PNAS Plus Significance Statements

^{15}N detection harnesses the slow relaxation property of nitrogen: Delivering enhanced resolution for intrinsically disordered proteins

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Intrinsically disordered proteins (IDPs) have attracted significant attention due to their roles in crucial cellular processes. NMR is the only technique that allows the study of IDPs at atomic-level resolution. However, narrow chemical shift dispersion, rapid exchange with solvent, and high proline content challenge conventional ^{1}H-detected experiments. Here, we report the development of a suite of 3D experiments based on ^{15}N direct detection that harnesses the slow relaxation and the larger chemical shift dispersion of ^{15}N nuclei for complete backbone assignment of IDPs, including proline residues, which are critical to the study of IDPs. Using this approach, we have assigned the regulatory domain of NFATC2 and have identified a likely mechanism by which 14-3-3 proteins regulate NFAT nuclear translocation. (See pp. E1710–E1719.)

Maturity of nearby faults influences seismic hazard from hydraulic fracturing

Maria Kozłowska, Michael R. Brudzinski, Paul Friberg, Robert J. Skoumal, Nicholas D. Baxter, and Brian S. Currie

Recent studies have focused on how wastewater disposal wells have caused dramatic increases in eastern US earthquakes. We focused instead on less common cases where hydraulic fracturing alone has caused earthquakes and found seismicity separated into two depth zones: a shallow zone on younger faults, with more small-magnitude earthquakes than expected, and a deeper zone on older faults, with larger magnitude earthquakes and seismicity continuing after fracturing stops. Hence, inducing deeper seismicity creates more hazard. Our observations are consistent with prior geologic, laboratory, and theoretical work indicating that age and maturity of faults causes the different seismicity patterns. We utilize data from well operators to demonstrate that both fluid pressure changes and rock stress transfer are needed to explain our observations. (See pp. E1720–E1729.)

Assessment of the Legionnaires' disease outbreak in Flint, Michigan

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Unresolved is the etiology of the 2014–2015 Legionnaires' disease outbreak in Genesee County, MI. Flint is the most populous city in Genesee County, and the outbreak coincided with damaged water infrastructure and the subsequent Flint water crisis. The unprecedented disturbance in water quality within Flint's drinking water distribution system allowed the evaluation of the statistical relationship between free chlorine residual and Legionnaires' disease risk within a full-scale drinking water system. Through the integration of multiple datasets, results from numerous causal inference tests implicate changes in water quality, as reflected by changes in free chlorine residual, in the City of Flint as responsible for the outbreak. These findings provide public health professionals and engineers unparalleled scientific evidence to reduce waterborne disease. (See pp. E1730–E1739.)

A big data analysis of the relationship between future thinking and decision-making

Robert Thorstad and Phillip Wolff

The way that people think about the future can affect their decisions. Our results suggest that individuals who think far into the future make a variety of future-oriented decisions, such as investing in the future and avoiding future harms. Our results also suggest that future thinking may affect decisions by making the future seem more connected to the present. More broadly, our results show the viability of using automated analysis of social media text to measure psychological constructs. Automated analyses of social media are naturalistic (increasing sensitivity to a range of future events), unsolicited (reducing the effects of experimenter prompting), and scalable to millions of tweets generated by tens of thousands of individuals. (See pp. E1740–E1748.)

Uterine influences on conceptus development in fertility-classified animals

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Successful pregnancy establishment requires synchronous interactions of the conceptus with the endometrium of the uterus. This study of pregnancy outcome after assisted reproduction in fertility-classified cattle determined how the uterine environment impacts and programs conceptus survival and development. The study found that ripple effects of dysregulated conceptus–endometrial interactions elicit postelongation pregnancy loss in subfertile animals during the implantation period. This research enhances our understanding of the mechanisms that lead to pregnancy loss in both natural and assisted reproduction and has

wide implications for improving pregnancy success in domestic animals and humans. (See pp. E1749–E1758.)

PARP-1–dependent recruitment of cold-inducible RNA-binding protein promotes double-strand break repair and genome stability

Jung-Kuei Chen, Wen-Ling Lin, Zhang Chen, and Hung-wen Liu

The repair of DNA double-strand breaks (DSBs) entails complex and highly coordinated machinery, the detailed molecular organization of which remains to be fully understood. In this study, the cold-inducible RNA-binding protein (CIRBP) was identified as an active contributor during DSB repair. On DNA damage, CIRBP was found to temporarily accumulate at DNA damage sites through an interaction with poly(ADP ribose) polymerase-1 (PARP-1)-generated poly(ADP-ribose). CIRBP was also shown to modulate association of the MRN (Mre11, Rad50, and NBS1) complex and ATM kinase with chromatin and to reduce the activation of downstream signaling. The complex interactions among CIRBP, PARP-1, ATM kinase, and MRN provide compelling evidence supporting a role for CIRBP in the regulation of DSB repair and genome stability. (See pp. E1759–E1768.)

SLC39A14 deficiency alters manganese homeostasis and excretion resulting in brain manganese accumulation and motor deficits in mice

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Manganese (Mn) is an essential nutrient that is toxic in excess. Exposure to excess Mn can result in Mn accumulation in the brain and neurological and motor disturbances resembling Parkinson disease. Here, we demonstrate that the transmembrane metal-ion transporter solute carrier family 39, member 14 (SLC39A14) is essential for Mn homeostasis. We provide evidence that SLC39A14 is required for efficient Mn uptake by the liver and pancreas, two organs that are known to actively participate in Mn excretion from the body. Accordingly, loss of SLC39A14 impairs Mn excretion, leading to Mn accumulation in the brain and most other extrahepatic tissues. *Slc39a14*-deficient mice, similar to *SLC39A14*-deficient human patients, display motor deficits, and thus offer a convenient model to study Mn/SLC39A14-related neurotoxicity. (See pp. E1769–E1778.)

A posttranslational modification of the mitotic kinesin Eg5 that enhances its mechanochemical coupling and alters its mitotic function

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Members of the kinesin superfamily serve a wide variety of functions, and a dominant narrative for these molecular motors has been that each member of the superfamily is uniquely specialized to serve a very limited set of functions. However, it is now appreciated that many members of this group serve several distinct physiological roles, and it has been unclear how these kinesins accomplish this functional flexibility. In this report, we describe a posttranslational modification of the kinesin 5 family member Eg5 that dramatically alters its chemomechanical behavior to make it function much more efficiently under load and in ensembles. This work provides the biophysical context required to mechanistically understand the effects of modified Eg5 in dividing cells. (See pp. E1779–E1788.)

Single-channel recordings of RyR1 at microsecond resolution in CMOS-suspended membranes

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We present a method for measuring the conductance of ion channels at bandwidths up to 500 kHz by fabricating lipid membranes directly on the surface of a custom amplifier chip. We apply this approach to the RyR1 receptor, enabling us to identify additional closed states for calcium-dependent inactivation at microsecond temporal resolutions. Additional data analysis using extended beta distributions allows us to detect gating events as short as 35 ns, a timescale that approaches that of single-file ion translocation. These measurement techniques hold the promise of reaching timescales for studying the kinetics of ion channels, achievable now only with computer-based molecular dynamics simulations. (See pp. E1789–E1798.)

Lipid bilayer composition modulates the unfolding free energy of a knotted α-helical membrane protein

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Cells in our bodies sense and communicate with the outside world via proteins embedded in membranes that surround the cells. As with all proteins, a fundamental parameter governing their biological function is the inherent, thermodynamic stability of the folded state. Surprisingly, there is no measure of this thermodynamic stability in a lipid membrane for the ubiquitous class of membrane proteins with structures based on α-helices. We remedy this through the study of a protein related to the physiologically important membrane proteins that are partly responsible for transmitting signals in the nervous system. We identify key properties of the surrounding lipid membrane that regulate the thermodynamic stability of the protein. (See pp. E1799–E1808.)

Molecular model of the mitochondrial genome segregation machinery in *Trypanosoma brucei*

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Mitochondrial genome replication and segregation are essential processes in most eukaryotic cells. While replication has been studied in some detail, much less is known about the molecular machinery required to distribute the replicated genomes. Using superresolution microscopy in combination with molecular biology and biochemistry, we show in which order the segregation machinery is assembled and that it is likely assembled de novo rather than in a semiconservative fashion in the single-celled parasite *Trypanosoma brucei*. Furthermore, we demonstrate that the mitochondrial genome itself is not required for assembly to occur. It seems that the physical connection of the mitochondrial genome to cytoskeletal elements is a conserved feature in most eukaryotes; however, the molecular components are highly diverse. (See pp. E1809–E1818.)

Bacteriocyte cell death in the pea aphid/*Buchnera* symbiotic system

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Beneficial symbiotic associations, ubiquitously found in nature, have led to the emergence of eukaryotic cells, the bacteriocytes,

specialized in harboring microbial partners. One of the most fundamental questions concerning these enigmatic cells is how organismal homeostasis controls their elimination. Here we report that aphid bacteriocytes have evolved a form of cell death distinct from the conserved cell-death mechanisms hitherto characterized. This cell-death mechanism is a nonapoptotic multistep process that starts with the hypervacuolation of the endoplasmic reticulum, followed by a cascade of cellular stress responses. Our findings provide a framework to study biological functioning of bacteriocytes and the cellular mechanisms associated with symbiosis and contribute to the understanding of eukaryotic cell-death diversity. (See pp. E1819–E1828.)

General amino acid control in fission yeast is regulated by a nonconserved transcription factor, with functions analogous to Gcn4/Atf4

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Eukaryotic cells respond to stress conditions by down-regulating general translation while selectively activating translation of genes required to cope with the stress (often encoding bZIP-family transcription factors, such as Gcn4 in *Saccharomyces cerevisiae* and Atf4 in mammals). Although the signal transduction pathways that mediate these responses are highly conserved, we report that the downstream transcriptional regulators are not: In the fission yeast *Schizosaccharomyces pombe*, this response is mediated by a GATA-type transcription factor (Fil1). Surprisingly, although Fil1 lacks any sequence homology to Atf4 and Gcn4, it regulates similar genes and is itself regulated in a similar manner. These results suggest that extensive rewiring has taken place during the evolution of this key response and highlights the plasticity of transcriptional networks. (See pp. E1829–E1838.)

Global changes of H3K27me3 domains and Polycomb group protein distribution in the absence of recruiters Spps or Pho

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How Polycomb group (PcG) proteins are precisely recruited to their target genes remains poorly understood. In *Drosophila*, PcG proteins are recruited to Polycomb response elements (PREs), composed of binding sites for multiple DNA-binding proteins. To understand how PcG proteins are recruited to and maintained at PREs, we systematically investigated PcG binding, associated H3K27me3, and transcriptome in wild type and mutants for three PRE-binding proteins. We show two factors are essential for high levels of H3K27me3 in PcG domains. Loss of H3K27me3 does not automatically result in gene expression. Different PREs respond differently to the loss of one factor. Many Polycomb domains contain different types of PREs. The diverse and combinatorial nature of PREs contributes to the remarkable resiliency of PcG repression. (See pp. E1839–E1848.)

16p11.2 transcription factor *MAZ* is a dosage-sensitive regulator of genitourinary development

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Copy number gains and losses have long been studied as the potential causes of various congenital defects. Urogenital birth defects account for as many at 30% of structural anomalies, and the 16p11.2 genetic locus is among the most common copy variant regions. Herein is shown that variation in copy number of the transcription factor *MAZ*, located within the 16p11.2 locus, contributes significantly to a wide range of the urogenital birth defects associated with this genomic syndrome, including structural abnormalities of the developing kidneys, ureters, and bladder. Understanding large chromosomal aberrations by studying the functions of individual developmental genes at each variant locus will pave the way for future targeted therapies in affected pregnancies. (See pp. E1849–E1858.)

Damaging de novo mutations diminish motor skills in children on the autism spectrum

Andreas Buja, Natalia Volfovsky, Abba M. Krieger, Catherine Lord, Alex E. Lash, Michael Wigler, and Ivan Iossifov

Genetics is a major determining factor in autism spectrum disorder (ASD). To date, only the most severe class of de novo mutation, likely gene disruptive (LGD), has been correlated with IQ, a phenotypic characteristic associated with ASD, but not a core feature. A less severe class of de novo mutation, missense, while enriched in individuals with ASD, has been refractory to correlation with any ASD phenotypic feature. In this report, we demonstrate that de novo LGD and missense mutations scored by target gene vulnerability both show significant associations with diminished motor skills. (See pp. E1859–E1866.)

5-Azacytidine prevents relapse and produces long-term complete remissions in leukemia xenografts treated with Moxetumomab pasudotox

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Moxetumomab pasudotox is a fusion protein of an anti-CD22 Fv and *Pseudomonas* exotoxin. It is highly active against leukemia in vitro but acute lymphoblastic leukemia (ALL) patients often are resistant. Studies with cultured cells showed resistance is caused by reduced diphthamide, the intracellular target of *Pseudomonas* exotoxin, but diphthamide is not reduced in most cells from most ALL patients. To study how resistance develops in animals, we injected ALL cells into mice and found that resistant cells occur in discrete bone marrow niches and contain major chromosomal and transcriptional changes. Mice pretreated with 5-azacytidine show greatly improved responses, supporting a trial of the combination in leukemia patients. (See pp. E1867–E1875.)

NAD^+ supplementation normalizes key Alzheimer's features and DNA damage responses in a new AD mouse model with introduced DNA repair deficiency

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Alzheimer's disease (AD) is the most common form of dementia, and there is no cure. DNA repair activity is deficient in AD patient brains, especially DNA polymerase β (Polβ), a key protein in DNA base excision. NAD^+ is a cellular metabolite critical for mitochondrial health and biogenesis, stem cell self-renewal, and neuronal stress resistance. This study shows that NAD^+ levels were decreased in a new AD mouse model with introduced DNA repair deficiency (3xTgAD/Polβ$^{+/-}$), and NAD^+ supplementation with nicotinamide riboside significantly normalized neuroinflammation, synaptic transmission, phosphorylated Tau, and DNA damage as well as improved learning and memory and motor function. This has implications for human AD intervention. (See pp. E1876–E1885.)

Septal cholinergic neurons gate hippocampal output to entorhinal cortex via oriens lacunosum moleculare interneurons

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Memory formation is a complex process that involves information transfer to the hippocampus for temporary storage (i.e., encoding) and the reciprocal circuit that relays the temporary information back to the neocortex for long-term storage (i.e., consolidation). Acetylcholine has been shown to play a critical role in memory function by differentially modulating encoding and consolidation, but the underlying mechanism is yet unclear. We found that acetylcholine suppresses the hippocampus–entorhinal cortex pathway, which is the gateway to the consolidation pathway. We show that this inhibition is mediated by oriens lacunosum moleculare interneurons and that the ablation of these interneurons impairs proper memory encoding. We provide evidence that demonstrates how acetylcholine tones down the memory consolidation pathway for efficient memory encoding. (See pp. E1886–E1895.)

Normal aging induces A1-like astrocyte reactivity

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In aging, the brain becomes vulnerable to injury and cognitive function declines, but the mechanisms responsible are unknown. Astrocytes, the most abundant class of glial cells, are vital for the proper function of the central nervous system, and impairment of astrocyte function has been implicated in disease. Here we perform RNA sequencing of astrocytes from different brain regions across the lifespan of the mouse to identify age-related transcriptional changes that could contribute to cognitive decline. We find that aged astrocytes take on a reactive phenotype characteristic of neuroinflammatory reactive astrocytes, and that microglia play a role in inducing astrocyte activation. The aging astrocyte RNA sequencing profiles provide an important new resource for future studies exploring the role of astrocytes in cognitive decline. (See pp. E1896–E1905.)

Regulation of *Arabidopsis* brassinosteroid receptor BRI1 endocytosis and degradation by plant U-box PUB12/PUB13-mediated ubiquitination

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The brassinosteroid (BR) receptor BRI1 provides a paradigm for understanding receptor-mediated signaling in plants. Different post-translational modifications have been implicated in the regulation of BRI1 activity. Here, we show that BR perception promotes BRI1 association with plant U-box E3 ubiquitin ligases PUB12 and PUB13, which in turn directly ubiquitinate BRI1. Importantly, the BRI1 protein abundance and plasma membrane-residence time are increased while the endosomal pool of BRI1 is reduced in the *pub12pub13* mutant, indicating that PUB12/PUB13-mediated ubiquitination regulates BRI1 endocytosis and degradation. BRI1 phosphorylates PUB13 on a specific residue to enhance its association with BRI1, suggesting a unique regulatory circuit of phosphorylation-regulated E3 ligase–substrate association. Our study elucidates a mechanism of BRI1 internalization through E3 ubiquitin ligase-mediated ubiquitination. (See pp. E1906–E1915.)

Circadian clock-dependent and -independent posttranscriptional regulation underlies temporal mRNA accumulation in mouse liver

Jingkui Wang, Laura Symul, Jake Yeung, Cédric Gobet, Jonathan Sobel, Sarah Lück, Pål O. Westermark, Nacho Molina, and Felix Naef

Rhythms in gene expression propelled by the circadian clock and environmental signals are ubiquitous across cells and tissues. In particular, in mouse tissues, thousands of transcripts show oscillations with a period of 24 hours. Keys question are how such rhythms propagate and eventually exert functions, but also how these are generated. Here, we developed a mathematical model based on total RNA-seq to classify genes according to the respective contributions of transcriptional and posttranscriptional regulation toward mRNA expression profiles. We found that about one-third of rhythmically accumulating mRNA are under post-transcriptional regulation. Such regulation is only partially dependent on the circadian clock, showing that systemic pathways and feeding patterns contribute important posttranscriptional control of gene expression in liver. (See pp. E1916–E1925.)

Endocytosis as a stabilizing mechanism for tissue homeostasis

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Many tissues in the body constantly turn over as cells divide and are replaced within weeks. Despite this turnover, tissues are able to keep proper ratios of their different cell types. How tissues attain this balance, called homeostasis, is unclear. Here we show that homeostasis can be achieved by circuits of cells that signal to each other using diffusible signals. A key negative feedback loop that stabilizes these circuits is endocytosis, a common feature of biological signaling in which a cell takes up and degrades the signal molecule that makes it divide and survive. Thus, the more of that cell type the less its numbers increase. (See pp. E1926–E1935.)