PNAS Plus Significance Statements

On the existence of thermodynamically stable rigid solids

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While common sense says that solids are rigid, careful arguments show that all solids under infinitesimal strain must eventually flow. Resolution of this paradoxical result lies at the core of our understanding of the behavior of solids under deformation. We provide a framework within which the paradox is reconciled and extract conditions wherein stable, rigid, crystalline solids are possible. Failure of ideal crystals is determined by a kinetic process similar to the decay of supercooled phases following quenches across a first-order phase boundary. This fresh conceptual viewpoint curiously allows us to study failure of perfect crystalline solids in quantitative detail without invoking specifics of many-body, defect–defect interactions, raising hope of a more unified description of materials in the future. (See pp. E4322–E4329.)

Status threat, not economic hardship, explains the 2016 presidential vote

Diana C. Mutz

Support for Donald J. Trump in the 2016 election was widely attributed to citizens who were "left behind" economically. These claims were based on the strong cross-sectional relationship between Trump support and lacking a college education. Using a representative panel from 2012 to 2016, I find that change in financial wellbeing had little impact on candidate preference. Instead, changing preferences were related to changes in the party's positions on issues related to American global dominance and the rise of a majority–minority America: issues that threaten white Americans' sense of dominant group status. Results highlight the importance of looking beyond theories emphasizing changes in issue salience to better understand the meaning of election outcomes when public preferences and candidates' positions are changing. (See pp. E4330–E4339.)

Rapid acquisition and model-based analysis of cell-free transcription–translation reactions from nonmodel bacteria

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Nonmodel bacteria have essential roles to play in the future development of biotechnology by providing new sources of biocatalysts, antibiotics, hosts for bioproduction, and engineered "living therapies." The characterization of such hosts can be challenging, as many are not tractable to standard molecular biology techniques. This paper presents a rapid and automated methodology for characterizing new DNA parts from a nonmodel bacterium using cell-free transcription–translation. Data analysis was performed with Bayesian parameter inference to provide an understanding of gene-expression dynamics and resource sharing. We suggest that our integrated approach is expandable to a whole range of nonmodel bacteria for the characterization of new DNA parts within a native cell-free background for new biotechnology applications. (See pp. E4340–E4349.)

Characterization and engineering of a plastic-degrading aromatic polyesterase

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Synthetic polymers are ubiquitous in the modern world but pose a global environmental problem. While plastics such as poly(ethylene terephthalate) (PET) are highly versatile, their resistance to natural degradation presents a serious, growing risk to fauna and flora, particularly in marine environments. Here, we have characterized the 3D structure of a newly discovered enzyme that can digest highly crystalline PET, the primary material used in

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the manufacture of single-use plastic beverage bottles, in some clothing, and in carpets. We engineer this enzyme for improved PET degradation capacity and further demonstrate that it can also degrade an important PET replacement, polyethylene-2,5-furandicarboxylate, providing new opportunities for biobased plastics recycling. (See pp. E4350–E4357.)

Elucidation of the trigonelline degradation pathway reveals previously undescribed enzymes and metabolites

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The experimental dissection of novel metabolic pathways, from genes and enzymes to metabolites, is a key issue for improving our knowledge of the enzymatic capabilities of the microbial world and providing accurate functional annotation of genomes. We used an integrative methodology combining the phenotyping of a complete genome-scale mutant collection of *Acinetobacter baylyi* ADP1 with an untargeted liquid chromatography/MS-based approach to uncover the degradation pathway of trigonelline (TG), a widespread osmolyte. We provide extensive information about this unusual *N*-heterocyclic aromatic degradation route that expands the metabolite repertoire. The occurrence of conserved gene clusters for TG dissimilation in soil, plant-associated, and marine bacteria underlines its environmental abundance. (See pp. E4358–E4367.)

Live-cell analysis of endogenous GFP-RPB1 uncovers rapid turnover of initiating and promoter-paused RNA Polymerase II

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Transcription by RNA Polymerase II (Pol II) is a highly dynamic process that is tightly regulated at each step of the transcription cycle. We generated GFP-RPB1 knockin cells and developed photobleaching of endogenous Pol II combined with computational modeling to study the in vivo dynamics of Pol II in real time. This approach allowed us to dissect promoter-paused Pol II from initiating and elongating Pol II and showed that initiation and promoter proximal pausing are surprisingly dynamic events, due to premature termination of Pol II. Our study provides new insights into Pol II dynamics and suggests that the iterative release and reinitiation of promoter-bound Pol II is an important component of transcriptional regulation. (See pp. E4368–E4376.)

Myosin IIA interacts with the spectrin-actin membrane skeleton to control red blood cell membrane curvature and deformability

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The biconcave disk shape and deformability of the mammalian RBC are vital to its circulatory function and rely upon a 2D viscoelastic spectrin–F-actin network attached to the membrane. A role for nonmuscle myosin II (NMII) contractility in generating tension in this network and controlling RBC shape has not been tested. We show that NMIIA forms bipolar filaments in RBCs, which associate with F-actin at the membrane. NMIIA motor activity regulates interactions with the spectrin–F-actin network to control RBC biconcave shape and deformability. These results provide a previously undescribed mechanism for actomyosin force generation at the plasma membrane, and may apply to spectrin–F-actin–based membrane skeleton networks in other cell types, such as neurons and polarized epithelial cells. (See pp. E4377–E4385.)

Interaction between cardiac myosin-binding protein C and formin Fhod3

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The actin cytoskeleton in living cells is not static but undergoes dynamic reorganization. Actin-containing thin filaments in cardiac sarcomeres are no exception; they exhibit exchange of actin subunits at the ends within actively contracting cardiomyocytes. Fhod3, an actin organizer in cardiac sarcomeres, is implicated in regulation of actin assembly in cardiomyocytes, although the mechanism is largely unknown. We discovered a direct molecular link between Fhod3 and cMyBP-C, a thick myosin filament-associated protein that modulates myocardial contraction via cross-bridge arrangement. Because Fhod3 adversely affected cardiac function in the absence of cMyBP-C, the interaction may serve to control the Fhod3-mediated actin reorganization at the cross-bridge region. Our results provide insight into actin reorganization in cardiac sarcomeres with implications for cardiac function. (See pp. E4386–E4395.)

Molecular mechanism to recruit galectin-3 into multivesicular bodies for polarized exosomal secretion

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Lacking a canonical signal peptide for translocation into the endoplasmic reticulum, galectin-3 (Gal3) is exported by unconventional secretion to play key roles in various cellular processes of fundamental importance. However, molecular details about the sorting components, the signals involved, and the underlying mechanism have remained elusive. Here, we identify a highly conserved tetrapeptide motif P(S/T)AP in the amino-terminal domain of Gal3 that directly interacts with the endosomal sorting complex required for transport (ESCRT) component Tsg101, resulting in exosomal release. This study thus defines a unique molecular mechanism based on a late domain-like motif known from many viruses by which an endogenous non-ESCRT protein is secreted in exosomes. (See pp. E4396–E4405.)

Spatial and temporal organization of cadherin in punctate adherens junctions

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Adherens junctions (AJs) are major intercellular adhesive structures in vertebrates. Despite the critical role of AJs in tissue integrity and morphogenesis, the detailed organization of their

key protein E-cadherin, inside and outside of AJs, remains controversial. Using superresolution microscopy approaches, we show that AJs can reach more than 1 μm in length and consist of tightly packed E-cadherin clusters with crystal-like density interspersed within sparser cadherin regions. No clusters were found outside of AJs. E-cadherin tracking showed that these crystal-like pAJ clusters are transient and their cadherin is reused for new clusters. Our results thus modify the classical view of AJs by depicting them as mosaics of cadherin clusters, whose short lifetimes enable stable overall morphology combined with rapid internal rearrangements. (See pp. E4406–E4415.)

Unexpected metabolic disorders induced by endocrine disruptors in *Xenopus tropicalis* provide new lead for understanding amphibian decline

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By performing a controlled exposure of an amphibian model to endocrine disruptors (EDs) at concentrations within the range of safe drinking water, we provide evidence of the role played by these widespread contaminants in amphibian population decline through metabolic disruption. In frogs exposed throughout their life cycle, this disruption induces a metabolic syndrome characteristic of a prediabetes state. Exposed animals produce progeny that metamorphose later, are smaller and lighter at the adult stage, and have reduced reproductive success. These transgenerational effects of EDs may impact overwintering survival, recruitment for reproduction, and fitness, each representing possible triggers of population decline. (See pp. E4416–E4425.)

Environmental selection during the last ice age on the mother-to-infant transmission of vitamin D and fatty acids through breast milk

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The frequency of the human-specific *EDAR V370A* isoform is highly elevated in North and East Asian populations. The gene is known to have several pleiotropic effects, among which are sweat gland density and ductal branching in the mammary gland. The former has led some geneticists to argue that the near-fixation of this allele was caused by selection for modulation of thermoregulatory sweating. We provide an alternative hypothesis, that selection instead acted on the allele's effect of increasing ductal branching in the mammary gland, thereby amplifying the transfer of critical nutrients to infants via mother's milk. This is likely to have occurred during the Last Glacial Maximum when a human population was genetically isolated in the high-latitude environment of the Beringia. (See pp. E4426–E4432.)

Recurrent structural variation, clustered sites of selection, and disease risk for the complement factor H (*CFH*) gene family

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Genetic variation of the complement factor H (*CFH*) gene family is associated with several complex diseases. Here, we have performed both long- and short-read sequencing of multiple humans and nonhuman primates in an effort to understand its complex evolutionary history. We find that this locus has evolved predominantly through incomplete segmental duplication and identify recurrent reuse of donor and acceptor duplications leading to *CFHR* fusion genes with diverse functions. Investigation of a large cohort of patients with age-related macular degeneration revealed multiple structural variation breakpoints and mutational burdens that cluster in specific domains of the CFH protein. These domains overlap sites showing signatures of natural selection, providing strong evidence for the shared role of selective pressure on diversity and disease. (See pp. E4433–E4442.)

Interdependent and separable functions of *Caenorhabditis elegans* MRN-C complex members couple formation and repair of meiotic DSBs

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Double-strand breaks (DSBs) are deleterious DNA lesions, and impairment of the DSB repair machinery can lead to devastating diseases, such as Nijmegen Breakage Syndrome (NBS). During meiosis, DSBs represent a "necessary evil": they are required to promote formation of crossovers between homologous chromosomes. Crossovers, in turn, ensure correct chromosome inheritance during gamete formation, which is essential for viability and normal development of embryos. During meiosis, numerous DSBs are actively created, so meiotic cells must ensure that all breaks are properly repaired to ensure crossover formation and restore genomic integrity. Here, we identify *Caenorhabditis elegans* NBS-1 as essential to properly process meiotic DSBs to both promote crossover formation and antagonize an error-prone DSB repair pathway, thereby ensuring faithful chromosome inheritance. (See pp. E4443–E4452.)

Induction of antitumor cytotoxic lymphocytes using engineered human primary blood dendritic cells

Long Wu, Huan Zhang, Yixing Jiang, Robert C. Gallo, and Hua Cheng

The dendritic cell (DC) is the master regulator of host immunity. The results of our study bring significant technical improvements in DC methodology. First, a method was developed to expand primary blood DCs at unlimited amounts. Second, the established DCs are constitutively activated and readily available to prime naïve T cells. Third, the DCs can be genetically modified to deliver given tumor antigens in high efficiency and to express activating molecules in driving simultaneous production of antigen-specific T cells and natural killer (NK) cells. Fourth, introducing two allogeneic DRB1 molecules into the DCs improves generation of tumor antigen-specific T cells. Further, the DC-activated cytotoxic T lymphocytes and NK cells potently suppress tumor growth and metastasis in human lung cancer mouse models. (See pp. E4453–E4462.)

Fully reduced HMGB1 accelerates the regeneration of multiple tissues by transitioning stem cells to G_{Alert}

Geoffrey Lee, Ana Isabel Espirito Santo, Stefan Zwingenberger, Lawrence Cai, Thomas Vogl, Marc Feldmann, Nicole J. Horwood, James K. Chan, and Jagdeep Nanchahal

While stem cell therapy has become the standard of care for hematological disorders, challenges remain for the treatment of solid organ injuries. Targeting endogenous cells would overcome many hurdles associated with exogenous stem cell therapy. Alarmins are released upon tissue damage, and here we describe how upregulation of a physiological pathway by exogenous

administration of a single dose of HMGB1, either locally or systemically, promotes tissue repair by targeting endogenous stem cells. We show that HMGB1 complexed with CXCL12 transitions stem cells that express CXCR4 from G_0 to G_{Alert}. These primed cells rapidly respond to appropriate activating factors released upon injury. HMGB1 promotes healing even if administered 2 wk before injury, thereby expanding its translational benefit for diverse clinical scenarios. (See pp. E4463–E4472.)

Systemic surfaceome profiling identifies target antigens for immune-based therapy in subtypes of advanced prostate cancer

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Advanced prostate cancer is a deadly disease made up of multiple cancer subtypes that evolve during its natural history. Unfortunately, antibody- and cell-based therapies in development that target single tumor antigens found in conventional prostate cancer do not account for this heterogeneity. Here, we show that two major subtypes of advanced prostate cancer, prostate adenocarcinoma (PrAd) and neuroendocrine prostate cancer (NEPC), exhibit distinct cell-surface expression profiles. Integrated analysis of gene expression and cell-surface protein expression of prostate cancer nominated multiple subtype-specific cell-surface antigens. We specifically characterize FXYD3 and CEACAM5 as targets for immune-based therapies in PrAd and NEPC and provide preliminary evidence of the antigen-specific cytotoxic activity of CEACAM5-directed chimeric antigen receptor T cells in NEPC. (See pp. E4473–E4482.)

Reduction in adaptor amounts establishes degradation hierarchy among protease substrates

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A given protease typically degrades multiple substrates that serve different functions and may be required in variable amounts under specific cellular conditions. Some substrates require adaptor proteins to be delivered to the protease. We find that changes in the abundance of an adaptor can lead to differential degradation of substrates that have different affinities for the adaptor. Thus, adaptor amounts can have a broad impact, as illustrated by the increase in antibiotic persister bacteria when the abundance of the ClpS adaptor is decreased. Specific growth conditions decrease ClpS abundance decreasing proteolysis of a subset of ClpSAP substrates. (See pp. E4483–E4492.)

Descending pathway facilitates undulatory wave propagation in *Caenorhabditis elegans* through gap junctions

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A deep understanding of the neural basis of motor behaviors must integrate neuromuscular dynamics, mechanosensory feedback, as well as global command signals, to predict behavioral dynamics. Here, we report on an integrative approach to define the circuit logic underlying locomotion in *Caenorhabditis elegans*. Our combined experimental and computational analyses revealed that (*i*) motor neurons in *C. elegans* function as oscillators; (*ii*) descending interneuron inputs and proprioceptive coupling between motor neurons work synergistically to facilitate the sequential activation of motor neuron activities, allowing bending waves to propagate efficiently along the body. Our work represents a key step toward an integrative view of animal locomotion. (See pp. E4493–E4502.)

ZINC-FINGER interactions mediate transcriptional regulation of hypocotyl growth in *Arabidopsis*

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Light coordinates energy production, growth, and survival throughout plant development. In *Arabidopsis*, light stimulates transcriptional reprogramming during developmental transitions such as photomorphogenesis and flowering through the action of photoreceptors, transcription factors, and signaling components. Here we assign a function to a member of the zinc-finger homeodomain (ZFHD) transcription factor family in regulating light-induced development. Our findings reveal ZFHD10 to be a missing link in understanding how the recently discovered integrator of light and photoperiodic flowering, TANDEM ZINC-FINGER PLUS3 (TZP), controls the expression of growth-promoting transcriptional regulators via direct association with light-regulated promoter elements. Elucidating how such novel protein complexes coordinate gene expression will allow scientists and breeders to optimize plant growth and development in response to unfavorable environmental conditions. (See pp. E4503–E4511.)

An atypical N-ethylmaleimide sensitive factor enables the viability of nematode-resistant *Rhg1* soybeans

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N-ethylmaleimide sensitive factor (NSF) and α-soluble NSF attachment protein (α-SNAP) are key components of vesicle trafficking systems and are conserved across eukaryotes. This study shows that these two essential housekeeping proteins have coevolved toward atypical forms in soybean to confer resistance to a highly damaging nematode pathogen while balancing plant fitness. We report discovery of a naturally occurring NSF variant carrying unusual polymorphisms that enhance interaction with and assuage the cytotoxicity of the *Rhg1* resistance-associated α-SNAPs. Pathogen selection pressure has apparently driven this rewiring of multiple components of the conserved SNARE recycling machinery. Useful introduction of the agriculturally valuable *Rhg1* resistance source into other plants is likely to require a cofunctional NSF protein partner. (See pp. E4512–E4521.)

Abscisic acid-induced degradation of *Arabidopsis* guanine nucleotide exchange factor requires calcium-dependent protein kinases

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Arabidopsis RopGEF1 acts as a negative regulator of signal transduction by the plant hormone abscisic acid (ABA). In turn, ABA treatment causes subcellular translocation and degradation of RopGEF1 protein. Interestingly, PP2C protein phosphatases, the core negative regulators of ABA signal transduction, protect RopGEF1 from degradation. This suggests that protein kinases may be involved in RopGEF1 protein removal. We find that calcium-dependent protein kinases (CPKs) including CPK4 phosphorylate RopGEF1. CPK4 promotes RopGEF1 degradation in *Arabidopsis*. CPK4 also negatively regulates RopGEF1

activities in root hair development. Furthermore, phosphorylation of serine residues at the N terminus of RopGEF1 is important for RopGEF1 degradation. We further discuss possible abiotic stress-triggered repression of plant growth via CPK-mediated removal of RopGEF. (See pp. E4522–E4531.)

Cognitive underpinnings of nationalistic ideology in the context of Brexit

Leor Zmigrod, Peter J. Rentfrow, and Trevor W. Robbins

Belief in rigid distinctions between the nationalistic ingroup and outgroup has been a motivating force in citizens' voting behavior, as evident in the United Kingdom's 2016 EU referendum. We found that individuals with strongly nationalistic attitudes tend to process information in a more categorical manner, even when tested on neutral cognitive tasks that are unrelated to their political beliefs. The relationship between these psychological characteristics and strong nationalistic attitudes was mediated by a tendency to support authoritarian, nationalistic, conservative, and system-justifying ideologies. This suggests flexible cognitive styles are related to less nationalistic identities and attitudes. (See pp. E4532–E4540.)