PNAS Plus Significance Statements

Classification of topological phonons in linear mechanical metamaterials

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A mechanical metamaterial is an engineered material that is characterized by properties that go beyond the properties of its microscopic building blocks. Specifically, in topological metamaterials, one makes use of surface or boundary modes that are stable against imperfections or environmental influences and hence constitute reliable building blocks for various applications. In our work, we provide a classification scheme of possible topological metamaterials and an extensive number of examples illustrating this scheme. Our classification can serve as an important blueprint for many future applications that target such stable boundary modes for engineering purposes. (See pp. E4767–E4775.)

Innate immunity kinase TAK1 phosphorylates Rab1 on a hotspot for posttranslational modifications by host and pathogen

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Rab GTPases regulate vesicle traffic within the cell by switching between active (GTP-bound) and inactive (GDP-bound) states. The switch II region of Rab proteins undergoes a significant conformational change to switch between states. Rab1 is hijacked during intracellular *Legionella pneumophila* infection by bacterial effector-mediated posttranslational modifications of the switch II region, a unique mechanism for regulation of Rab function. We present new evidence that Rab1 is endogenously modified within switch II by TGF-β activated kinase 1 (TAK1), a kinase crucial for responding to infection. We show phosphorylation of Rab1 is necessary for normal Rab1 function. Interestingly, phosphorylation of Rab1 is competed during *Legionella* infection, adding to evidence that *Legionella* target substrates of the innate immunity kinase TAK1. (See pp. E4776–E4783.)

Distinct cellular properties of oncogenic KIT receptor tyrosine kinase mutants enable alternative courses of cancer cell inhibition

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A common practice in modern clinics is to identify a match between a mutated oncogenic protein that functions as a "driver" of a particular cancer with a known or new cancer drug from available targeted therapies. To understand mechanisms underlying the differential clinical impact of various targeted therapies on cancers driven by the receptor tyrosine kinase KIT, we analyzed a variety of biochemical and cellular properties of the most common KIT somatic mutations identified in human cancers. Surprisingly, each of the six major KIT oncogenic mutants exhibits distinct properties and responds differently to targeted therapies. These experiments show that detailed biochemical and cellular analyses of oncogenic mutations are required to optimize precision medicine for cancer treatment. (See pp. E4784–E4793.)

Dynamic periplasmic chaperone reservoir facilitates biogenesis of outer membrane proteins

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The study of bacterial outer membrane proteins (OMPs) is critical to understanding cellular communication, metabolic transport across membranes, and pathogenesis. We used a holistic computational approach to examine how the OMP biogenesis machinery maintains cellular proteostasis under biologically relevant conditions. This treatment overcomes common limitations of both in vitro and in vivo experiments because we can simultaneously investigate unfolded OMP (uOMP) transport and folding trajectories at a microscopic level and phenotypes at a macroscopic level. This analysis provides global insight into the dynamic process of periplasmic uOMP transport and highlights the unique contributions of individual chaperones in the maintenance of outer membrane and periplasmic proteostasis. (See pp. E4794–E4800.)

Gambogic acid identifies an isoform-specific druggable pocket in the middle domain of Hsp90β

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The molecular chaperone heat-shock protein 90 (Hsp90) is a key member of the cellular proteostasis network, and as such helps to protect cells against proteotoxic stress. Cancer cells have up-regulated members of this network, including Hsp90, to promote their survival and growth. Several Hsp90 inhibitors have undergone clinical trials, but these drugs, which bind to a shared nucleotide pocket in the N-terminal domain, do not differentiate between the four Hsp90 family members [Hsp90α, Hsp90β, GRP94 (glucose-regulated protein 94 kDa), and

TRAP1 (tumor necrosis receptor-associated protein 1)]. In this report, we identify a pharmacophore contained within the natural product gambogic acid that binds uniquely to a site in Hsp90β, thus identifying this compound as a prototype of a new class of isoform-specific Hsp90 inhibitors. (See pp. E4801–E4809.)

Selectivity of ORC binding sites and the relation to replication timing, fragile sites, and deletions in cancers

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The origin recognition complex (ORC) binds sites from which DNA replication is initiated. By mapping binding sites in human cells, we show that ORC binds selectively to open (DNase I-hypersensitive) regions containing active chromatin marks. There are far more ORC sites in early replicating regions of the genome, and computational simulation based on ORC binding indicates that replication timing is due primarily to ORC density and stochastic initiation of DNA replication from origins. Large genomic regions with a paucity of ORC sites are strongly associated with common fragile sites and recurrent deletions in cancers. Thus, replication origins, replication timing, and replication-dependent chromosome breaks are determined ultimately by the genomic distribution of activator proteins at enhancers and promoters. (See pp. E4810–E4819.)

Nuclear repartitioning of galectin-1 by an extracellular glycan switch regulates mammary morphogenesis

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Malignant cells of breast carcinoma and nonmalignant epithelia of branching mammary glands share the ability to migrate through their surroundings. To form the mammary tree-like architecture, nonmalignant epithelia must migrate in a controlled fashion, integrating cues from their microenvironment, notably, the glycan appendages on extracellular proteins and lipids. Here, we show that Galectin-1, a glycan-binding protein, is able to sense glycan signatures on mammary gland epithelia, transmit this information to epithelial nuclei by direct translocation, and drive branching migration. Nuclear galectin-1 is regulated by the relative levels of α2,6–sialic acids and *N*-acetyllactosamine on extracellular glycans. Similar lectin–glycan signatures were observed in malignant breast cells and suggest cancer cells use this pathway during their invasion. (See pp. E4820–E4827.)

microRNA-309 targets the Homeobox gene *SIX4* and controls ovarian development in the mosquito *Aedes aegypti*

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We report here that microRNA-309 (miR-309) plays a critical role in ovarian development of female *Aedes aegypti* mosquitoes. The genetic disruption of miR-309 by CRISPR/Cas9 system displays a failure of ovarian primary follicle formation, and several pathways associated with ovarian development are down-regulated after miR-309 depletion. Comprehensive screening and functional identification have revealed that SIX homeobox 4 protein (SIX4) is a direct target of miR-309. miR-309–targeted degradation of *SIX4* mRNA is required for appropriate commencement of the preparatory phase and initiation of the blood feeding-triggered phase of ovarian development. Thus, miR-309 serves as a regulatory switch permitting a stage-specific degradation of the ovarian *SIX4* mRNA, allowing a shift from previtellogenic to postvitellogenic phases of ovarian development. (See pp. E4828–E4836.)

Regulation of eosinophilia and allergic airway inflammation by the glycan-binding protein galectin-1

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Allergic asthma is a chronic airway disease, and the number of individuals with asthma continues to grow. Eosinophils recruited to allergic airways contribute significantly to airway inflammation via release of proinflammatory mediators that cause epithelial tissue damage, bronchoconstriction, and airway remodeling. Here we show that galectin-1 (Gal-1), an endogenous immunoregulatory lectin, binds to eosinophil-expressed surface glycans to inhibit cell migration and induce apoptosis. Using a mouse model of allergic asthma, we show that mice lacking Gal-1 exhibit increased airway eosinophils and airway hyperresponsiveness compared with wild-type mice. Because Gal-1 plays an important role in regulating airway inflammation, identifying pathways to induce Gal-1 synthesis and/or favor its biological activity might enable exploitation of its proresolving function to suppress allergic asthma. (See pp. E4837–E4846.)

CCN1/CYR61-mediated meticulous patrolling by Ly6C^{low} monocytes fuels vascular inflammation

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Upon infection, circulating leukocytes leave the bloodstream and migrate into the inflammatory site. Neutrophils are the first leukocytes to be recruited within a few hours, followed by inflammatory lymphocyte antigen 6 complex (Ly6C)-positive monocytes. This study refines the model of the leukocyte recruitment cascade. We demonstrate that upon Toll-like receptor 7/8-mediated vascular inflammation, platelet activation drives the rapid mobilization of Ly6C^{low} monocytes to the luminal side of the endothelium. Accumulated Ly6C^{low} monocytes do not extravasate into the tissue. Instead, they meticulously patrol the endothelium and control the subsequent recruitment of neutrophils. Moreover, we show that endothelium-bound cyteine-rich protein 61 (CYR61)/CYR61 connective tissue growth factor nephroblastoma overexpressed 1 (CCN1) protein provides a molecular support for adequate patrolling of Ly6C^{low} monocytes in the steady state and under inflammatory conditions. (See pp. E4847–E4856.)

Site-specific phosphorylation and microtubule dynamics control Pyrin inflammasome activation

Wenqing Gao, Jieling Yang, Wang Liu, Yupeng Wang, and Feng Shao

Pyrin, encoded by the *MEFV* gene, is causative for familial Mediterranean fever (FMF), an autoinflammatory disease. Pyrin responds to bacterial modifications/inactivation of Rho GTPases by assembling an inflammasome complex for activating caspase-1. Pyrin is a unique immune sensor because it senses bacterial virulence rather than recognizing microbial products. We found that Pyrin is phosphorylated on two serine sites which keep Pyrin inactive through binding by 14-3-3 proteins. Toxin stimulation and bacterial infection trigger Pyrin dephosphorylation and 14-3-3 dissociation, allowing Pyrin inflammasome activation. Colchicine, a microtubule-disrupting drug used to treat FMF, inhibits Pyrin activation downstream of dephosphorylation and 14-3-3 dissociation. These findings not only help us understand FMF pathogenesis/treatment but also

shed mechanistic insights into cytosolic immunity. (See pp. E4857–E4866.)

The stringent response regulates adaptation to darkness in the cyanobacterium *Synechococcus elongatus*

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Cyanobacteria are an important group of photosynthetic bacteria that rely upon light energy for growth but frequently must adapt to darkness. Cells stop growing and decrease overall rates of gene expression and protein synthesis in the dark, but the molecular mechanisms behind these observations remain unknown. We find that a widespread bacterial stress response, the stringent response, helps cells conserve resources during darkness. In the dark, cells produce higher levels of the stringent response signaling molecule guanosine 3′-diphosphate 5′-diphosphate (ppGpp), thereby altering gene expression patterns and affecting the protein synthesis machinery. These results help explain previous observations in the cyanobacterial literature and extend our knowledge of how the same signaling pathway has been adapted to different bacterial lifestyles and metabolisms. (See pp. E4867–E4876.)

Learning to soar in turbulent environments

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Thermals are ascending currents that typically extend from the ground up to the base of the clouds. Birds and gliders piggyback thermals to fly with a reduced expenditure of energy, for example, during migration, and to extend their flying range. Flow in the thermals is highly turbulent, which poses the challenge of the orientation in strongly fluctuating environments. We combine numerical simulations of atmospheric flow with reinforcement learning methods to identify strategies of navigation that can cope with and even exploit turbulent fluctuations. Specifically, we show how the strategies evolve as the level of turbulent fluctuations increase, and we identify those sensorimotor cues that are effective at directing turbulent navigation. (See pp. E4877–E4884.)

Neural mechanisms of transient neocortical beta rhythms: Converging evidence from humans, computational modeling, monkeys, and mice

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Neocortical beta is one of the most prominent signatures of neural activity measured noninvasively in humans. Beta expression is a strong predictor of healthy and pathological perceptual and motor performance. However, there is considerable debate as to whether beta itself is causally important in information and disease processes. Key to resolving this debate is understanding the neural mechanisms inducing beta. Here, building on prior work, we combined human magnetoencephalography, computational modeling, and laminar recordings in mice and monkeys to establish and test a new theory explaining the emergence of spontaneous transient neocortical beta events in somatosensory and frontal cortex. Our results enable a principled understanding of neocortical beta and can help guide studies seeking to understand its relation to function. (See pp. E4885–E4894.)

Leaky RyR2 channels unleash a brainstem spreading depolarization mechanism of sudden cardiac death

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Gain-of-function "leaky" ryanodine receptor-2 (RyR2) mutations are detected in many cases of human sudden cardiac death and sudden unexpected death in epilepsy. The early lethality is considered to be due to arrhythmogenic behavior of cardiomyocytes caused by excess intracellular calcium ions released from internal stores. In this study, we find that the leaky RyR2 mutation also modulates neurotransmitter release from neurons in brainstem autonomic centers and facilitates spreading depolarization, which when provoked silences autonomic microcircuitry in the dorsal medulla. When seizures were generated in mice carrying a leaky RyR2 mutation, the mice experienced sudden cardiorespiratory collapse concomitant with brainstem spreading depolarization. These findings indicate that in addition to the myocardium, the brainstem is a target of leaky RyR2 mutations. (See pp. E4895–E4903.)

Pacemaker-neuron–dependent disturbance of the molecular clockwork by a *Drosophila* CLOCK mutant homologous to the mouse *Clock* mutation

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The circadian clock drives ~24-hour rhythms in behavior and physiology of organisms, and is dependent on transcriptional/translational feedback loops (TTFLs) at the cellular level. Pacemaker neurons in the brain control specific circadian behaviors in response to environmental timing cues such as light and temperature cycles. We show here that flies expressing dCLOCK (dCLK) lacking amino acids 657–707, homologous to the mouse *Clock* mutation, display pacemaker-neuron–dependent disturbance of the molecular clockwork. Specifically, the molecular rhythms in light-sensitive pacemaker neurons were significantly disrupted, but the molecular rhythms in temperature-sensitive pacemaker neurons were robust. Our results suggest that the dCLK-controlled TTFL operates differently in subsets of pacemaker neurons, which contributes to their specific functions, such as differential sensitivity to entraining cues. (See pp. E4904–E4913.)

Major neurotransmitter systems in dorsal hippocampus and basolateral amygdala control social recognition memory

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The hippocampus and basolateral amygdala—modulated by β-noradrenergic, D1/D5 dopaminergic, and H2-histaminergic receptors—control memory processing of many memories, but their role in social recognition memory (SRM) has been little studied. SRM is fundamental for the establishment of social relationships and, consequently, for the formation and stability of social groups. The social deficits of psychiatric disorders, such as autism and schizophrenia, are believed to be caused by alterations in SRM processing by the hippocampus and amygdala. Here we examine the involvement of the hippocampus and basolateral amygdala—and β-noradrenergic, D1/D5 dopaminergic, and H2-histaminergic receptors therein—in SRM consolidation. The results suggest an important and complex modulation of this process, which may help to elucidate the basis of

inappropriate social behavior in psychiatric patients. (See pp. E4914–E4919.)

Extensive phosphorylation of AMPA receptors in neurons

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Decades of research from many laboratories has established a model in which phosphorylation of the GluA1 AMPA-type glutamate receptor subunit plays a significant role modulating long-term potentiation and depression, homeostatic and neuromodulator-regulated plasticity, spatial memory, fear/extinction, and appetitive incentive learning. However, a recent study suggests that GluA1 phosphorylation is exceedingly low, even in synaptic fractions. Here, we address this controversy using in vitro and in vivo techniques. We find a large fraction of excitatory synapses are positive for phosphorylated GluA1. Moreover, phosphorylated species make up a significant fraction of the population and are highly responsive to numerous physiologically relevant stimuli. This characterization reaffirms a large body of work defining a prominent role of AMPA receptor phosphorylation in synapse biology and synaptic plasticity. (See pp. E4920–E4927.)