PNAS Plus Significance Statements

Electrochemical trapping of metastable Mn^{3+} ions for activation of MnO_2 oxygen evolution catalysts

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Manganese oxide films are desirable oxygen evolution reaction (OER) catalysts due to their stability in acidic solutions and viability as earth-abundant materials. Enhanced catalytic activity of MnO_2 incorporated with Mn^{3+} provides an imperative for understanding the structural and electronic effects giving rise to the superior OER catalysis. We show that (*i*) Mn^{3+} is stabilized kinetically in tetrahedral sites and (*ii*) its presence strains the oxide lattice, leading to a favorable disposition of oxide-based vs. metal-based energy levels that favors enhanced OER activity. The results herein offer a design concept of exploiting ion-induced lattice strain for creating superior metal oxide OER catalysts. (See pp. E5261–E5268.)

Stabilized single-injection inactivated polio vaccine elicits a strong neutralizing immune response

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Inactivated polio vaccine (IPV) must be administered two to three times, with a 1–2 month gap between administrations, for patients to be protected. However, in the developing world healthcare workers often have difficulty reaching their patients multiple times to administer booster shots. We formulated IPV into microspheres that need to be injected only once and will be released in pulses with the desired timing without needing additional visits by a healthcare worker. To achieve this, we stabilized IPV using biocompatible excipients that allow it to remain in its active conformation inside the particles for months, and showed that they elicited a strong neutralizing immune response in rats, similar to that elicited by two separate injections of the traditional vaccine. (See pp. E5269–E5278.)

Coordination of the leucine-sensing Rag GTPase cycle by leucyl-tRNA synthetase in the mTORC1 signaling pathway

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LRS, an enzyme involved in protein synthesis, and Sestrin2, a stress-induced metabolic protein, are suggested to function as leucine sensors for the mTORC1 pathway, a central regulator of cell metabolism, growth, proliferation, and survival. The Rag GTPase cycle regulates mTORC1; however, regulators of the Rag GTPase cycle and their coordination remain unknown. We show the dynamics of the RagD–RagB GTPase cycle during leucine signaling and describe contrasting yet complementary roles for LRS and Sestrin2 in the Rag GTPase–mTORC1 pathway, functioning as "ON" and "OFF" switches, respectively. Our results extend the current view of amino acid sensing by mTORC1 and will be invaluable for the development of novel approaches to combat mTORC1-related human diseases such as cancer. (See pp. E5279–E5288.)

cAMP-inducible coactivator CRTC3 attenuates brown adipose tissue thermogenesis

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Physiologic systems often maintain homeostasis through negative-feedback loops. Unlike most regulatory targets for the sympathetic nervous system, interscapular brown adipose tissue (BAT) lacks parasympathetic inputs that might otherwise counterbalance the stimulatory effects of catecholamines. We found that the cAMP response element-binding protein (CREB) coactivator cAMP-regulated transcriptional coactivator 3 (CRTC3) reduces BAT function by downregulating sympathetic nerve activity and vascularization. Mice with a knockout of CRTC3 in BAT have reduced adiposity and are more cold tolerant. CRTC3 inhibits BAT activity by disrupting the expression of neurotrophins and proangiogenic factors that otherwise promote sympathetic innervation and vascularization of BAT. These studies highlight an important feedback mechanism that maintains energy homeostasis via its effects in brown fat. (See pp. E5289–E5297.)

Xenoprotein engineering via synthetic libraries

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Combinatorial protein libraries—prepared via molecular biology-based approaches—are invaluable tools for protein engineering. The inclusion of noncanonical

amino acids in such libraries is of considerable interest. However, at present no approach competes with chemical synthesis in terms of the variety and number of noncanonical amino acids that can be simultaneously incorporated into a protein molecule. Here, we describe selection from synthetic libraries as a strategy for protein engineering. The approach enables identification of small (~30 aa), functional protein variants comprising a virtually unlimited variety of noncanonical amino acids. Increasing the throughput of synthetic library screening, which was achieved through this effort, is anticipated to improve the utility of synthetic libraries for identifying polypeptide-based ligands with de novo function. (See pp. E5298–E5306.)

Systematic prediction of genes functionally linked to CRISPR-Cas systems by gene neighborhood analysis

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The CRISPR-Cas systems that mediate adaptive immunity in bacteria and archaea encompass a small set of core *cas* genes that are essential in diverse systems belonging to the same subtype, type, or class. However, a much greater number of genes only sporadically co-occur with CRISPR-Cas, and for most of these, involvement in CRISPR-Cas functions has not been demonstrated. We developed a computational strategy that provides for systematic identification of CRISPR-linked proteins and prediction of their functional association with CRISPR-Cas systems, and employed it to identify 79 previously undetected, putative CRISPR-accessory proteins. A large fraction of these proteins are predicted to be membrane-associated, revealing a potentially unknown side of CRISPR biology. (See pp. E5307–E5316.)

Arginine methylation is required for canonical Wnt signaling and endolysosomal trafficking

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Endocytosis plays a fundamental role in Wnt signaling, leading to the sequestration of cytosolic GSK3 kinase inside multivesicular endosomes. Here we show an unexpected role for the enzyme protein arginine methyltransferase 1 (PRMT1) during Wnt signaling. In the case of the soluble tumor suppressor transcription factor Smad4, modification of a particular arginine was required before this protein could be phosphorylated by GSK3. Wnt3a addition caused rapid endocytosis that strikingly expanded the cellular liquid-phase compartment within 5–20 min. Wnt-induced vesicles contained arginine-methylated proteins, GSK3 and PRMT1 itself, within membrane-bound organelles. The translocation of these proteins into multivesicular endosomes via microautophagy was essential for canonical Wnt signaling. (See pp. E5317–E5325.)

Oocyte stage-specific effects of MTOR determine granulosa cell fate and oocyte quality in mice

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MTOR (mechanistic target of rapamycin), an integrator of pathways important for cellular metabolism, proliferation, and differentiation, is expressed at all stages of oocyte development. Primordial oocytes constitute a nonproliferating, nongrowing reserve of potential eggs maintained for the entire reproductive lifespan of mammalian females. Using conditional knockouts, we determined the role of MTOR in both primordial and growing oocytes. MTOR-dependent pathways in primordial oocytes are not needed to sustain the viability of the primordial oocyte pool or their recruitment into the cohort of growing oocytes but are essential later for maintenance of oocyte genomic integrity, sustaining ovarian follicular development, and fertility. In growing oocytes, MTOR-dependent pathways are required for processes that promote completion of meiosis and enable embryonic development. (See pp. E5326–E5333.)

Human plasma and serum extracellular small RNA reference profiles and their clinical utility

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Nucleic acids mediate storage and expression of genetic information. Extracellular DNA (exDNA) and exRNA are traces of nucleic acids released from cells into the extracellular environment. Their use as disease biomarkers has been limited by technical challenges in their isolation caused by abundant RNA- and DNA-degrading enzymes in biofluids. Using isolation protocols developed especially for biofluids, we generated plasma and serum exRNA reference profiles from 13 healthy volunteers over time and determined the effect of critical clinical parameters such as gender and fasting. Surprisingly, we encountered one participant with dramatically increased endocrine-origin exRNA contributions stable over 1 year and detectable in all of his samples, thereby demonstrating the robustness of this approach and the clinical potential of circulating RNAs as biomarkers. (See pp. E5334–E5343.)

Encephalitis is mediated by ROP18 of *Toxoplasma gondii*, a severe pathogen in AIDS patients

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As a neurotropic parasite, *Toxoplasma* predominantly interacts with neurons throughout CNS infection and causes severe damage to the brain. In patients with severe immune dysfunction, dormant encysted bradyzoites can reactivate into fast-replicating tachyzoites and may cause damage to the brain. We report that an endoplasmic reticulum (ER)-associated protein, RTN1-C, is a substrate of ROP18 kinase. ROP18 phosphorylation of RTN1-C triggers ER stress-mediated apoptosis. Phosphorylated RTN1-C enhances GRP78 acetylation via attenuating the activity of histone deacetylases and is associated with neural apoptosis. These results link *Toxoplasma*'s effector ROP18 to RTN1-C, the CNS predominantly expressed protein, as well as ER stress-induced apoptosis via GRP78 acetylation, which may provide new insight into the preferential invasion of the central nervous system by *Toxoplasma*. (See pp. E5344–E5352.)

T7 phage factor required for managing RpoS in *Escherichia coli*

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Viruses that infect bacteria (phages) represent the most abundant living entities on the planet, and many aspects of our fundamental knowledge of phage–bacteria relationships have been derived in the context of exponentially growing bacteria. In the case of the prototypical *Escherichia coli* phage T7, specific inhibition of the housekeeping form of the RNA polymerase ($E\sigma^{70}$) by a T7 protein, called Gp2, is essential for the development of viral progeny. We now reveal that T7 uses a second specific inhibitor that selectively inhibits the stationary phase RNA polymerase ($E\sigma^{S}$), which enables T7 to develop well in exponentially growing and stationary phase bacteria. The results have broad implications for our understanding of phage–bacteria relationships and the therapeutic application of phages. (See pp. E5353–E5362.)

Activity-dependent aberrations in gene expression and alternative splicing in a mouse model of Rett syndrome

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Rett syndrome (RTT) is a severe neurological disease affecting girls in their early childhood. The underlying cause of most RTT cases is mutations in the gene Methyl-CpG-Binding Protein 2 (*MECP2*). How the loss of MeCP2 function in the brain due to these mutations causes such severe neurological symptoms remains a mystery. Here, we show widespread aberrations in gene expression and anomalous patterns of alternative splicing, specifically when neurons of RTT mice are stimulated. Furthermore, these aberrations occur in conjunction with higher seizure susceptibility in response to neuronal stimulation in these RTT mice. Our findings suggest that MeCP2 is required for adjusting the robust changes in gene transcription and for proper regulation of alternative splicing during neuronal stimulation. (See pp. E5363–E5372.)

Postsynaptic δ1 glutamate receptor assembles and maintains hippocampal synapses via Cbln2 and neurexin

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The delta glutamate receptors (GluD1 and GluD2) shared high homology with ionotropic glutamate receptors but, surprisingly, are not gated by glutamate, or any other known ligand. GluD2 is only expressed in cerebellar Purkinje cells, where it forms a scaffolding complex with Cbln1 and presynaptic neurexin 1β (+S4). Might other synapses in the brain use a similar mechanism? We have found that GluD1, a long-neglected receptor subtype, powerfully and specifically regulates synapses in the hippocampus. We show that Cbln2 interacts with GluD1 and presynaptic neurexin 1β (+S4). This tripartite complex plays critical roles in both synaptogenesis and maintenance. Finally, we provide evidence that our results likely apply to synapses throughout the forebrain where GluD1 is widely expressed. (See pp. E5373–E5381.)

Deletion of *LRRTM1 and LRRTM2* in adult mice impairs basal AMPA receptor transmission and LTP in hippocampal CA1 pyramidal neurons

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Brain function requires high-fidelity transmission of information between individual brain cells at synapses, physical contacts that contain a specialized machinery for passing and receiving signals. Synaptic cell adhesion proteins form physical bridges between neurons and are critical for fine-tuning synaptic properties. Because of their roles in synaptic transmission, mutations in these proteins contribute to a wide range of neuropsychiatric diseases. Here, we study the role of two synaptic cell adhesion proteins, LRRTM1 and LRRTM2, in signaling at excitatory synapses utilizing a conditional knockout mouse line. Genetically deleting these proteins in mature neurons dramatically impairs basal synaptic transmission and disrupts long-term potentiation, a prominent form of synaptic plasticity that is critical for learning and memory. (See pp. E5382–E5389.)

Mistimed food intake and sleep alters 24-hour time-of-day patterns of the human plasma proteome

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Circadian misalignment (i.e., behavioral processes such as food intake or sleep occurring at inappropriate endogenous circadian times) commonly occurs during shift work and is associated with health problems. Identifying mechanisms underlying health problems associated with circadian misalignment will help develop precision medicine countermeasures. Thus, we investigated the impact of circadian misalignment on the human plasma proteome using a simulated nightshift protocol in healthy volunteers. We demonstrate that circadian and/or behavioral wake–sleep/food intake–fasting cycles regulate 24-h time-of-day patterns of the human plasma proteome. Further, we show that proteins altered during circadian misalignment are associated with biological pathways involved in immune function, metabolism, and cancer and with altered glucose and energy metabolism, identifying potential mechanisms contributing to metabolic dysregulation. (See pp. E5390–E5399.)

Epigenetic switch from repressive to permissive chromatin in response to cold stress

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Phenotypic adaptations of plants in response to changes in climate are well known to be mediated by molecular mechanisms, including activation or suppression of transcription factors that control target gene expression. However, the chromatin changes that are essential for the binding of transcription factors are much less understood. Gene derepression at the chromatin level is considered to be the starting point for gene transcription. We report a mechanism of gene derepression through which HOS15 promotes the degradation of histone deacetylase HD2C in a cold-dependent manner that correlates with increased levels of acetylated histones on *COR* gene chromatin. Moreover, HOS15 directly promotes *COR* gene transcription by association of CBF

transcription factors with the "open" state of the target *COR* chromatin. (See pp. E5400–E5409.)

Characterization of gossypol biosynthetic pathway

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Cotton is an important crop, and terpenoids form the largest group of natural products. Gossypol and related sesquiterpene aldehydes in cotton function as phytoalexins against pathogens and pests but pose human health concerns, as cotton oil is still widely used as vegetable oil. We report the isolation and identification of four enzymes and the recharacterization of one previously reported P450. We are now close to the completion of the gossypol pathway, an important progress in agricultural and plant sciences, and the data are beneficial to improving food safety. Among the six compounds (intermediates) isolated following gene silencing, one affected plant disease resistance significantly. Thus, these "hidden natural products" harbor interesting biological activities worthy of exploration. (See pp. E5410–E5418.)

Short-chain dehydrogenase/reductase governs steroidal specialized metabolites structural diversity and toxicity in the genus *Solanum*

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Plants synthesize a vast repertoire of steroidal specialized metabolites. These include the well-known class of antinutritional steroidal glycoalkaloids (SGAs), which act as defensive chemicals in the Solanaceae, and the pharmacologically important and widespread steroidal saponins. Here, we uncover an elusive enzymatic step that acts on unsaturated steroidal metabolites. We find that GLYCOALKALOID METABOLISM25 (GAME25) acts at a key branch point in the biosynthesis pathways of steroidal specialized metabolites. The activity of GAME25 not only affects the enormous diversity of SGAs and steroidal saponins, which are produced in hundreds of plant species, but also modulates the molecules' toxic effects. This work helps explain the extensive structural diversity in specialized metabolism through a relatively simple chemical modification in a single metabolite backbone. (See pp. E5419–E5428.)