PNAS Plus Significance Statements

DNA and RNA topoisomerase activities of Top3β are promoted by mediator protein Tudor domain-containing protein 3

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Tudor domain-containing protein 3 (TDRD3), a multidomain scaffold protein functions as an epigenetic reader on nuclear chromatin and binds with fragile X mental retardation protein on mRNA. It forms a conserved complex with topoisomerase 3β (Top3β), a type IA topoisomerase, and participates in both transcription and translation. The mechanism of how TDRD3 acts as Top3β's partner in regulating these cellular processes is unknown. Here, we demonstrated that TDRD3 is able to stimulate Top3β's DNA and RNA topoisomerase catalytic activities, through binding and stabilizing single-stranded regions in DNA and RNA substrates. Because these regions are the preferred site for Top3β, TDRD3 therefore acts as a regulator to provide access of the enzyme to these nucleic acid substrates and to act upon them. (See pp. E5544–E5551.)

Coordinated autoinhibition of F-BAR domain membrane binding and WASp activation by Nervous Wreck

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Membrane-deforming proteins cooperate with the cytoskeleton to sculpt lipid bilayers into complex and dynamic geometries, but we still do not understand how their activities are temporally and spatially regulated in cells. Here we show that the neuronal membrane remodeling protein Nervous Wreck (Nwk) is autoinhibited by intramolecular interactions between its membrane binding F-BAR domain and its C-terminal SRC homology 3 (SH3) domains. These autoinhibitory interactions control Fes/Cip4 homology-Bin/Amphiphysin/Rvs167 (F-BAR)–mediated membrane remodeling and also, unexpectedly, inhibit SH3-mediated actin cytoskeleton assembly. Uncoupling these dual autoregulatory mechanisms in the fruit fly leads to excess neuronal synapse growth. Thus, coordinated autoregulation couples membrane remodeling and SH3 domain activities, and is critical for proper control of neuronal shape and size. (See pp. E5552–E5561.)

Selenoprotein H is an essential regulator of redox homeostasis that cooperates with p53 in development and tumorigenesis

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Dietary selenium and selenoproteins play important roles in regulating redox processes that impact human health. The human genome includes 25 genes for selenoproteins, which have diverse roles in redox homeostasis, thyroid hormone metabolism, endoplasmic reticulum quality control, selenium transport, and other functions. Selenoprotein H (*seph*) is a recently identified nucleolar oxidoreductase with DNA-binding properties whose function is not well understood. In this work, we used a unique combination of unbiased metabolomic and transcriptomic approaches in zebrafish to discover that *seph* is an essential regulator of redox homeostasis that regulates p53. In addition, we demonstrate the *seph*-deficient adults are prone to chemically induced carcinogenesis. Our results suggest that *seph* suppresses oxidative stress and DNA damage in the nucleolus. (See pp. E5562–E5571.)

Spiny plants, mammal browsers, and the origin of African savannas

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Africa hosts contrasting communities of mammal browsers and is, thus, the ideal background for testing their effect on plant communities and evolution. In this study at the continental scale, we reveal which mammal browsers are most closely associated with spiny communities of trees. We then show a remarkable convergence between the evolutionary histories of these browsers (the bovids) and spiny plants. Over the last 16 My, plants from unrelated lineages developed spines 55 times. These convergent patterns of evolution suggest that the arrival and diversification of bovids in Africa changed the rules for persisting in woody communities. Contrary to our current understanding, our data suggest that browsers predate fire by millions of years as agents driving the origin of savannas. (See pp. E5572–E5579.)

Explosive ice age diversification of kiwi

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The role of Pleistocene ice ages in driving a recent burst of diversification is controversial. We used thousands of loci to test the timing and rates of diversification in kiwi—a flightless avian group endemic to New Zealand. Not only did we discover many kiwi taxa—we found 16 or 17 genetically distinct lineages within the currently recognized five species—but we found that most diversification dates to the seven major glacial advances that characterized the latter half of the Pleistocene ice ages and that directly fragmented New Zealand into a series of glacial refugia. Rates at which new kiwi taxa originated increased fivefold during these major cycles, thus linking rapid kiwi diversification to glacial periods. (See pp. E5580–E5587.)

Emergent rules for codon choice elucidated by editing rare arginine codons in *Escherichia coli*

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This work presents the genome-wide replacement of all rare AGR (AGA and AGG) arginine codons in the essential genes of *Escherichia coli* with synonymous CGN alternatives. Synonymous codon substitutions can lethally impact noncoding function by disrupting mRNA secondary structure and ribosomal binding site-like motifs. Here we quantitatively define the range of tolerable deviation in these metrics and use this relationship to provide critical insight into codon choice in recoded genomes. This work demonstrates that genome-wide removal of AGR is likely to be possible and provides a framework for designing genomes with radically altered genetic codes. (See pp. E5588–E5597.)

Mutations in mitochondrial enzyme GPT2 cause metabolic dysfunction and neurological disease with developmental and progressive features

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We report autosomal recessive mutations in the enzyme glutamate pyruvate transaminase 2 (GPT2) in a neurological syndrome involving intellectual disability, reduced brain growth, and progressive motor symptoms. We show that the mutations inactivate the enzyme. GPT2 catalyzes the reversible addition of an amino group from glutamate to pyruvate, yielding alanine and α-ketoglutarate. The *GPT2* gene demonstrates expression in brain postnatally, and the protein localizes to mitochondria. As in humans, *Gpt2*-null mice exhibit reduced brain growth. Furthermore, mutant mouse brains show abnormal metabolite levels, including in pathways involving amino acid metabolism, the TCA cycle, and neuroprotective mechanisms. Our study identifies GPT2 as an important mitochondrial enzyme in disease that has general relevance to developmental and potentially to neurodegenerative mechanisms. (See pp. E5598–E5607.)

Class II major histocompatibility complex mutant mice to study the germ-line bias of T-cell antigen receptors

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The evolutionary hypothesis for T-cell antigen receptor–peptide major histocompatibility complex (TCR–pMHC) interaction posits the existence of germ-line–encoded rules by which the TCR is biased toward recognition of the MHC. Understanding these rules is important for our knowledge of how to manipulate this important interaction at the center of adaptive immunity. In this study, we highlight the flexibility of thymic selection as well as the existence of these rules by generating knockin mutant MHC mice and extensively studying the TCR repertoires of T cells selected on the mutant MHC molecules. Identifying novel TCR subfamilies that are most evolutionarily conserved to recognize specific areas of the MHC is the first step in advancing our knowledge of this central interaction. (See pp. E5608–E5617.)

Pericyte–fibroblast transition promotes tumor growth and metastasis

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We show that vascular pericytes significantly contribute to cancer invasion and metastasis by the mechanism of the pericyte–fibroblast transition (PFT). This study proposes this concept and indicates the vascular pericyte's role. Vascular pericytes were considered to remodel tumor vessels toward a mature phenotype. However, once dissociated from tumor vessels their functions within the tumor tissue are not known. In the present study, we show that pericytes, once detached from tumor microvasculatures, underwent differentiation to become stromal fibroblasts, which are known to contribute to tumor invasion and metastasis. Our results show that vascular pericytes are the important source of stromal fibroblasts and targeting PFT may offer a new treatment option in cancer metastasis. (See pp. E5618–E5627.)

Pathological Ace2-to-Ace enzyme switch in the stressed heart is transcriptionally controlled by the endothelial Brg1–FoxM1 complex

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Angiotensin-converting enzymes Ace and Ace2 counteract each other to control the metabolism of angiotensin peptides and heart function. When the heart is pathologically stressed, Ace is up-regulated whereas Ace2 is down-regulated, leading to a pathological Ace2-to-Ace switch and increased production of angiotensin II, which promotes hypertrophy and fibrosis. The mechanism of Ace2-to-Ace switch is unknown. In this study, we discovered that the Ace/Ace2 switch occurs at the transcription level and defined a chromatin-based endothelial mechanism that triggers Ace/Ace2 transcription switch and heart failure. Human tissue studies suggest that this mechanism is evolutionarily conserved. Our studies reveal a pharmacological method to simultaneously inhibit pathogenic Ace and activate cardioprotective Ace2. This finding provides new insights and methods for heart failure therapy. (See pp. E5628–E5635.)

$CD4^+$ T-cell–independent mechanisms suppress reactivation of latent tuberculosis in a macaque model of HIV coinfection

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According to the World Health Organization, one in three humans is latently infected with *Mycobacterium tuberculosis* and 10% of these individuals risk developing active, clinical tuberculosis (TB) over their lifetimes. Coinfection with human immunodeficiency virus increases this risk substantially, with depletion of $CD4^+$ T cells believed to drive disease progression. Although a minority of coinfected individuals can control the infection, the mechanisms underlying this phenomenon remain unknown. Modeling coinfection using macaques, we discovered that one-third of the animals maintained latency despite complete ablation of lung $CD4^+$ T cells. We report that protective immune responses mediated by $CD8^+$ T cells and B cells correlate with TB control. These findings have important implications in development of both prophylactic and therapeutic measures against TB and acquired immunodeficiency syndrome. (See pp. E5636–E5644.)

GluA1 signal peptide determines the spatial assembly of heteromeric AMPA receptors

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In the brain, AMPA-type glutamate receptors, especially heteromeric GluA1/A2s, are the major postsynaptic receptors mediating fast excitatory neurotransmission. Recently, the crystal structure of GluA2 homomeric AMPA-type glutamate receptors (AMPARs) revealed some interesting features, such as the four subunits in each AMPAR are of two different conformations. However, what the heteromeric GluA1/A2 receptors look like is unknown. In this study, we used a biochemical technique called cysteine cross-linking assay to analyze the spatial architecture of GluA1/A2s. We determined that GluA1/GluA2s have preferred spatial assembly. Surprisingly, this spatial assembly pattern is dictated by the excisable signal peptides, but not the intrinsic sequences of the subunit proteins. (See pp. E5645–E5654.)

TRiC subunits enhance BDNF axonal transport and rescue striatal atrophy in Huntington's disease

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Degeneration of the corticostriatal circuit is a key neuropathological and clinical feature of Huntington's disease (HD). To define disease mechanisms and explore treatments, we recreated the corticostriatal circuit in microfluidic chambers using neurons from the BACHD mouse model of HD and WT controls. We showed that expression of mutant huntingtin (mHTT) induced defects in brain-derived neurotrophic factor (BDNF) transport in BACHD cortical axons that resulted in atrophy of striatal target neurons. Introducing subunits of the cytosolic chaperonin T-complex 1 (TCP-1) ring complex (TRiC) into BACHD cortical neurons reduced mHTT, rescued defects in BDNF transport, and normalized the size of striatal neurons. These findings encourage studies to explore a role for TRiC reagents as possible treatments for HD. (See pp. E5655–E5664.)

Infiltrating monocytes promote brain inflammation and exacerbate neuronal damage after status epilepticus

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Status epilepticus is a frequent neurological emergency. These unabated seizures reduce quality of life, promote the development of epilepsy, and can cause death. Activation of microglia, the brain's resident immune cells, is an invariable feature of seizure activity. However, the involvement of blood-borne immune cells in the brain's inflammatory reaction after seizures remains unresolved. Here we identify a blood cell not normally encountered in the healthy brain, called a monocyte, which invades brain tissue after seizures and contributes to inflammation. Blocking brain entry of the blood monocytes was beneficial, reducing neuronal damage and accelerating weight regain. Treatment strategies aimed at inhibiting peripheral immune cells from entering the brain after seizures could be beneficial. (See pp. E5665–E5674.)

Accelerated structure-based design of chemically diverse allosteric modulators of a muscarinic G protein-coupled receptor

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Chemical diversity has recently risen as key structural feature for the discovery of novel selective drugs of G protein-coupled receptors (GPCRs). However, the traditional drug discovery technique of combinatorial chemistry coupled to high-throughput screening has become less attractive because of its immense financial impact. To address this problem, we implemented a computer-aided drug design approach, using the M_2 muscarinic acetylcholine receptor (mAChR) as a GPCR model, and performed computational enhanced sampling simulations to account for the receptor flexibility. Through iterative molecular docking and experimental testing, half of the 38 computationally selected National Cancer Institute compounds were validated as allosteric modulators of the M_2 mAChR. Our method successfully identified positive and negative allosteric modulators of M_2 mAChR with unprecedented chemical diversity. (See pp. E5675–E5684.)

SCAP/SREBP pathway is required for the full steroidogenic response to cyclic AMP

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Luteinizing hormone stimulates production of testosterone and other steroids largely through a surge in the second messenger cAMP and subsequent activation of protein kinase A (PKA) in target cells. Rates of steroidogenesis are also dependent on the availability of cholesterol, a steroid building block. We propose, based on our results, that cAMP/PKA coordinates the functions of multiple pathways to regulate cellular cholesterol handling and synthesis and downstream steroid output. Activation of the cholesterol-sensing SCAP–SREBP2 pathway plays an important role in cAMP/PKA coordination of steroidogenesis. These cAMP/PKA-induced pathways are likely to be major regulators of sterol biosynthesis and cholesterol recharging in steroid hormone synthetic and other tissues. Cyclic nucleotide phosphodiesterases can be targeted to promote steroidogenesis and cholesterol metabolism. (See pp. E5685–E5693.)