PNAS Plus Significance Statements

Histone tails decrease N7-methyl-2′-deoxyguanosine depuration and yield DNA–protein cross-links in nucleosome core particles and cells

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DNA modification gives rise to diverse outcomes, including cancer and cell death. Understanding the chemical and biochemical effects of DNA modification contributes to the fundamental understanding of the etiology and treatment of cancer. 2′-Deoxyguanosine methylation at the N7 position (MdG) is a major mechanism of action of some DNA alkylating agents. We examined MdG in nucleosome core particles (NCPs). Abasic site formation from MdG is suppressed in NCPs. Furthermore, MdG and histone proteins form cross-links [DNA–protein cross-links (DPCs)], a deleterious type of DNA damage. DPCs are also formed in cells treated with monofunctional alkylating agent. DPC formation from MdG is a previously unrecognized process that could have significant effects on cells and may play a role in the cytotoxicity of DNA alkylating agents. (See pp. E11212–E11220.)

Simplicial closure and higher-order link prediction

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Networks provide a powerful abstraction for complex systems throughout the sciences by representing the underlying set of pairwise interactions, but much of the structure within these systems involves interactions that take place among more than two nodes at once. While these higher-order interactions are ubiquitous, an evaluation of the basic properties and organizational principles in such systems is missing. Here we study 19 datasets from biology, medicine, social networks, and the web and characterize how higher-order structure emerges and differs between domains. We then propose a general framework for evaluating higher-order data models based on link prediction, a task in which we seek to predict future interactions from a system's structure and past history. (See pp. E11221–E11230.)

Remote optimization of an ultracold atoms experiment by experts and citizen scientists

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The emerging field of gamified citizen science continually probes the fault line between human and artificial intelligence. A better understanding of citizen scientists' search strategies may lead to cognitive insights and provide inspiration for algorithmic improvements. Our project remotely engages both the general public and experts in the real-time optimization of an experimental laboratory setting. In this citizen science project the game and data acquisition are designed as a social science experiment aimed at extracting the collective search behavior of the players. A further understanding of these human skills will be a crucial challenge in the coming years, as hybrid intelligence solutions are pursued in corporate and research environments. (See pp. E11231–E11237.)

Training in cognitive strategies reduces eating and improves food choice

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Despite public health interventions, most individuals in the United States are overweight or obese. Here, we provide evidence for a mechanism-based technique to improve food choices in an "obesogenic" environment filled with temptation. Across two studies, we demonstrate that cognitive strategies decrease craving for unhealthy foods, increase craving for healthy foods, and modulate subjective valuation. Importantly, across four additional studies we show that brief training in such cognitive strategies increases subsequent healthy food choices in the presence of unhealthy alternatives, without explicit instructions to use the strategies, and across individual differences in weight. Furthermore, this training significantly reduces food consumption. Thus, training in cognitive strategies might ultimately advance clinical treatment and public health interventions aiming to prevent and reduce obesity. (See pp. E11238–E11247.)

Bronze Age population dynamics and the rise of dairy pastoralism on the eastern Eurasian steppe

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Since the Bronze Age, pastoralism has been a dominant subsistence mode on the Western steppe, but the origins of this tradition on the Eastern steppe are poorly understood. Here we investigate a putative early pastoralist population in northern Mongolia and find that dairy production was established on the Eastern steppe by 1300 BCE. Milk proteins preserved in dental calculus indicate an early focus on Western domesticated ruminants rather than local species, but genetic ancestry analysis indicates minimal admixture with Western steppe herders, suggesting that dairy pastoralism was introduced through adoption by local hunter-gatherers rather than population replacement. (See pp. E11248–E11255.)

Polygenic adaptation and convergent evolution on growth and cardiac genetic pathways in African and Asian rainforest hunter-gatherers

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The "pygmy" phenotype is a classic example of convergent adaptation in humans, having evolved independently in both African and Asian rainforest hunter-gatherers. By focusing on indications of subtle allele-frequency changes occurring in aggregate across variants in many genes (polygenic adaptation), we observed signatures of positive natural selection on the same growth-related pathways in rainforest hunter-gatherer populations from both continents. Unexpectedly, we also observed signatures of convergent positive selection on heart development pathway genes. We hypothesize that the heart pathway result may reflect compensatory changes following height-related adaptation in the GH/IGF1 pathway, which in addition to general growth processes also affects heart development. Our results exemplify the insights that can be gained from comparative studies of diverse human populations. (See pp. E11256–E11263.)

Histone H3 lysine 4 methylation signature associated with human undernutrition

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The early life environment can exert a profound effect on long-term health. However, differences in developmental epigenetic patterns in response to environmental challenges are not well understood in humans, where nutrient insufficiency and pathogen exposure in early infancy can impact immune system function and metabolic health into adulthood. Here we report a comprehensive global picture of the patterns of the epigenetic modification histone H3 lysine 4 trimethylation (H3K4me3) in undernourished infants and their mothers. Comparisons of the emergent patterns of H3K4me3 within the first year of life reveal large-scale changes consistent with the impact of a poor environment, and uncovered a candidate gene with a role in the response, which was validated in a mouse model. (See pp. E11264–E11273.)

Dynamic interactions of type I cohesin modules fine-tune the structure of the cellulosome of *Clostridium thermocellum*

Anders Barth, Jelle Hendrix, Daniel Fried, Yoav Barak, Edward A. Bayer, and Don C. Lamb

Cellulosomes are large, multienzyme complexes that efficiently degrade plant cell walls. Their central building blocks are cohesin modules that serve as attachment sites for enzymes. We study the dynamic structural organization by investigating a dyad of cohesin modules connected by a flexible linker using a combination of single-molecule FRET experiments and molecular dynamics simulations. We show that cohesin modules engage in intermodular interactions on the submillisecond timescale, which persist in the presence of the catalytic modules of the cellulosome. We propose that cohesin–cohesin interactions are important for the fine-tuning of the structure of cellulosomes for precise positioning of the catalytic enzymes, while their structural flexibility is facilitated by the flexible linkers. (See pp. E11274–E11283.)

Folding pathway of an Ig domain is conserved on and off the ribosome

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Most proteins need to fold into a specific 3D structure to function. The mechanism by which isolated proteins fold has been thoroughly studied by experiment and theory. However, in the cell proteins do not fold in isolation but are synthesized as linear chains by the ribosome during translation. It is therefore natural to ask at which point during synthesis proteins fold, and whether this differs from the folding of isolated protein molecules. By studying folding of a well-characterized protein domain, titin I27, stalled at different points during translation, we show that it already folds in the mouth of the ribosome exit tunnel and that the mechanism is almost identical to that of the isolated protein. (See pp. E11284–E11293.)

Monomerization of far-red fluorescent proteins

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All known naturally occurring red fluorescent proteins (RFPs), a class that is desirable for biological imaging, are tetrameric, limiting their usefulness as molecular fusion tags in in vivo model systems. Here we explore protein variant libraries targeted at monomerizing far-red RFP variants and describe a generalizable method to monomerize RFPs of interest. This method preserves the fluorescence of the molecule throughout its monomerization, in contrast to break–fix methods, allowing selective enrichment of bright, far-red monomeric variants. Furthermore, we report four bright monomeric RFPs here, which are among the most red-shifted of any monomeric *Aequorea victoria*-class FPs. (See pp. E11294–E11301.)

Long-range regulation of p53 DNA binding by its intrinsically disordered N-terminal transactivation domain

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The tumor suppressor p53 plays a central role in mediating the cellular response to stress and DNA damage. p53 is a tetramer containing both structured and intrinsically disordered domains

that function synergistically to regulate p53 activity. Using intein technology and NMR spectroscopy, we show that the disordered N-terminal transactivation domain of p53 makes intramolecular interactions with the structured DNA-binding domain. These interactions impair binding of p53 to nonspecific DNA sequences but not to p53-specific DNA sequences, providing a means whereby p53 can discriminate more effectively between cognate sites and the vastly more abundant noncognate sites in the genome. (See pp. E11302–E11310.)

Chromatin modifiers Mdm2 and RNF2 prevent RNA:DNA hybrids that impair DNA replication

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Accurate DNA replication is a prerequisite for cell proliferation and genetic stability, but obstacles to smooth replication fork progression are frequent. The oncogenic activity of Mdm2 has been largely ascribed to its ability of antagonizing the tumor suppressor p53. This report, however, points out a p53-independent activity of Mdm2 in suppressing R loops, a structure that includes DNA:RNA hybrids and has recently emerged as a key obstacle to DNA replication. Accordingly, Mdm2 is required for sustaining DNA replication. Our results also reveal that Mdm2 and the polycomb repressor complexes act in parallel to not only modify histones but also support DNA replication. Thus, chromatin modifiers that were traditionally implicated in transcription regulation are enabling unperturbed DNA replication as well. (See pp. E11311–E11320.)

Global impacts of chromosomal imbalance on gene expression in *Arabidopsis* and other taxa

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The phenomenon of genetic balance is characterized by the finding that addition or subtraction of a single chromosome to the whole set is more detrimental than altering the dosage of the complete complement. The molecular basis of this principle has not been studied previously in a comprehensive manner. In this study, the set of all five trisomic chromosomes and a ploidy series of diploid, triploid, and tetraploid in *Arabidopsis* were examined for global gene expression modulations. The results indicate an impact of genomic stoichiometry on the landscape of gene expression, which has implications for how gene expression operates, the evolution of duplicate genes, and the underlying basis of quantitative traits. (See pp. E11321–E11330.)

Themis-associated phosphatase activity controls signaling in T cell development

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Thymocyte-expressed molecule involved in selection (Themis) regulates T cell selection. Absence of Themis leads to severely reduced numbers of CD4 and CD8 T cells, indicating a defect in T cell selection. The molecular mechanism of Themis involvement is not clear. Themis was shown to bind to Src-homology domain containing phosphatase-1 (Shp1), which is a known negative regulator of T cell receptor signaling. Here, using a very sensitive technique to measure phosphatase activity from immunoprecipitated proteins, we find that Themis positively regulates

Shp1 phosphatase activity in thymocytes. Shp1 activity is reduced in the absence of Themis, thus providing an explanation for why Themis-deficient thymocytes respond more strongly to positive-selecting ligands, resulting in fewer thymocytes reaching maturity. (See pp. E11331–E11340.)

Relationship between intact HIV-1 proviruses in circulating CD4+ T cells and rebound viruses emerging during treatment interruption

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The HIV-1 latent reservoir is the major barrier to cure. Analysis of the replication competent latent reservoir that can be induced in viral outgrowth assays (VOAs) showed little or no overlap with HIV viruses that emerge in plasma after treatment interruption. To determine whether intact proviruses amplified from DNA are more closely related to rebound viruses than those obtained from VOA, we sequenced HIV proviral genomes from CD4+ T cells of individuals who underwent analytical treatment interruption. We find that intact proviruses obtained from DNA overlap in part with those obtained by VOA, but do not overlap with rebound viruses. However, nearly half of all rebound sequences could be accounted for in part by recombination of intact near full-length sequences. (See pp. E11341–E11348.)

Genetic variant at coronary artery disease and ischemic stroke locus 1p32.2 regulates endothelial responses to hemodynamics

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Biomechanical stimuli control major cellular functions and play critical roles in human diseases. Although studies have implicated genetic variation in regulating key biological functions, whether human genetic variants participate in the processes by which cells sense and respond to biomechanical cues remains unclear. This study provides a line of evidence supporting an underappreciated role of genetic predisposition in cellular mechanotransduction. Using genetics approaches and genome editing, our data demonstrate that rs17114036, a common noncoding polymorphism implicated in coronary artery disease and ischemic stroke by genome-wide association studies, dynamically regulates endothelial responses to atherosclerosis-related blood flow (hemodynamics) via a noncoding DNA region important for transcription activation (enhancer). These results provide molecular insights linking disease-associated genetic variants to cellular mechanobiology. (See pp. E11349–E11358.)

Permanent neuroglial remodeling of the retina following infiltration of CSF1R inhibition-resistant peripheral monocytes

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This work contributes to the understanding of the enigmatic progressive retinal damage following acute ocular surface injury. Clinical findings in patients suggest that such injuries can adversely affect the retina. This study demonstrates that corneal injury leads to rapid infiltration of blood-derived monocytes into the retina and to subsequent remodeling of the neuroglial system. In contrast to previously held belief, this study shows that

the blood-derived monocytes engraft permanently into the retina and differentiate into microglia-like cells. Although these cells are morphologically indistinguishable from native microglia, they retain a distinct signature and insensitivity to CSF1R inhibition and exhibit a reactive phenotype which persists long after the noxious stimuli is removed, ultimately contributing to progressive neuroretinal degeneration. (See pp. E11359–E11368.)

Role of humoral immunity against hepatitis B virus core antigen in the pathogenesis of acute liver failure

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Hepatitis B virus (HBV)-associated acute liver failure (ALF), also known as fulminant hepatitis B, is a rare but often fatal complication of acute HBV infection. The pathogenesis of ALF is still largely unknown due to the lack of experimental systems and the difficulties in obtaining liver samples. Our comprehensive study of both liver tissue and serum samples from ALF patients using cutting-edge technologies allowed us to identify viral and host factors uniquely associated with this disease. In contrast to classic acute hepatitis B where the liver damage appears to be T cell-mediated, this study demonstrates a major role of the humoral immunity in the pathogenesis of HBV-associated ALF, which may open new avenues for the diagnosis and treatment of this dramatic disease. (See pp. E11369–E11378.)

Epstein–Barr virus enhances genome maintenance of Kaposi sarcoma-associated herpesvirus

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Primary effusion lymphoma (PEL) is a B cell lymphoma; the prognosis is poor, with a median survival around 6 months. PEL is always associated with the presence of Kaposi's sarcoma-associated herpesvirus and in most cases is coinfected with Epstein–Barr virus (EBV); however, the role of EBV in the pathogenesis of the tumor is still not clear. This study demonstrates the intricate interaction between the two herpesviruses, which exacerbate tumorigenesis by mutually reinforcing the persistence of each latent genome and by altering cell proliferation. It establishes curing EBV and inhibiting Epstein–Barr nuclear antigen 1 as a potential treatment for PEL. (See pp. E11379–E11387.)

Klotho controls the brain–immune system interface in the choroid plexus

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Global depletion of klotho accelerates aging, whereas klotho overexpression counteracts aging-related impairments. Why klotho is expressed at much higher levels in the choroid plexus than in other brain regions is unknown. We demonstrate in mice that aging is associated with klotho depletion in the choroid plexus. Reducing klotho selectively within the choroid plexus triggered inflammation within this structure and enhanced activation of innate immune cells within an adjacent brain region following a peripheral immune challenge. In cell culture, we identified a signaling pathway by which klotho suppresses

activation of macrophages. Our findings shed light on klotho functions in the choroid plexus and provide a plausible mechanism by which klotho depletion from this structure promotes brain inflammation during the aging process. (See pp. E11388–E11396.)

Regulatory discrimination of mRNAs by FMRP controls mouse adult neural stem cell differentiation

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Fragile X syndrome (FXS) is the most common form of inherited intellectual disability and autism. FXS results from the loss of functional fragile X mental retardation protein (FMRP), an RNA binding protein involved in translational regulation. However, the impact of FMRP on gene expression has not been evaluated comprehensively. Here, we present simultaneous high-resolution ribosome profiling and RNA-sequencing data from the same wild-type and FMRP-deficient adult neural stem cells. We find remarkable and heretofore unknown forms of regulation by FMRP critical for neural differentiation. Importantly, our data also show that FMRP controls RNA expression in six distinct ways. Thus, we have uncovered a molecular foundation for pathophysiologies associated with FXS. (See pp. E11397–E11405.)

Site occupancy calibration of taxane pharmacology in live cells and tissues

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Taxanes are important for treating cancer and as tools in mitosis research, but mechanistic understanding has been limited by their complex pharmacology. They cause multiple biological actions, and it has been unclear how much drug bound to microtubules is needed for each action. We developed microscopy-based assays for measuring the fraction of specific binding sites occupied by taxanes in living cells as a function of drug concentration, and, in parallel, the different biological activities they cause. These assays will be useful for new drug development and suggest that the most important anticancer action of taxanes is postmitotic micronucleation. (See pp. E11406–E11414.)

Cell size control driven by the circadian clock and environment in cyanobacteria

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When and at what size to divide are critical decisions, requiring cells to integrate internal and external cues. While it is known that the 24-h circadian clock and the environment modulate division timings across organisms, how these signals combine to set the size at which cells divide is not understood. Iterating between modeling and experiments, we show that, in both constant and light–dark conditions, the cyanobacterial clock produces distinctly sized and timed subpopulations. These arise from continuous coupling of the clock to the cell cycle, which, in light–dark cycles, steers cell divisions away from dawn and dusk. Stochastic modeling allows us to predict how these effects emerge from the complex interactions between the environment, clock, and cell size control. (See pp. E11415–E11424.)