PNAS Plus Significance Statements

Anti-inflammatory ω-3 endocannabinoid epoxides

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The health benefits of ω-3 fatty acids are mediated, in part, through metabolic conversion to bioactive epoxides. Here we detail the discovery and initial characterization of naturally occurring ω-3–derived endocannabinoid epoxides that are formed via enzymatic oxidation of ω-3 endocannabinoids by cytochrome P450s. These dual functional ω-3 endocannabinoid epoxides are anti-inflammatory and vasodilatory and reciprocally modulate platelet aggregation. By virtue of their physiological properties, they are expected to play important roles in neuroinflammation and in cerebrovascular diseases such as stroke. (See pp. E6034–E6043.)

Structural insights into lipoprotein N-acylation by *Escherichia coli* apolipoprotein N-acyltransferase

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Lipoprotein biosynthesis is crucial for Gram-negative bacterial viability and involves the activities of three essential integral membrane proteins embedded in the inner membrane (Lgt, LspA, and Lnt). These enzymes function sequentially to produce mature triacylated lipoproteins, many of which are then transported to the outer membrane. Lnt is responsible for catalyzing the addition of palmitate to the N terminus of diacylated apolipoproteins. Despite a number of studies that have biochemically characterized *Escherichia coli* Lnt, the structural basis for substrate engagement and catalysis remains unclear. Here we present the crystal structures of wild-type *E. coli* Lnt and a C387S active-site mutant. These structures provide insights into the molecular mechanisms of apolipoprotein N-acylation by Lnt and shed further light on the mechanism of lipoprotein biosynthesis by these essential bacterial enzymes. (See pp. E6044–E6053.)

G9a coordinates with the RPA complex to promote DNA damage repair and cell survival

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The G9a histone methyltransferase primarily regulates the expression of genes associated with cancer development in cancer cells, but it has also been implicated in mediating the DNA damage response. Here, we confirmed a role for G9a in DNA damage repair following double-strand breaks. G9a is recruited to chromatin as a result of casein kinase 2-mediated phosphorylation, where it directly interacts with replication protein A (RPA). G9a binding to RPA modulates RPA and Rad51 foci formation and permits efficient homologous recombination. This molecular mechanism renders cancer cells more resistant to radiation and chemotherapeutics. Our improved understanding of the molecular function of G9a may help with the future design of G9a inhibitors and G9a-based DNA damage agents as cancer therapeutics. (See pp. E6054–E6063.)

Size and mobility of lipid domains tuned by geometrical constraints

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Diffusion of lipids and proteins organized in finite-sized domains plays a pivotal role in biological membranes enhancing biomolecular signaling efficiency. The impact of membrane geometry and spatial boundaries on the lateral mobility of domains in membranes remains, however, still unclear. By using pore-spanning membranes, we were able to control the length scale and dynamics of lipid domains by an underlying porous mesh serving both as a confinement and as a source of additional friction that slows down the mobility of domains by several orders of magnitude. We could show that increased hydrodynamic drag acting on liquid-ordered domains due to the confined geometry is responsible for the reduction of diffusion constants. (See pp. E6064–E6071.)

Functional characterization of human pluripotent stem cell-derived arterial endothelial cells

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Generating fully functional arterial endothelial cells is a critical problem for vascular development and disease research. Currently, the arterial endothelial cells derived from human pluripotent stem cells lack the range of arterial-specific functions in vitro and the protective function for ischemic tissues in vivo. Here, we combine single-cell RNA sequencing and CRISPR-Cas9 technology to identify pathways for regulating arterial endothelial cell differentiation. We then manipulate

these pathways and generate arterial endothelial cells that demonstrate unprecedented arterial-specific functions as well as improve survival of myocardial infarction. These findings facilitate the understanding of vascular development and disease and provide a source of cells that have broad applications for vascular disease modeling and regenerative medicine. (See pp. E6072–E6078.)

TRPM7 senses oxidative stress to release Zn^{2+} from unique intracellular vesicles

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TRPM7 (transient receptor potential cation channel subfamily M member 7) is required for normal organ development but also mediates anoxic neuronal death. TRPM7 contains a channel that conducts cations into the cytosol and C-terminal kinase that can phosphorylate multiple substrates. The kinase is cleaved to regulate gene expression and apoptosis. The link between TRPM7's channel and its organismal function remains the least understood aspect of TRPM7. Here, we identify intracellular Zn^{2+} storage vesicles that contain the majority of TRPM7 protein. TRPM7 senses reactive oxygen species (ROS) to release Zn^{2+} from these vesicles. Just as the endoplasmic reticulum sequesters and releases Ca^{2+}, we propose that these vesicles fulfill a similar function for Zn^{2+} and that TRPM7 coordinates fluctuations in cellular [Zn^{2+}] and ROS during development and injury. (See pp. E6079–E6088.)

Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines

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The strong focus on species extinctions, a critical aspect of the contemporary pulse of biological extinction, leads to a common misimpression that Earth's biota is not immediately threatened, just slowly entering an episode of major biodiversity loss. This view overlooks the current trends of population declines and extinctions. Using a sample of 27,600 terrestrial vertebrate species, and a more detailed analysis of 177 mammal species, we show the extremely high degree of population decay in vertebrates, even in common "species of low concern." Dwindling population sizes and range shrinkages amount to a massive anthropogenic erosion of biodiversity and of the ecosystem services essential to civilization. This "biological annihilation" underlines the seriousness for humanity of Earth's ongoing sixth mass extinction event. (See pp. E6089–E6096.)

Continuous immunotypes describe human immune variation and predict diverse responses

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The human immune system consists of many different white blood cells that coordinate their actions to fight infections. The balance between these cell populations is determined by direct interactions and soluble factors such as cytokines, which serve as messengers between cells. Understanding how the interactions between cell populations influence the function of the immune system as a whole will allow us to better distinguish patients most at risk for specific infections or immune-mediated diseases and inform vaccination strategies. Here, we determine key collective interactions between white blood cells present in blood samples taken from healthy individuals. This perspective allows us to predict functional responses and describe previously unappreciated differences between age groups and in individuals carrying cytomegalovirus. (See pp. E6097–E6106.)

Influenza infection triggers disease in a genetic model of experimental autoimmune encephalomyelitis

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Peripheral infections exacerbate symptoms of many neurological diseases, including the most common autoimmune demyelinating disease of the central nervous system (CNS), multiple sclerosis (MS). We demonstrate that influenza viral infection of autoimmune-prone mice triggers clinical and histological disease. We further show that influenza infection alters the transcriptome of the central nervous system and facilitates immune cell trafficking to the brain. Finally, we identified a specific chemokine that is upregulated in the CNS during infection that is also increased in the cerebrospinal fluid of MS patients during relapse. These observations improve our understanding of how peripheral infection may act to exacerbate neurological diseases such as multiple sclerosis. (See pp. E6107–E6116.)

Suboptimal T-cell receptor signaling compromises protein translation, ribosome biogenesis, and proliferation of mouse CD8 T cells

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Optimal antigenic stimulation through T-cell receptors is required by T lymphocytes to exert full expansion, effector functions, and memory cell differentiation. Suboptimal TCR stimulation influences both transcription of genes and synthesis of subsets of proteins in a nonconcordant manner. Detailed polysome profiling revealed that weakly activated cells prioritized mRNA translation so that specific transcripts were translationally sequestered. Strikingly, ribosome biogenesis was compromised at both transcriptional and translational levels after weak stimulation, which still allowed the cells to undergo initial cell division, but proliferation was not sustained. Our work has demonstrated that T cells respond to environmental signals and use specific components of the translation machinery to regulate the translation of activation-dependent mRNAs. (See pp. E6117–E6126.)

Loss-of-function mutation in *Mirta22/Emc10* rescues specific schizophrenia-related phenotypes in a mouse model of the 22q11.2 deletion

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Despite substantial progress in the field of schizophrenia (SCZ) genetics, the heterogeneity of genetic etiology and neural complexity has rendered the task of developing improved treatments inauspicious. Thus, there is need to identify convergent neural substrates and underlying molecular mechanisms that can serve the prevention or reversal of disease progression. Our extensive characterization of an animal model of the 22q11.2 deletion, one of the strongest genetic risk factors for SCZ, when combined with a loss-of-function (LoF) mutation for a microRNA-dependent upregulated target, offers a proof of principle for such approaches. Hence, identification of variants that confer protection against disease by disabling protein function via LoF effects holds great promise for devising

therapeutic schemes to restore or prevent disease symptoms. (See pp. E6127–E6136.)

Combined epigenetic and differentiation-based treatment inhibits neuroblastoma tumor growth and links HIF2α to tumor suppression

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High-risk neuroblastoma remains a therapeutic challenge, and adjuvant retinoic acid (RA) treatment shows poor efficacy. We demonstrate that combined treatment with 5-Aza-deoxycytidine (AZA) and RA impedes neuroblastoma growth and induces a transcriptional response characterized by high levels of the HIF2α transcription factor. This approach targets high-risk neuroblastoma that responds poorly to RA. In addition, genome-wide analysis of treated tumors and patient data links HIF2α to tumor suppression, which is supported by a HIF2α-specific small molecule inhibitor-mediated block of the tumor response to AZA+RA treatment. (See pp. E6137–E6146.)

Multiplexed RNAi therapy against brain tumor-initiating cells via lipopolymeric nanoparticle infusion delays glioblastoma progression

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Glioblastoma is a deadly brain tumor with no cure. Brain tumor-initiating cells (BTICs) have been recognized as the key driver behind the unstoppable malignant growth, therapy resistance, and recurrence. BTICs are exceptionally difficult to target because of heterogeneous genetic and epigenetic aberrations that are challenging to reverse therapeutically using conventional pharmaceuticals or biologics. Here we report a lipopolymeric nanoparticle (LPNP) formulation that demonstrates a surprisingly high affinity for BTICs and the capacity to encapsulate multiple siRNAs for potent and targeted anti-BTIC therapy. We show that direct infusion of LPNP siRNAs to brain tumors effectively impedes tumor growth in mouse and provides encouraging survival benefits. This multiplexed nanomedicine platform carries strong potential for personalized anti-BTIC therapies. (See pp. E6147–E6156.)

Stem cell-released oncolytic herpes simplex virus has therapeutic efficacy in brain metastatic melanomas

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This study provides an insight into stem cell-based oncolytic virus therapies for advanced melanoma tumors that have metastasized into the brain by developing clinically relevant mouse tumor models and testing the fate and efficacy of oncolytic herpes simplex virus-armed mesenchymal stem cells in such models. This study therefore overcomes the hurdles of systemic delivery of oncolytic viruses and provides a clinically applicable therapeutic platform to target melanoma brain metastasis. (See pp. E6157–E6165.)

Intestinal virome changes precede autoimmunity in type I diabetes-susceptible children

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Type 1 diabetes (T1D) is a major autoimmune disease with increasing incidence in recent years. In this study, we found that the intestinal viromes of cases were less diverse than those of controls. We identified eukaryotic viruses and bacteriophage contigs that are associated with the presence or absence of autoimmunity. These viruses provide targets for future mechanistic studies to differentiate causal and incidental associations between the virome and protection against the development of T1D. (See pp. E6166–E6175.)

Two dynamin-like proteins stabilize FtsZ rings during *Streptomyces* sporulation

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Bacterial dynamins were discovered ∼10 y ago and the explosion in genome sequencing has shown that they radiate throughout the bacteria, being present in >1,000 species. In eukaryotes, dynamins play critical roles in the detachment of endocytic vesicles from the plasma membrane, the division of chloroplasts and peroxisomes, and both the fusion and fission of mitochondria. However, in evolutionary terms, dynamins are of bacterial origin, and yet the biological functions of bacterial dynamins remain poorly understood. Here we demonstrate a critical role for dynamins in bacterial cytokinesis, reminiscent of the essential role of eukaryotic dynamins in the division of chloroplasts and mitochondria. (See pp. E6176–E6183.)

Bifunctionality of a biofilm matrix protein controlled by redox state

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The biofilm matrix is a critical target in the hunt for novel strategies to destabilize or stabilize biofilms. Knowledge of the processes controlling matrix assembly is therefore an essential prerequisite to exploitation. Here, we highlight that the complexity of the biofilm matrix is even higher than anticipated, with one matrix component making two independent functional contributions to the community. The influence the protein exerts is dependent on the local environmental properties, providing another dimension to consider during analysis. These findings add to the evidence that bacteria can evolve multifunctional uses for the extracellular matrix components. (See pp. E6184–E6191.)

Attention model of binocular rivalry

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Binocular rivalry provides a unique opportunity to characterize intrinsic neural dynamics of cortical processing. A computational model was developed as a parsimonious explanation of the empirical phenomena of rivalry for which there was no previous explanation. The key idea in the model is that rivalry relies on interactions between sensory processing and attentional modulation with distinct dynamics and selectivity. Bifurcation theory was used to identify the parameter regime in which the behavior of the model was consistent with empirical findings. The model explained a wide range of phenomena, including (*i*) that binocular rivalry requires attention, (*ii*) that different perceptual states emerge when the two images are swapped between the eyes, and (*iii*) how dominance duration changes as a function of stimulus input strength. (See pp. E6192–E6201.)

Acetylcholine-producing NK cells attenuate CNS inflammation via modulation of infiltrating monocytes/macrophages

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Acetylcholine (ACh) produced by neurons performs an array of functions that control cardiac, gastrointestinal, and other biosystems. Here we discovered that lymphocytic natural killer (NK) cells bear machinery that produces ACh. The activity of ACh-producing NK cells up-regulates during the disease flare of multiple sclerosis (MS) and may, therefore, reflect the pathologic state. In the mouse model of MS, experimental autoimmune encephalomyelitis, these ACh-producing NK cells can reduce the intensity of inflammation and autoimmune responses in the brain and spinal cord. Therefore, the nonneural cholinergic system, as reflected by ACh-producing NK cells, appeared to counteract aberrant immune responses and lessen brain damage. This observation offers insight into the therapeutic mechanisms of the Food and Drug Administration-approved drug daclizumab high-yield process for MS. (See pp. E6202–E6211.)

Synaptic properties of the lemniscal and paralemniscal pathways to the mouse somatosensory thalamus

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Functional mapping of the routes of information processing is essential to understand how the brain operates. The main carriers of information are excitatory (glutamatergic) neurons, which form synapses that can either reliably deliver information or modify it. Using the somatosensory system in the mouse, we show that the two parallel glutamatergic routes from periphery to cortex should not be considered equal in carrying somatosensory information. Instead, one route has synapses solely suited for fast information transfer—the Lemniscal pathway—and the other suited for mainly modifying such information—the Paralemniscal pathway. This is an example of synaptic and anatomical analyses that inform circuit function and build on a framework for feedforward and feedback processing. (See pp. E6212–E6221.)

Corticogeniculate feedback sharpens the temporal precision and spatial resolution of visual signals in the ferret

J. Michael Hasse and Farran Briggs

The functional role of corticothalamic circuits, connecting the cortex to the thalamus in the feedback direction, has remained a fundamental mystery in neuroscience. In spite of the fact that corticothalamic inputs are numerous, their influence on thalamic activity is modest. We used an innovative combination of virus-mediated gene delivery and optogenetics to probe the function of corticothalamic feedback in vision. We found that corticothalamic feedback does not alter the visual response properties of thalamic neurons, but instead controls the timing and fidelity of their responses to incoming visual inputs. Thus, our results provide an answer to a long-standing question in neuroscience: A key function of corticothalamic feedback is to control the timing and precision of thalamic responses. (See pp. E6222–E6230.)

Prediction of intracellular exposure bridges the gap between target- and cell-based drug discovery

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Exposure at the site of action has been identified as one of the three most important factors for success in drug discovery and the design of chemical probes. Modern drug discovery programs have, to a great extent, shifted to intracellular targets, but methods to determine intracellular drug concentrations have been lacking. Here, we use a methodology for predicting intracellular exposure of small-molecule drugs to understand their potency toward intracellular targets. We show that our approach is generally applicable to multiple targets, cell types, and therapeutic areas. We expect that routine measurements of intracellular drug concentration will contribute to reducing the high attrition observed in drug discovery and the design of both better chemical probes and medicines. (See pp. E6231–E6239.)

Analyses of PDE-regulated phosphoproteomes reveal unique and specific cAMP-signaling modules in T cells

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We have coupled mass spectrometry-based phosphoproteomic analyses with treatment using various selective PDE inhibitors to characterize the PDE-regulated phosphoproteome of CD3/CD28-stimulated Jurkat cells. Predictive algorithms were used to identify likely upstream regulatory kinases, metabolic pathways, and biological processes that can be regulated by different PDEs. Here we compare the phosphoproteomes of different functional compartments subserved by combinations of individual PDE isozymes in a T-cell model. We observed unique phosphoproteomes associated with specific combinations of PDEs. These data allow one to prioritize future experiments to understand further how these pathways are regulated by specific PDEs. The results also have substantial implications for the design

and use of selective PDE inhibitors in clinical practice. (See pp. E6240–E6249.)

Loss of mouse cardiomyocyte talin-1 and talin-2 leads to β-1 integrin reduction, costameric instability, and dilated cardiomyopathy

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Heart failure is a significant health problem with a poorly understood molecular basis. Continuous contraction–relaxation cycles of the heart and its cardiomyocytes (CMs) require strong interactions between intracellular proteins and the surrounding extracellular matrix to maintain normal heart function. We exhaustively studied the function of the cytoskeletal protein talin (Tln), and its two isoforms, Tln1 and Tln2, in CMs and the intact heart. Both Tln isoforms can independently support costamere, CM, and whole-heart function. Yet, combined deletion of CM Tln1 and Tln2 destabilized the myocardium, leading to heart failure. This study significantly advances knowledge about the basic biology of talin, particularly given its study in vivo, and advances understanding of Tln's role in maintenance of normal heart muscle function. (See pp. E6250–E6259.)

Caffeine induces gastric acid secretion via bitter taste signaling in gastric parietal cells

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This study shows that caffeine's effect on gastric acid secretion (GAS) is more complex than has been previously thought. Oral and gastric bitter taste receptors are involved in the regulation of GAS in humans. This regulatory process can be modified by the bitter-masking compound homoeriodictyol. Practical applications of the results may include treatment of gastroesophageal reflux disease or peptic ulcer by manipulating gastric pH by means of bitter tastants and inhibitors. (See pp. E6260–E6269.)