two-hit hypothesis" of amplification of microglial activation—nerve injury being the first "hit," morphine the second. The implications of such potent microglial "priming" has fundamental clinical implications for pain and may extend to many chronic neurological disorders. (See pp. E3441–E3450.)

Thyroid hormones inhibit TGF-β signaling and attenuate fibrotic responses

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We show here that binding of the thyroid hormone triiodothyronine to the thyroid hormone receptors (TRs) antagonizes TGF-β/SMAD (mothers against decapentaplegic)-dependent transcription. Transcriptionally inactive TR mutants that do not bind coactivators retained most of the capacity of suppressing transactivation by TGF-β/SMAD, whereas selective mutations in the DNA binding domain abolished this action. TGF-β is a major profibrogenic cytokine, and through this transcriptional mechanism, the hormone-bound TRs act as an endogenous barrier to moderate liver and skin fibrosis. These antagonistic actions on TGF-β/SMAD transcription suggest that TR ligands might be used to block the progression of fibrotic diseases. The natural hormone cannot be used clinically because of severe adverse effects, but novel synthetic ligands with fewer effects might be potentially developed and used. (See pp. E3451–E3460.)

Mass spectrometry-based absolute quantification reveals rhythmic variation of mouse circadian clock proteins

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A method for absolute quantification of proteins for targeted proteomics is developed. It introduces a simple and high-throughput synthesis of internal standards for peptide quantification and thereby facilitates both multiplexed and sensitive absolute quantification of proteins. Application of this method to the systems-level dynamic analysis of core circadian clock proteins and detection of internal body time using quantified values of circadian clock proteins is shown. The results demonstrate the validity of the developed method in which quantified values from wild-type mice can predict the endogenous state of the circadian clock. (See pp. E3461–E3467.)