PNAS Plus Significance Statements

Bayesian chronological analyses consistent with synchronous age of 12,835–12,735 Cal B.P. for Younger Dryas boundary on four continents

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A cosmic impact event at ~12,800 Cal B.P. formed the Younger Dryas boundary (YDB) layer, containing peak abundances in multiple, high-temperature, impact-related proxies, including spherules, melt glass, and nanodiamonds. Bayesian statistical analyses of 354 dates from 23 sedimentary sequences over four continents established a modeled YDB age range of 12,835 Cal B.P. to 12,735 Cal B.P., supporting synchroneity of the YDB layer at high probability (95%). This range overlaps that of a platinum peak recorded in the Greenland Ice Sheet and of the onset of the Younger Dryas climate episode in six key records, suggesting a causal connection between the impact event and the Younger Dryas. Due to its rarity and distinctive characteristics, the YDB layer is proposed as a widespread correlation datum. (See pp. E4344–E4353.)

Multitarget, quantitative nanoplasmonic electrical field-enhanced resonating device (NE^2RD) for diagnostics

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Biosensing technologies have significant impact on medical diagnostics but difficulties in the handling of complex biospecimens, portability, and nonlinearity in dynamic detection range present considerable technical bottlenecks in their translation into clinical settings. Here, we present the nanoplasmonic electrical field-enhanced resonating device (NE^2RD) that detects and quantifies multiple biotargets from distinct clinical specimens (i.e., saliva, serum, and whole blood) with a broad linear dynamic range. Unlike conventional platforms, the NE^2RD does not require lengthy sample-preparation steps, skilled personnel, or expensive infrastructure. Further, as a model clinical validation study, we monitored chemotherapy effects on viral load for coinfected patients on a single platform. Therefore, the portable NE^2RD can be broadly applied to primary care and point-of-care settings with multiple clinical applications. (See pp. E4354–E4363.)

Baculovirus protein PK2 subverts eIF2α kinase function by mimicry of its kinase domain C-lobe

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Many pathogens use molecular mimicry to subvert key cellular processes of the host. RNA-dependent protein kinase (PKR), a member of the eukaryotic translation initiation factor 2α (eIF2α) kinase family, is an important component of innate immunity in vertebrates and has often been subjected to inhibition by viral mimicry. In this study we show that the paradigm of host–virus mimicry extends to invertebrates where there is no discernable PKR homologue. We characterize an eIF2α kinase-mimic protein called "PK2," encoded by baculoviruses, that inhibits a heme-regulated inhibitor kinase (HRI)-like eIF2α kinase, possibly through a lobe-swap mechanism. The inhibition of the HRI-like kinase confers a growth advantage to the baculovirus during infection of its insect host. These experiments suggest the independent emergence of eIF2α kinase antiviral defense mechanisms in vertebrates and invertebrates. (See pp. E4364–E4373.)

Two transcription pause elements underlie a σ^{70}-dependent pause cycle

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During transcription, RNA polymerase encounters DNA-encoded signals that interrupt RNA synthesis and cause the transcription elongation complex to pause. An important function of transcription pausing in gene regulation is the halting of transcription to facilitate assembly of regulatory factors into complex with RNA polymerase. An example of this function is found in the bacteriophage λ late-gene transcription, in which the initiation factor σ^{70} induces a pause that mediates recruitment of the antitermination factor Q^{λ}, thereby allowing the expression of genes downstream of an intrinsic terminator. In this study, we characterize the contributions of two pause signals to σ^{70}-dependent pause kinetics and propose a comprehensive model for σ^{70}-dependent pausing. (See pp. E4374–E4380.)

Mechanics of torque generation in the bacterial flagellar motor

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Locomotion in many bacterial species is driven by the rotation of one or more long flagellar filaments, each powered by a bacterial flagellar motor (BFM) at its base. The BFM, then, plays a central role in processes such as chemotaxis, bacterial pathogenicity, and biofilm formation. Using information from structural and biophysical experiments on the BFM, we construct a testable model for the mechanism of torque generation. Our model is, to our knowledge, the first to propose and test a specific physical mechanism for this process, and it provides a mechanical explanation for several fundamental properties of the BFM. In addition to fitting current experimental results, model predictions suggest further experiments to shed light on various aspects of motor function. (See pp. E4381–E4389.)

Live-cell superresolution microscopy reveals the organization of RNA polymerase in the bacterial nucleoid

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Transcription is one of the most fundamental processes for life. In eukaryotic cells, transcriptional activity is regulated to a large degree by chromosome packaging. In bacteria, despite the absence of a nuclear envelope and many of the DNA-packaging proteins of eukaryotes, the chromosome is still highly condensed into a structured object, the nucleoid. The spatial organization of transcription within the nucleoid and the effect of transcription on DNA organization remain poorly understood. In this work, we characterize how RNA polymerase accesses transcription sites on DNA, and show that active transcription can cause spatial reorganization of the nucleoid, with movement of gene loci out of the bulk of DNA as levels of transcription increase. (See pp. E4390–E4399.)

Accumulation of non-outer segment proteins in the outer segment underlies photoreceptor degeneration in Bardet–Biedl syndrome

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The photoreceptor outer segment (OS) is a cellular compartment that senses light in the eye. Structural and functional defects in the OS are common causes of inherited blindness. Bardet–Biedl syndrome (BBS) is a human genetic disease associated with defective protein trafficking and blindness. However, it is not well understood why or how photoreceptors die in BBS. In this article, we show that the primary cause of photoreceptor degeneration in BBS is likely aberrant accumulation of non-OS proteins in the OS, which is accompanied by OS disorganization and deficiencies of certain proteins in the cell body, resulting from their sequestration in the OS. Our study provides important clues to the pathogenic mechanisms of BBS and the molecular functions of BBS proteins in vivo. (See pp. E4400–E4409.)

Metabolite profiling stratifies pancreatic ductal adenocarcinomas into subtypes with distinct sensitivities to metabolic inhibitors

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Targeting cancer metabolism requires personalized diagnostics for clinical success. Pancreatic ductal adenocarcinoma (PDAC) is characterized by metabolism addiction. To identify metabolic dependencies within PDAC, we conducted broad metabolite profiling and identified three subtypes that showed distinct metabolite profiles associated with glycolysis, lipogenesis, and redox pathways. Importantly, these profiles significantly correlated with enriched sensitivity to a variety of metabolic inhibitors including inhibitors targeting glycolysis, glutaminolysis, lipogenesis, and redox balance. In primary PDAC tumor samples, the lipid subtype was strongly associated with an epithelial phenotype, whereas the glycolytic subtype was strongly associated with a mesenchymal phenotype, suggesting functional relevance in disease progression. Our findings will provide valuable predictive utility for a number of metabolic inhibitors currently undergoing phase I testing. (See pp. E4410–E4417.)

AAV9 delivering a modified human Mullerian inhibiting substance as a gene therapy in patient-derived xenografts of ovarian cancer

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To improve ovarian cancer patient survival, effective treatments addressing chemoresistant recurrences are particularly needed. Mullerian inhibiting substance (MIS) has been shown to inhibit the growth of a stem-like population of ovarian cancer cells. To test this protein therapeutic, malignant ascites from patients with highly resistant recurrent ovarian cancer were used to create patient-derived ovarian cancer xenografts. Mice bearing tumors were treated with adeno-associated virus serotype 9 gene therapy delivering MIS, which inhibited three of five models without signs of toxicity. Finally, we found that 88% of serous tumors express MIS type II receptor by immunohistochemistry. These preclinical data suggest that gene therapy with MIS provides a potentially well-tolerated and effective treatment strategy for chemoresistant serous ovarian cancer. (See pp. E4418–E4427.)

MIR retrotransposon sequences provide insulators to the human genome

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Insulators are genome sequence elements that help to organize eukaryotic genomes into coherent regulatory domains. Insulators can encode both enhancer-blocking activity, which prevents the interaction between enhancers and promoters located in distinct regulatory domains, and/or chromatin barrier activity that helps to delineate active and repressive chromatin domains. The origins and functional characteristics of insulator sequence elements are important, open questions in molecular biology and genomics. This report provides insight into these questions by demonstrating the origins of a number of human insulator sequences from a family of transposable-element–derived repetitive sequence elements: mammalian-wide interspersed repeats (MIRs). Human MIR-derived insulators are characterized by distinct sequence, expression, and chromatin features that provide clues as to their potential mechanisms of action. (See pp. E4428–E4437.)

Novel serologic biomarkers provide accurate estimates of recent *Plasmodium falciparum* exposure for individuals and communities

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Widely available accurate estimates of malaria exposure are essential for targeting and evaluation of public health interventions. Antibody responses to the malaria parasite can provide information on past exposure, but to date, most such measurements have been based on responses to a small number of parasite proteins chosen by convenience rather than utility and have not provided quantitative information on an individual's exposure. Our results generated by screening hundreds of responses in children with known exposure histories indicate that responses to a few appropriately selected antigens can provide such information. This new approach can be transformed into high-throughput, low-cost, field-based assays useful for surveillance of malaria and has the potential to be translated into similar tools for other infectious diseases. (See pp. E4438–E4447.)

Brd4 bridges the transcriptional regulators, Aire and P-TEFb, to promote elongation of peripheral-tissue antigen transcripts in thymic stromal cells

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Aire is an enigmatic transcription factor that controls immunologic tolerance by inducing, specifically in the thymus, a battery of transcripts encoding proteins not usually encountered until the periphery, thereby promoting negative selection of self-reactive thymocytes and positive selection of regulatory T cells. We document a striking correspondence between those genes induced by Aire and those inhibited by a small-molecule inhibitor of the bromodomain protein Brd4. Aire and Brd4 directly interact, dependent on an orchestrated series of phosphorylation and acetylation events. Aire:Brd4 engagement draws in P-TEFb, mobilizing the transcription and splicing machineries and inducing transcription. Blocking the Aire:Brd4 interaction inhibits negative selection of self-reactive T cells in mice, and point-mutations of Aire that abrogate this association give rise to autoimmune disease. (See pp. E4448–E4457.)

Requirement of Fra proteins for communication channels between cells in the filamentous nitrogen-fixing cyanobacterium *Anabaena* sp. PCC 7120

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Cellular communication along the filaments of heterocyst-forming, nitrogen-fixing cyanobacteria has been discussed for at least 50 y but how this might be accomplished is not fully understood. We recently showed that the septum between heterocysts and vegetative cells is pierced by channels 12 nm in diameter and 20 nm long. Here, we show that three proteins, FraC, FraD, and FraG, participate in the formation of the channels although none of them appears to be a structural component of the channels. Moreover, using gold particle-labeled antibody, FraG was found around the cyanophycin plug as well as associated with the cytoplasmic membrane in the neighborhood of the peptidoglycan that forms the septum. (See pp. E4458–E4464.)

Na, K-ATPase α3 is a death target of Alzheimer patient amyloid-β assembly

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Alzheimer's disease (AD) involves neuron dysfunction and loss. This brain damage is thought to be caused by a small protein, the amyloid β-protein (Aβ), which forms aggregates that are neurotoxic. This neurotoxicity has been explained by multiple mechanisms. We reveal here a new neurotoxic mechanism that involves the interaction between patient-derived Aβ assemblies, termed amylospheroids, and the neuron-specific Na^+/K^+-ATPase α3 subunit. This interaction causes neurodegeneration through pre-synaptic calcium overload, which explains earlier observations that such neuronal hyperactivation is an early indicator of AD-related neurodegeneration. Importantly, amylospheroid concentrations correlate with disease severity and progression in AD patients. Amylospheroid:neuron-specific Na^+/K^+-ATPase α3 subunit interactions may be a useful therapeutic target for AD. (See pp. E4465–E4474.)

Activity-dependent BDNF release via endocytic pathways is regulated by synaptotagmin-6 and complexin

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Brain-derived neurotrophic factor (BDNF) is a secreted neurotrophin known to mediate activity-dependent synaptic plasticity. Endogenously synthesized BDNF is normally stored and transported in dense core vesicles and secreted at synapses in response to activity. However, secreted BDNF may also be endocytosed by neurons and transported within neuronal cytoplasm in the form of endosomes. By monitoring the endocytosed BDNF bound to fluorescent quantum dots (BDNF-QDs), we found that endocytosed BDNF-QDs could be preferentially localized to postsynaptic sites in cultured hippocampal neurons and became exocytosed in response to synaptic activity. This synaptic release of endocytic BDNF requires a synaptotagmin isoform distinct from that regulates the secretion of dense core vesicles, and may serve as a source for activity-dependent secretion of synaptic BDNF. (See pp. E4475–E4484.)

Action potentials and amphetamine release antipsychotic drug from dopamine neuron synaptic VMAT vesicles

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Antipsychotic drugs (APDs) are dopamine (DA) receptor antagonists, whose action has been hypothesized to be affected by acidic trapping in and release by synaptic vesicles. However, acidic trapping of APDs has not been detected directly. Therefore, multiphoton microscopy was used to image an APD directly in living brain slices containing DA neurons. The APD accumulated by acidic trapping, and in the striatum, it was selectively depleted from DA synaptic vesicles by an amphetamine. Action potentials evoked Ca^{2+}-dependent striatal APD depletion, suggesting release by vesicle exocytosis. Therefore, APDs concentrate by acidic trapping in DA synaptic vesicles so that these antagonists will be coreleased with the native transmitter when and where physiological and pharmacological stimuli evoke dopaminergic transmission. (See pp. E4485–E4494.)

Optogenetic determination of the myocardial requirements for extrasystoles by cell type-specific targeting of ChannelRhodopsin-2

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Arrhythmias are potentially life-threatening electrical heart diseases that are difficult to predict and are mostly treated by empirical interventions. The mechanisms of arrhythmia initiation (triggers) are still poorly understood, mostly due to the technical limitations of conventional experimental methods in cardiac electrophysiology. Here, we use optogenetics, a technique based on the expression of photoactivated proteins such as ChannelRhodopsin-2 (ChR2), which allows for non-invasive control of the cell membrane potential through illumination with blue light. By developing mice with cell-specific expression of ChR2, we investigated the role of the different cardiomyocyte types present in the heart and determined the factors triggering arrhythmic beats in the normal heart and during myocardial ischemia, a condition frequently associated with lethal arrhythmias causing sudden cardiac death. (See pp. E4495–E4504.)