PNAS Plus Significance Statements

Generating carbon schwarzites via zeolite-templating

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Nanocarbons can be characterized by their curvature—that is, positively curved fullerenes, zero-curved graphene, and negatively curved schwarzites. Schwartzites are fascinating materials but have not been synthesized yet, although disordered materials with local properties similar to schwarzites ("random schwarzites") have been isolated. A promising synthetic method allows for the interior surfaces of zeolites to be templated with sp^2 carbon, but theoretical study of these zeolite-templated carbons (ZTCs) has been limited because of their noncrystalline structures. In this work, we develop an improved molecular description of ZTCs, show that they are equivalent to schwartzites, and thus make the experimental discovery of schwarzites ex post facto. Our topological characterization of ZTCs lends insights into how template choice allows for the tunability of ordered microporous carbons. (See pp. E8116–E8124.)

General methodology for inferring failure-spreading dynamics in networks

Xiangyang Guan and Cynthia Chen

Failure spreading widely exists in many systems, but methodologies devised to understand its dynamics so far are domainconstrained and demonstrate limited applicability across different systems. This paper tackles this issue from a reverse perspective of failure-spreading processes: It takes the spreading outcomes as inputs and seeks to infer the spreading process that gives rise to the outcomes, instead of the other way around as the prevalent approaches do. Because failure-spreading outcomes are commonly observed for different systems, we envision that this approach is generally applicable and provides a promising avenue to potentially unify research on spreading dynamics across disciplines. This research will facilitate understanding system dynamics and developing control techniques for them at different systems, scales, and dimensions. (See pp. E8125–E8134.)

Self-organized criticality and pattern emergence through the lens of tropical geometry

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A simple geometric continuous model of self-organized criticality (SOC) is proposed. This model belongs to the field of tropical geometry and appears as a scaling limit of the classical sandpile model. We expect that our observation will connect the study of SOC and pattern formation to other fields (such as algebraic geometry, topology, string theory, and many practical applications) where tropical geometry has already been successfully used. (See pp. E8135–E8142.)

Deciphering the super relaxed state of human β-cardiac myosin and the mode of action of mavacamten from myosin molecules to muscle fibers

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Cardiac muscle contraction is powered by ATP hydrolysis during cycles of interaction between myosin-containing thick filaments and actin-containing thin filaments. This generates force in the cardiac muscle necessary for pumping blood through the body. Mutations in myosin alter this force generation leading to hypercontractility and hypertrophic cardiomyopathy (HCM). An energy-conserving, super relaxed state (SRX) of myosin, which has a very low ATPase activity, has previously been described in muscle fibers. Destabilization of the SRX has been proposed to be a chief cause of HCM. This work sheds light on the biochemical and molecular nature of SRX and demonstrates the mechanism of action of mavacamten, a cardiac inhibitor in phase 2 clinical trials. Mavacamten exerts its effects primarily by stabilizing the SRX of β-cardiac myosin. (See pp. E8143–E8152.)

The consequences of cavity creation on the folding landscape of a repeat protein depend upon context

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Extant protein sequences are the result of evolutionary pressure. Eliminating core packing interactions by mutation, regardless of position, generally results in similar perturbations to global stability. In contrast, we find that cavity creation has highly varied consequences for a protein folding landscape, depending upon the context in which the cavities are introduced. These observations have implications for interpreting evolutionary adaptation, as it is likely that proteins

have evolved to exhibit optimal levels of conformational heterogeneity and dynamics. These results should also inform protein engineering efforts, as they provide insight into how sequence can modulate the population of functionally important excited states, as well as states that lead to secondary, undesirable reactions such as oligomerization, aggregation, surface activity, and phase separation. (See pp. E8153–E8161.)

Altered conformational landscape and dimerization dependency underpins the activation of EGFR by αC–β4 loop insertion mutations

Zheng Ruan and Natarajan Kannan

The αC–β4 loop is a conserved structural motif present in all eukaryotic protein kinases. This study focuses on epidermal growth factor receptor (EGFR), a receptor tyrosine kinase that harbors many recurrent insertion mutations in the αC–β4 loop. We show that EGFR activity and drug sensitivity are strongly dependent on the nature, size, and location of mutations in the loop and that even subtle variations in the loop have a dramatic effect on kinase dynamics and free-energy landscape. These findings inform ongoing drug-discovery efforts on EGFR kinases and provide a structural framework for investigating the role of the understudied αC–β4 loop in kinase regulation, dynamics, and evolution. (See pp. E8162–E8171.)

Proteomic analysis of monolayer-integrated proteins on lipid droplets identifies amphipathic interfacial α-helical membrane anchors

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Biological membranes are semipermeable barriers that are composed primarily of phospholipid bilayers or monolayers and proteins. Proteins embedded within membranes are extremely diverse in structure and function. Some membrane-integrated proteins do not fully span phospholipid bilayers but play key roles in organizing membrane surfaces. How these monolayer-integrated proteins interact with membranes has not been systematically investigated, primarily because they are very difficult to distinguish from the far more common class of proteins that fully traverse the phospholipid bilayer. We describe an approach to systematically identify monolayer-integrated proteins and demonstrate that interfacial α-helices are a structural motif that directs membrane integration for this important class of membrane protein. (See pp. E8172–E8180.)

JUM is a computational method for comprehensive annotation-free analysis of alternative pre-mRNA splicing patterns

Qingqing Wang and Donald C. Rio

Alternative pre-mRNA splicing (AS) is a critical gene regulatory mechanism to produce diverse, tissue-specific, and functionally distinct protein profiles in eukaryotes to maintain normal cellular functions. Aberrant AS patterns are constantly associated with many human diseases, including cancer. The exceptional complexity of AS imposes a major challenge to analyzing AS across various tissues and cell types. Here we present a computational algorithm to profile and quantitate tissue-specific AS profiles from RNA-sequencing data without any prior knowledge of the host transcriptome. The junction usage model shows consistent superior performance in both specificity and sensitivity compared with other currently available AS analysis methods, and can be readily applied to a wide range of RNA samples from different organisms for accurate and comprehensive analyses of AS. (See pp. E8181–E8190.)

Rev7 dimerization is important for assembly and function of the Rev1/Polζ translesion synthesis complex

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We describe a class of protein–protein interactions mediated by the HORMA (Hop1, Rev7, Mad2) dimerization interface of Rev7, a multitasking scaffolding protein involved in translesion synthesis (TLS), repair of double-strand breaks, and mitosis. Biochemical and structural analyses of Rev7 dimerization reveal an unexpected architecture of the Rev1/Polζ TLS complex, which plays a central role in replication of damaged DNA, and describe the mechanism of Rev7 interactions with HORMA proteins from other pathways. Assays in $Rev7^{-/-}$ cells complemented with mutant Rev7 provide evidence that protein–protein interactions mediated by the Rev7 HORMA interface are important for the DNA damage response. These results contribute to the structural biology of DNA replication and repair and to understanding of the important class of HORMA proteins. (See pp. E8191–E8200.)

Structure of the mammalian TRPM7, a magnesium channel required during embryonic development

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Ion channels are pore-forming proteins spanning biological membranes. Transient receptor potential ion channels are a subclass of ion channel proteins, characterized by nonselective permeability to cations such as sodium, calcium, magnesium, and zinc, and little voltage sensitivity; their gating is still an area of active investigation. TRPM6 and TRPM7 are ubiquitously expressed with prominent roles in early embryonic development. Uniquely, these channels also include an active kinase domain. The functions of TRPM6 and TRPM7 are correlated with proteolytic cleavage of the kinase domain, which is then translocated to the nucleus to phosphorylate histones and regulate gene expression. Here we describe the structure of the TRPM7 transmembrane regions and compare its features to other ion channels. (See pp. E8201–E8210.)

Cellular function given parametric variation in the Hodgkin and Huxley model of excitability

Hillel Ori, Eve Marder, and Shimon Marom

Macroscopic cellular function is maintained despite extensive variations in underlying elementary constituents, including the size of the cell, and the number, distribution, and kinetics of their proteins. Here, we take advantage of the sound theoretical and experimental basis of action potential generation to analyze macroscopic cellular invariance given microscopic variation. This analysis points to a significant gap between the high-dimensional level of description captured by biophysical measurements of channel function and the lower, physiological dimensionality, to which cellular function is sensitive. When examined in a lower dimension, a simple rule that relies on sodium channel slow inactivation provides a powerful homeostatic control mechanism that maintains excitability amid changes in protein concentrations and their kinetics. (See pp. E8211–E8218.)

Control of the Restriction Point by Rb and p21

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The canonical Restriction Point model suggests that cells are born into a state in which they are uncommitted to the cell cycle, but will activate cyclin-dependent kinase 2 and cross the Restriction Point several hours later if sufficient nutrients are available. However, recent single-cell studies have challenged aspects of this model. This work examines the Restriction Point in cancerous and noncancerous cells and shows that, in six cases tested, the cell populations split such that only a subset of cells is born into a pre-Restriction Point state, while the remainder immediately commits to another cell cycle. This shows that even cancer cells can experience significant heterogeneity in this cell fate decision, which may be exploitable for therapeutic gain. (See pp. E8219–E8227.)

Tyrosyl-tRNA synthetase stimulates thrombopoietin-independent hematopoiesis accelerating recovery from thrombocytopenia

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Aminoacyl-tRNA synthetases (aaRSs) catalyze aminoacylation of tRNAs in the first step of protein synthesis in the cytoplasm. However, in higher eukaryotes, they acquired additional functions beyond translation. In the present study, we show that an activated form of tyrosyl-tRNA synthetase (YRS^{ACT}) functions to enhance megakaryopoiesis and platelet production in vitro and in vivo. These findings were confirmed with human megakaryocytes differentiated from peripheral blood $CD34^+$ hematopoietic stem cells and with human induced pluripotent stem (iPS) cells. The activity of YRS^{ACT} is independent of thrombopoietin (TPO), as evidenced by expansion of the megakaryocytes from iPS cell-derived hematopoietic stem cells from a patient deficient in TPO signaling. These findings demonstrate a previously unrecognized function of an aaRS which may have implications for therapeutic interventions. (See pp. E8228–E8235.)

Regulation of axon repulsion by MAX-1 SUMOylation and AP-3

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During neural development, growing axons navigate over long distances to reach their targets. A critical step in this process is the regulation of its surface receptors on the axon's growth cone in response to environmental cues. We focus on how the UNC-5 receptor in *Caenorhabditis elegans* motor axons is regulated during axon repulsion. By combining *C. elegans* genetics, biochemistry, and imaging, we found that MAX-1 SUMOylation and AP-3 complex have significant roles in UNC-5–mediated axon repulsion. Our findings reveal how SUMOylation and AP-3–mediated trafficking and degradation interact to help the growing axon find its final target. (See pp. E8236–E8245.)

Oligodendrocyte precursor survival and differentiation requires chromatin remodeling by Chd7 and Chd8

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Oligodendrocyte precursor cells (OPCs) constitute the main proliferative cells in the adult brain and deregulation of OPC proliferation-differentiation balance results in either glioma formation or defective (re)myelination. Mutations in chromatin remodelers *CHD7* and *CHD8* are the cause of CHARGE syndrome and some autism spectrum disorders (ASD). Here we show that Chd7 protects OPCs from apoptosis by chromatin closing and gene repression of *p53*, while Chd7 induces chromatin opening and gene activation of OPC-differentiation regulators. Chd7 is, however, dispensable for oligodendrocyte stage progression, consistent with Chd8 compensatory function, as suggested by their common chromatin-binding profiles, including ASD-risk–associated genes. Our results thus involve oligodendroglia in ASD and CHARGE and offer new avenues to understand and modulate CHD7/CHD8 functions in normal and pathological brain development. (See pp. E8246–E8255.)

Differences in neural stem cell identity and differentiation capacity drive divergent regenerative outcomes in lizards and salamanders

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The evolutionary changes behind the loss in regenerative potential from salamanders to mammals remain largely elusive. Lizards, representing an intermediary species between the two, possess a limited ability to regenerate their tails. Here, we probe the mechanisms behind the differing regenerative patterns between lizards and salamanders, and we find that neural stem cells within the regenerated spinal cords are distinct cell populations that regulate divergent tail regeneration patterns. This finding sheds light on the factors that govern regenerative ability as well as the loss of this capability and brings us one step closer to eventually elucidating strategies to allow for mammalian regeneration. (See pp. E8256–E8265.)

Oceanographic boundaries constrain microbial diversity gradients in the South Pacific Ocean

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High-resolution data covering marine microbes and microeukaryotes are sparse, even though these organisms control global biogeochemical cycles. Here we present a dataset describing the microbial pro- and eukaryotic diversity along a 7,000-km transect from the Antarctic ice edge to the equator in the South Pacific Ocean. We show that (*i*) temperature is not a primary driver of richness gradients, (*ii*) prokaryotic richness increases with productivity, and (*iii*) oceanographic features can structure the diversity of pro- and eukaryotes. Our data have given us a better understanding of how diversity relates to dissolved inorganic nitrogen and productivity as well as insights into the potential shifts in the geographical range of marine microbe communities in light of the rapidly changing climate. (See pp. E8266–E8275.)

Deep mutational scanning of hemagglutinin helps predict evolutionary fates of human H3N2 influenza variants

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A key goal in the study of influenza virus evolution is to forecast which viral strains will persist and which ones will die out. Here we experimentally measure the effects of all amino acid mutations to the hemagglutinin protein from a human H3N2 influenza strain

on viral growth in cell culture. We show that these measurements have utility for distinguishing among viral strains that do and do not succeed in nature. Overall, our work suggests that new high-throughput experimental approaches may be useful for understanding virus evolution in nature. (See pp. E8276–E8285.)

RNF169 limits 53BP1 deposition at DSBs to stimulate single-strand annealing repair

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53BP1 restrains DNA end resection, and its dosage imbalance upsets DNA double-strand break (DSB) repair pathway choice. Here, by monitoring 53BP1 distribution on DSB-flanking chromatin, we have established a dose-dependent role of the RING finger protein RNF169 in limiting 53BP1 DSB deposition. Moreover, we found that forced expression of RNF169 overcomes 53BP1 activity and stimulates mutagenic DSB repair via the single-strand annealing pathway. Our findings suggest that aberrant expression of RNF169 may represent a deleterious factor in DSB repair control and in maintenance of genome stability. (See pp. E8286–E8295.)

Thioredoxin-like2/2-Cys peroxiredoxin redox cascade supports oxidative thiol modulation in chloroplasts

Keisuke Yoshida, Ayaka Hara, Kazunori Sugiura, Yuki Fukaya, and Toru Hisabori

To ensure efficient photosynthetic carbon gain, plant chloroplasts have to adjust their own physiology toward changes in light environments. Specific chloroplast proteins are reversibly activated–inactivated during light–dark cycles by switching the reduction–oxidation states of their Cys residues, which is termed redox regulation. A long-standing issue in plant biology is the manner in which redox-regulated proteins are reoxidized upon the interruption of light exposure. In this study, we identified the thioredoxin-like2 (TrxL2)/2-Cys peroxiredoxin (2CP) redox cascade as a molecular basis for oxidative thiol modulation in chloroplasts. This finding dissects the "dark side" of chloroplast redox regulation, providing an insight into how plants rest their photosynthetic activity at night. (See pp. E8296–E8304.)

Phosphoinositides control the localization of HOPS subunit VPS41, which together with VPS33 mediates vacuole fusion in plants

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Plant vacuoles are essential organelles and occupy up to 90% of the cell volume. Their roles include regulation of stomata movements, protein storage in seeds, gravity sensing, and ion homeostasis. Vacuole or lysosome fusion in eukaryotes is mediated by two multisubunit complexes, SNARE and homotypic fusion and vacuolar protein sorting (HOPS), but only the SNARE complex is well characterized in plants. Here, we show that, similar to other eukaryotes, HOPS mediates vacuole fusion in plants by interaction with SNAREs and that the HOPS subunit VPS33 and the SNARE protein SYP22 display the sites for interaction between these complexes. In contrast to other eukaryotes, however, plant HOPS recruitment to liposomes is inhibited by phosphoinositides, which appear to define strict rules for regulating fusion and fragmentation of dynamic vacuoles. (See pp. E8305–E8314.)

Seed genome hypomethylated regions are enriched in transcription factor genes

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We scanned soybean and *Arabidopsis* seed genomes for hypomethylated regions, or DNA methylation valleys (DMVs), present in mammalian cells. Seeds contain DMV regions that have <5% bulk DNA methylation or, in many cases, no detectable DNA methylation. Methylation levels of seed DMVs do not vary detectably during seed development and are present prior to fertilization. Seed DMVs are enriched in transcription factor (TF) genes and are decorated with histone marks that fluctuate developmentally, resembling their animal counterparts in significant ways. We conclude that many genes playing important roles in seed formation are regulated without detectable DNA methylation events and suggest that selective action of TFs, as well as chromatin epigenetic events, play important roles in making a seed. (See pp. E8315–E8322.)