PNAS Plus Significance Statements

Nature of ground and electronic excited states of higher acenes

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Higher acenes are promising organic semiconductors with versatile electronic properties. A better understanding of their ground- and electronic excited states will benefit further molecular design and future applications. However, their instability and multireference character have impeded experimental and theoretical studies. Here, we use the recently developed particle–particle random-phase approximation in combination with a diradical analysis to unveil the nature of their ground- and electronic excited states. The excitation energies are presented, along with a detailed description of the bonding nature, which switches from regular molecules to full diradicals, and then even to polyradicals. The likelihood of singlet fission is briefly discussed from an energetic perspective. (See pp. E5098–E5107.)

Mouse MORC3 is a GHKL ATPase that localizes to H3K4me3 marked chromatin

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The Microrchidia (MORC) family of ATPases are important regulators of gene silencing in multiple organisms but little is known about their molecular behavior. In this study, we used crystallography and native mass spectrometry to show that MORC3 forms dimers when it binds to nonhydrolyzable ATP analogues. We also determined that the CW zinc finger-like domain of MORC3 can bind euchromatic histone H3 lysine 4 (H3K4) methylation and that MORC3 localizes to H3K4me3-marked chromatin. The MORC3 crystal structure provides details as to the intermolecular interactions that allow dimerization and the binding to ATP and histones. This work reveals key molecular activities of MORC3 that might apply to other MORC family members in eukaryotic organisms. (See pp. E5108–E5116.)

Curvature–undulation coupling as a basis for curvature sensing and generation in bilayer membranes

Ryan P. Bradley and Ravi Radhakrishnan

Most intracellular trafficking and many intercellular communications are orchestrated by curvature-driven or curvature-associated cellular processes. Hence, understanding how proteins sculpt lipid bilayers is vital to our understanding of how cell membranes modulate cell signaling pathways and consequent cell fate. In this study, we present coarse-grained molecular dynamics simulations of the epsin N-terminal homology domain interacting with a lipid bilayer and demonstrate a cooperative and fluctuation-mediated mechanism by which these proteins can generate curvature in bilayer membranes as well as sense curvature at large distances compared with their size. (See pp. E5117–E5124.)

Structural conservation in the template/pseudoknot domain of vertebrate telomerase RNA from teleost fish to human

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Telomerase synthesizes the telomeric DNA at the 3′ ends of chromosomes and maintains genome integrity. Telomerase RNA (TR) provides the template for telomere-repeat synthesis within a template/pseudoknot (t/PK) domain that is essential for activity. We investigated the structure and dynamics of the t/PK from medaka fish, which contain the smallest vertebrate TR, using NMR and modeling. Despite differences in length, sequence, and predicted secondary structure with human TR, the remarkable similarities between subdomains, including one newly identified in medaka, reveal a conserved architecture for vertebrate t/PK. Combining our model of the full-length pseudoknot and information from the 9-Å structure of *Tetrahymena* telomerase, we propose models for the interaction of medaka and human t/PK with telomerase reverse transcriptase, providing insight into function. (See pp. E5125–E5134.)

Cilium transition zone proteome reveals compartmentalization and differential dynamics of ciliopathy complexes

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Cilia are highly conserved organelles present in most eukaryotic cell types. The transition zone (TZ) is a ciliary subdomain that acts as a "gate" to control the composition of the cilium. The importance of the TZ is reflected in the many human diseases (termed ciliopathies) that are caused by mutations in TZ complexes. Here, we use a new proteomics technique to find new components of the African trypanosome TZ. We leverage the extraordinary tractability of this system to investigate TZ proteins, localizing them to distinct subdomains within the TZ, and demonstrating their

essential roles in building cilia. We show that while orthologs of some ciliopathy complexes show long-term association with the TZ, others are highly dynamic. (See pp. E5135–E5143.)

Regulation of intracellular heme trafficking revealed by subcellular reporters

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The intracellular and extracellular trafficking of heme, a hydrophobic and potentially cytotoxic cofactor in proteins such as hemoglobin, remains an underexplored area. While cellular heme can be derived exogenously or from de novo synthesis, it is unclear if there is differential trafficking of heme from these two sources. To critically examine this possibility, we developed peroxidase-based enzymatic reporters for heme and deployed them in subcellular compartments in mammalian cells and in several tissues in the *Caenorhabditis elegans* animal model. Our results show that extracellular and endogenous heme is trafficked to virtually all intracellular compartments via distinct cellular routes and that interorgan heme transport is essential for systemic regulation of heme homeostasis in *C. elegans*. (See pp. E5144–E5152.)

Notch-mediated lateral inhibition regulates proneural wave propagation when combined with EGF-mediated reaction diffusion

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Notch-mediated lateral inhibition regulates binary cell fate choice, resulting in salt and pepper patterns during various developmental processes. The wave of differentiation in the *Drosophila* visual center accompanies Notch activity that is propagated without the formation of a salt and pepper pattern, implying that Notch does not regulate lateral inhibition during this process. In this study, by combining mathematical modeling and genetic analysis, we showed that Notch-mediated lateral inhibition is implemented within the proneural wave. The combination of Notch-mediated lateral inhibition and EGF-mediated reaction diffusion enables a function of Notch signaling that regulates propagation of the wave of differentiation. (See pp. E5153–E5162.)

Extensive sequence divergence between the reference genomes of two elite *indica* rice varieties Zhenshan 97 and Minghui 63

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Indica rice accounts for >70% of total rice production worldwide, is genetically highly diverse, and can be divided into two major varietal groups independently bred and widely cultivated in China and Southeast Asia. Here, we generated high-quality genome sequences for two elite rice varieties, Zhenshan 97 and Minghui 63, representing the two groups of *indica* rice and the parents of a leading rice hybrid. Comparative analyses uncovered extensive structural differences between the two genomes and complementarity in their hybrid transcriptome. These findings have general implications for understanding intraspecific variations of organisms with complex genomes. The availability of the two genomes will serve as a foundation for future genome-based explorations in rice toward both basic and applied goals. (See pp. E5163–E5171.)

Patrolling monocytes promote intravascular neutrophil activation and glomerular injury in the acutely inflamed glomerulus

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Nonclassical monocytes patrol the microvascular lumen in numerous organs in behavior thought to represent a form of immune surveillance. However, the mechanisms whereby they promote inflammatory responses are unclear. Here, we show using in vivo imaging that, in the unique microvasculature of the glomerulus in the kidney, monocytes constitutively undergo interactions with intravascular migratory neutrophils. Upon induction of glomerular inflammation, neutrophils that interact with monocytes show increased retention in glomerular capillaries and increased propensity to generate reactive oxygen species, leading to renal injury. These findings of immune cell interactions occurring within the glomerular microvasculature indicate that cell–cell contact between neutrophils and nonclassical monocytes is a previously unrecognized intravascular inflammatory mechanism underpinning glomerular injury. (See pp. E5172–E5181.)

Sickle cell anemia mice develop a unique cardiomyopathy with restrictive physiology

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Sickle cell anemia (SCA) is a common monogenic disorder associated with significant morbidity and mortality and a high incidence of unexplained sudden death in young adults. With the prevention of infections, there is an increasing appreciation for cardiopulmonary complications and a cardiac phenotype that cannot be solely attributed to chronic anemia. We used mouse models of SCA and iron-deficient anemia to show distinct functional, pathological, ultrastructural, and molecular cardiac features causing a unique restrictive cardiomyopathy in SCA that predisposed the myocardium to electrophysiological abnormalities and sudden death. This is a comprehensive longitudinal analysis in preclinical mouse models that unifies the previously reported diverse cardiac phenotypes in SCA, and opens new avenues for early diagnostics and targeted therapies for human SCA-related cardiac disease. (See pp. E5182–E5191.)

p53 down-regulates SARS coronavirus replication and is targeted by the SARS-unique domain and PL^{pro} via E3 ubiquitin ligase RCHY1

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Severe acute respiratory syndrome coronavirus (SARS-CoV) is one of the most pathogenic human coronaviruses. Virulence is reflected in

the molecular interplay between virus and host cells. Here we show a strategy of how SARS-CoV antagonizes the host antiviral factor p53, which impairs viral replication. The papain-like protease of the nonstructural protein 3 of SARS-CoV and other coronaviruses physically interact with and stabilize E3 ubiquitin ligase ring-finger and CHY zinc-finger domain-containing 1 (RCHY1), thereby augmenting RCHY1-mediated degradation of p53. The SARS-unique domain (SUD) enhances these effects. Knockout of p53 promotes replication of SARS-CoV replicons and of infectious virus. Taken together we identify cellular p53 as antiviral measure of coronavirus-infected cells, which is counteracted via the stabilization of RCHY1 by viral SUD and papain-like protease (PL^{pro}) proteins and via ubiquitination of p53. (See pp. E5192–E5201.)

Two distinct mechanisms of transcriptional regulation by the redox sensor YodB

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Bacteria sense and protect themselves against oxidative stress using redox-sensing transcription regulators with cysteine residues. Here, we investigate at the molecular level how the YodB protein, a transcription repressor in *Bacillus subtilis*, monitors and responds to different oxidative stresses. Diamide stress leads to the formation of disulfide bonds between cysteine residues, whereas the more toxic quinone compound methyl-*p*-benzoquinone forms an adduct on a specific cysteine residue. These chemical modifications lead to considerably different changes in the YodB structure, causing the release of YodB from the DNA of antioxidant genes. The redox-sensing transcription regulator YodB allows *B. subtilis* to respond to multiple oxidative signals of differing toxicity by adopting different structures. (See pp. E5202–E5211.)

Holdase activity of secreted Hsp70 masks amyloid-β42 neurotoxicity in *Drosophila*

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Heat shock protein 70 (Hsp70) is a critical protein with many protective activities inside the cell. We demonstrate that forced secretion of Hsp70 is beneficial against the extracellular protein aggregates typical of Alzheimer's disease (AD). Engineering Hsp70 enables its interaction with the amyloid-β42 peptide, the main pathogenic agent in AD. This interaction suppresses amyloid-β toxicity in the eye, reduces cell death in brain neurons, and protects neuronal architecture and function. Interestingly, secreted Hsp70 exerts this protective activity without utilizing its refolding activity and without decreasing the levels and aggregation of amyloid-β42. These results suggest a protective mechanism mediated by direct binding to amyloid-β42, which blocks amyloid-β42 neurotoxicity. We discuss here the potential therapeutic benefits of secreted Hsp70. (See pp. E5212–E5221.)

Single-cell RNAseq reveals cell adhesion molecule profiles in electrophysiologically defined neurons

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Synapses functionally connect neurons in the brain and mediate information processing relevant to all aspects of life. Among others, synaptic connections are enabled by cell adhesion molecules, which connect presynaptic and postsynaptic membranes by binding to each other via the synaptic cleft. Mammalian genomes express hundreds of cell adhesion molecules whose combinatorial utilization is thought to contribute to the brain's "connectivity code." Such code could explain the versatility of synapses as well as the logic of connectivity between cell types. Here, we used single-cell RNA sequencing to analyze the expression of cell adhesion molecules and other signaling proteins in defined cell types, and found developmental patterns that potentially identify relevant elements of the connectivity code. (See pp. E5222–E5231.)

Regulatory network analysis reveals novel regulators of seed desiccation tolerance in *Arabidopsis thaliana*

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Seed desiccation tolerance (DT) is one of the most fascinating processes of higher plants, and has played a fundamental role in the evolution of land plants. DT allows plant seeds to remain viable in the dry state for years and even centuries. What the key transcription factors (TFs) are that activate the mechanisms that allow plant seeds to maintain cellular and DNA integrity for centuries remains largely unknown. In this paper, we report the identification of the TFs that act as major nodes of the transcriptional networks that regulate the acquisition of seed DT. We also report the functional validation of several of the major regulators of seed DT in plants. (See pp. E5232–E5241.)

Rapid hyperosmotic-induced Ca^{2+} responses in *Arabidopsis thaliana* exhibit sensory potentiation and involvement of plastidial KEA transporters

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How plant roots initially sense osmotic stress in an environment of dynamic water availabilities remains largely unknown. Plants can perceive water limitation imposed by soil salinity or, potentially, by drought in the form of osmotic stress. Rapid osmotic stress-induced intracellular calcium transients provide the opportunity to dissect quantitatively the sensory mechanisms that transmit osmotic stress under environmental and genetic perturbations in plants. We describe a phenomenon whereby prior exposure to osmotic stress increases the sensitivity of the rapid calcium responses to subsequent stress. Further, mutations in plastidial K^+ exchange antiporter (KEA)1/2 and KEA3 transporters were unexpectedly found to reduce the rapid osmotic stress-induced calcium elevation. These findings advance the understanding of the mechanisms underlying the rapid osmotic stress response in plants. (See pp. E5242–E5249.)