PNAS Plus Significance Statements

Higher thermoelectric performance of Zintl phases $(Eu_{0.5}Yb_{0.5})_{1-x}Ca_xMg_2Bi_2$ by band engineering and strain fluctuation

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The search for high-efficiency thermoelectric materials encompasses many classes of semiconductors. Zintl phases are attractive thermoelectric materials for thermoelectric applications. Here, we report the high thermoelectric performance of the rarely studied bismuth (Bi)-based Zintl phases $(Eu_{0.5}Yb_{0.5})_{1-x}Ca_xMg_2Bi_2$ with the record figure-of-merit *ZT* as high as 1.3 at 873 K. This *ZT* value is, to our knowledge, the highest ever reported in $CaAl_2Si_2$-based structures, especially compared with the best antimony (Sb)-based $YbZn_{0.4}Cd_{1.6}Sb_2$ compound. Because Sb-based Zintl compounds have been studied for many decades, this Bi-based Zintl phase with high thermoelectric properties could be a good thermoelectric material candidate in the future. (See pp. E4125–E4132.)

Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H1N1 influenza, and *Toxoplasma gondii* challenges with a single dose

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To respond better to evolving pathogens, sudden outbreaks, and individual patient needs, a flexible, safe, and efficient vaccine platform amenable to rapid production near the point of care is required. To this end, we created a fully synthetic, single-dose, adjuvant-free nanoparticle vaccine platform wherein modified dendrimer molecules nanoencapsulate antigen-expressing replicon mRNAs. Vaccines can be multiplexed and formed with multiple antigen-expressing replicons. After a single immunization, the rapid-production, contaminant-free vaccines elicit vital $CD8^+$ T-cell and antibody responses that fully protect against lethal exposures to several deadly pathogens, including Ebola virus, H1N1 influenza, and *Toxoplasma gondii*. We believe this technology may allow for rapid-response vaccines with broad efficacy that reduce the number and frequency of vaccinations, and healthcare worker burden. (See pp. E4133–E4142.)

In situ characterization of the mTORC1 during adipogenesis of human adult stem cells on chip

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Adipogenesis is vital for animals to maintain energy balance by storing lipid. As a crucial integrator of nutrient, energy, and metabolite signals, mammalian target of rapamycin (mTOR) reportedly regulates adipogenesis. However, conventional cell cultures are limited to mimicking in vivo conditions, and previous reports do not provide the localization information of mTOR during adipogenesis. Here, we developed an automated microfluidic large-scale integration platform for differentiating human adult stem cells into mature adipocytes with standardized nutrient availability over weeks. With an integrated multiplexed immunoassay, we detected interaction, phosphorylation, and abundance changes of mTOR under the defined conditions. High-content analysis of single-cell data revealed that mTOR complex 1 changes its subcellular position in a temporally synchronized process with lysosomes during adipogenesis. (See pp. E4143–E4150.)

Structure and function of the yeast listerin (Ltn1) conserved N-terminal domain in binding to stalled 60S ribosomal subunits

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The listerin (Ltn1) E3 ubiquitin ligase ubiquitylates and promotes degradation of aberrant nascent chains that become stalled on ribosomal 60S subunits. Ltn1-dependent nascent chain ubiquitylation was reconstituted in vitro using extracts of genetically manipulated *Neurospora* strains. Such extracts, supplemented or not with recombinant factors (such as Ltn1 from *Saccharomyces cerevisiae*), represent a new system to study ribosome-associated protein quality control. Utilizing this system, we show that mutations in Ltn1's conserved N-terminal domain result in defective 60S binding and nascent chain ubiquitylation, without affecting Ltn1's intrinsic E3 activity. Furthermore, we have solved the crystal structure of Ltn1's N-terminal domain, which provides detailed information and insights into how Ltn1 interacts with stalled 60S subunits. Our observations shed light on how cells handle protein quality control substrates. (See pp. E4151–E4160.)

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Assignment of function to a domain of unknown function: DUF1537 is a new kinase family in catabolic pathways for acid sugars

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Domain of unknown function (DUF) families constitute 3,892 of the 16,295 families in the Pfam database (release 29.0). Given their biological importance, large-scale strategies are required to accomplish their functional assignments. Here, we illustrate an integrated "genomic enzymology" strategy to identify diverse functions within the DUF1537 family (PF07005). We combined high-throughput ligand screening results for transport system solute binding proteins with the synergetic analysis of sequence similarity networks and genome neighborhood networks to establish that the members of the DUF1537 family are novel ATP-dependent four-carbon sugar kinases. This study illustrates the utility of this strategy and enhances our knowledge of bacterial carbohydrate catabolism. (See pp. E4161–E4169.)

Structural basis for sulfation-dependent self-glycan recognition by the human immune-inhibitory receptor Siglec-8

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Siglec-8 downregulates eosinophil- and mast cell-mediated inflammatory responses upon engagement by specific self-glycans. We used solution NMR spectroscopy to determine the structure of the N-terminal lectin domain of human Siglec-8 in complex with its preferred glycan target 6′-sulfo sialyl Lewis^x. Quantitative binding studies with differently sulfated glycans and structure-based mutants demonstrate that Siglec-8 simultaneously recognizes a terminal *N*-acetylneuraminic acid (sialic acid) and an underlying 6-*O*–sulfated galactose, yielding a tight and unique specificity. We offer direct structural and mechanistic insights into how the self-glycan code is deciphered by Siglec-8, emphasize the crucial role of glycan sulfation in immunological control of inflammation, and provide a rational framework for designing Siglec-8 agonists to harness its signaling pathway in allergic and inflammatory disorders. (See pp. E4170–E4179.)

Generalized nucleation and looping model for epigenetic memory of histone modifications

Fabian Erdel and Eric C. Greene

The genome-wide distribution of histone modifications influences cellular processes that involve access to the DNA. How cells establish and maintain these patterns is currently under debate. Understanding the underlying mechanisms is a prerequisite for predicting the cellular response to epigenetic drugs or programmed epigenetic editing. Here we simulated linear and looping-driven propagation mechanisms and compared the results to the spatiotemporal methylation profiles recently observed in fission yeast. We found that looping-driven spreading, which arises from productive collisions among nucleosomes and chromatin-bound histone modifiers, explains several key observations on engineered methylation domains. These findings point to important roles of chromatin-bound enzymes and chromatin dynamics in controlling modification patterns and epigenetic memory, which might not be restricted to repressive histone methylation. (See pp. E4180–E4189.)

Unfolding the mechanism of the AAA+ unfoldase VAT by a combined cryo-EM, solution NMR study

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Cellular function is tightly regulated by large molecular assemblies such as the proteasome, which is involved in the removal of damaged or misfolded proteins. Proteasome substrates are unfolded by complexes such as valosin-containing protein-like ATPase of *Thermoplasma acidophilum* (VAT) via a process that is coupled to ATP hydrolysis. We used a combined electron cryomicroscopy (cryo-EM) and NMR analysis to show that VAT undergoes large, previously unidentified, conformational changes that are essential for substrate unfolding and to suggest a model by which the energy released upon ATP hydrolysis can be coupled to the unfolding process. Our approach demonstrates that cryo-EM/NMR studies offer the exciting potential of obtaining both structural and dynamic information that, together, can provide a detailed understanding of how molecular machines function. (See pp. E4190–E4199.)

Neighboring genes for DNA-binding proteins rescue male sterility in *Drosophila* hybrids

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Hybrid sterility is a frequent outcome of crosses between closely related plant and animal species because of incompatibilities that have evolved in the parental genomes. Here, we show that a small region associated with hybrid male sterility between two closely related species of *Drosophila* contains two genes, both encoding DNA-binding proteins, each of which contributes to the hybrid male sterility. These results emphasize that hybrid incompatibility between well-established species is the result of numerous genetic factors, each contributing quantitatively to the incompatibility. Among these factors, DNA-binding proteins are disproportionately represented. Each incompatibility is complex, resulting from interactions between nucleotide sites in different regions of the gene, and is likely to have evolved long after the initial establishment of reproductive isolation. (See pp. E4200–E4207.)

Ascorbate attenuates pulmonary emphysema by inhibiting tobacco smoke and Rtp801-triggered lung protein modification and proteolysis

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Tobacco smoking causes emphysema, a fatal disease involving extensive structural damage of the lung. Besides directly oxidizing lung proteins, tobacco smoke activates Rtp801, a proinflammatory cellular factor that induces overproduction of NO by inducible NOS and consequent lung protein nitration and damage. Such oxidized or nitrated lung proteins become susceptible to breakdown by lung proteases causing emphysema. On interacting with the antioxidant vitamin C, tobacco smoke loses its ability to cause lung protein oxidation or damage, indicating that its oxidant(s) are primarily responsible for triggering lung injury. Vitamin C provides comprehensive protection against tobacco smoke-induced emphysema by attenuating direct damage of the lung by its oxidant(s) and their ability to activate cellular damage machineries including Rtp801 and lung proteases. (See pp. E4208–E4217.)

piRNA pathway is not required for antiviral defense in *Drosophila melanogaster*

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In animals, one of the main forms of RNA interference involves Piwi-interacting RNAs (piRNAs), which protect genomes against the activity of transposable elements. Several groups have recently described piRNAs from viruses in mosquitoes and suggested their involvement in antiviral defense. To understand the extent to which the piRNA pathway contributes to antiviral defense in insects, we used *Drosophila melanogaster* and different viruses. Using high-throughput sequencing, we were unable to find any evidence of piRNAs from viruses in flies. Furthermore, flies lacking components of the piRNA pathway were not unusually susceptible to viral infection. Taken together, our results indicate that fundamental differences have arisen between the antiviral defenses of flies and mosquitoes since they last shared a common ancestor >200 Mya. (See pp. E4218–E4227.)

Deletion of a dehydratase important for intracellular growth and cording renders rough *Mycobacterium abscessus* avirulent

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Mycobacterium abscessus is currently the most frequently isolated rapid-growing mycobacterium in human pathology and is responsible for devastating pulmonary infections in cystic fibrosis patients. It commutes from a nonvirulent smooth to a virulent rough morphotype. The latter produces characteristic serpentine cords that often associate with severe infections, but the molecular basis and contribution of cording in the physiopathology of the infection remain obscure. Herein, we characterized a dehydratase and found it to be required for cording. We demonstrate that the absence of this dehydratase correlates with an extremely attenuated phenotype in immunocompetent and immunocompromised zebrafish. Therefore, targeting the dehydratase may open the way to antivirulence strategies to control *M. abscessus*, notorious for being one of the most drug-resistant mycobacterial species. (See pp. E4228–E4237.)

Mapping transcription factor interactome networks using HaloTag protein arrays

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Using a newly developed technology, HaloTag nucleic acid programmable protein array (HaloTag-NAPPA), we increase the capacity of in situ protein microarray technology several-fold, such that proteome-scale screening becomes feasible. Many examples of novel protein–protein interactions (PPIs) among plant signaling pathways were observed. With few exceptions, nearly all of these connections are undocumented in the existing literature. This study has resulted in an important new resource for the plant biology community—a plant transcription factor-anchored protein–protein interaction network map. Such transcription factor- and transcriptional regulator-based PPI networks may help in the identification of novel genes for use in the improvement of agronomic traits such as grain quality, disease resistance, and stress tolerance. (See pp. E4238–E4247.)

Methylation interactions in *Arabidopsis* hybrids require RNA-directed DNA methylation and are influenced by genetic variation

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The epigenome influences gene regulation and genome evolution. The DNA methylomes of *Arabidopisis* hybrids are distinct from both parents; however, how the parental methylomes interact in hybrids is poorly understood. We discovered pervasive, nonadditive DNA methylation changes ("methylation interactions") throughout the genome in hybrids of Col and C24 *Arabidopsis* accessions. Methylation interactions correlated with high levels of small interfering RNAs, known components of the RNA-directed DNA methylation (RdDM) pathway. Indeed, abrogation of RdDM activity abolished methylation interactions in filial 1 (F1) hybrids. Methylation interactions have distinct polymorphism frequencies: Regions with increased methylation compared with the parents are highly conserved, whereas regions with decreased methylation are divergent. Our results show that RdDM is required for DNA methylation interactions in hybrids. (See pp. E4248–E4256.)