PNAS Plus Significance Statements

Estimates of the magnitudes of major marine mass extinctions in earth history

Steven M. Stanley

This paper shows that background extinction definitely preceded mass extinctions; introduces a mathematical method for estimating the amount of this background extinction and, by subtracting it from total extinction, correcting estimates of losses in mass extinctions; presents a method for estimating the amount of erroneous backward smearing of extinctions from mass extinction intervals; and introduces a method for calculating species losses in a mass extinction that takes into account clustering of losses. It concludes that the great terminal Permian crisis eliminated only about 81% of marine species, not the frequently quoted 90–96%. Life did not almost disappear at the end of the Permian, as has often been asserted. (See pp. E6325–E6334.)

Lifespan adversity and later adulthood telomere length in the nationally representative US Health and Retirement Study

Eli Puterman, Alison Gemmill, Deborah Karasek, David Weir, Nancy E. Adler, Aric A. Prather, and Elissa S. Epel

The gradual aging of the immune system is partly marked by shortened telomeres, the DNA–protein caps at the ends of chromosomes that protect genes from degradation. This study undertakes a lifespan approach to stress and leukocyte telomere length in a nationally representative sample of US residents. By using data from 16 y of the Health and Retirement Study, childhood and adulthood life stressors were examined for their individual and combined associations with increased odds of having short telomeres. Accumulated adverse experiences in childhood significantly predicted an increased likelihood of having short telomeres later in life, suggesting a potential pathway through which childhood experiences have been previously shown to predict adulthood morbidity and mortality. (See pp. E6335–E6342.)

Genomic charting of ribosomally synthesized natural product chemical space facilitates targeted mining

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Natural products and their derivatives are essential to the treatment of many diseases. Ribosomally synthesized and posttranslationally modified peptides (RiPPs) are a class of natural products noted for their bioactivities. Genome sequencing has revealed that most natural products remain undiscovered, but the complexity and diversity of RiPPs has challenged the systematic identification of these molecules from genomic data. Here, we present an algorithm for RiPP structure prediction from prokaryotic genomes and systematically investigate the chemical space occupied by genetically encoded RiPPs. We reveal widespread biosynthesis of RiPPs by prokaryotes, identify candidates for targeted discovery, and isolate a RiPP from a rare family. (See pp. E6343–E6351.)

Dissection of molecular assembly dynamics by tracking orientation and position of single molecules in live cells

Shalin B. Mehta, Molly McQuilken, Patrick J. La Riviere, Patricia Occhipinti, Amitabh Verma, Rudolf Oldenbourg, Amy S. Gladfelter, and Tomomi Tani

In living cells, the 3D architecture of molecular assemblies, such as chromosomes, lipid bilayers, and the cytoskeleton, is regulated through the interaction among their component molecules. Monitoring the position and orientation of constituent molecules is important for understanding the mechanisms that govern the structure and function of these assemblies. We have developed an instantaneous fluorescence polarization microscope to track the position and orientation of fluorescently labeled particles, including single molecules, which form micrometer-scale macromolecular assemblies in living cells. Our imaging approach is broadly applicable to the study of dynamic molecular interactions that underpin the function of micrometer-scale assemblies in living cells. (See pp. E6352–E6361.)

Interfibrillar stiffening of echinoderm mutable collagenous tissue demonstrated at the nanoscale

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Collagen plays crucial biomechanical roles in a wide array of animal tissues, but its mechanical properties remain largely static over short timescales. However, echinoderms (sea cucumbers, starfish) are striking exceptions to this rule, having "mutable collagenous tissue" with changeable mechanical properties, enabling complex locomotion, postural maintenance, defense, and reproductive strategies. Using a high-resolution

X-ray probe that measures how the building blocks—fibrils—of echinoderm connective tissue stretch, slide, or reorient in real time, we show that sea cucumbers achieve this remarkable property by changing the stiffness of the matrix between individual fibrils, rather than the properties of the fibrils themselves. Understanding the mechanisms of mutability in this unique tissue may help design novel mechanically tunable synthetic biomaterials. (See pp. E6362–E6371.)

Label-free imaging of the native, living cellular nanoarchitecture using partial-wave spectroscopic microscopy

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Chromatin is one of the most critical structures within the cell because it houses most genetic information. Its structure is well understood at the nucleosomal (<20-nm) and chromosomal (>200-nm) levels; however, due to the lack of quantitative imaging modalities to study this organization, little is known about the higher-order structure between these length scales in live cells. We present a label-free technique, live-cell partial-wave spectroscopic (PWS) microscopy, with sensitivity to structures between 20 and 200 nm that can quantify the nanoarchitecture in live cells. With this technique, we can detect DNA fragmentation and expand on the link between metabolic function and higher-order chromatin structure. Live-cell PWS allows high-throughput study of the relationship between nanoscale organization and molecular function. (See pp. E6372–E6381.)

Wnt/β-catenin signaling promotes self-renewal and inhibits the primed state transition in naïve human embryonic stem cells

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Pluripotent stem cells (PSCs) can exist in a naïve or primed pluripotency state. Naïve PSCs correspond to an earlier developmental state more closely related to cells from the preimplantation embryo and may have more robust developmental potential than primed PSCs. However, understanding the molecular mechanisms that regulate naïve PSC behaviors such as self-renewal, differentiation, or preservation of the naïve state is incomplete. Here, we report that Wnt/β-catenin signaling promotes the self-renewal of naïve human embryonic stem cells (hESCs). When grown in conditions that inhibit Wnt/β-catenin signaling, naïve hESCs remain undifferentiated but have a more primed-like protein expression profile. Our results suggest that Wnt/β-catenin signaling plays a critical role in regulating human naïve pluripotency. (See pp. E6382–E6390.)

Isotopic overprinting of nitrification on denitrification as a ubiquitous and unifying feature of environmental nitrogen cycling

Julie Granger and Scott D. Wankel

Stable isotopes of nitrate have long provided a tool for tracking environmental sources and biological transformations. However, divergent interpretations of fundamental nitrate isotope systematics exist among disciplinary divisions. In an effort to transcend disciplinary boundaries of terrestrial and marine biogeochemistry, we use a quantitative model for coupled nitrogen and oxygen isotopes of nitrate founded on benchmarks established from microbial cultures, to reconcile decades of nitrate isotopic measurements in freshwater and seawater and move toward a unified understanding of cycling processes and isotope systematics. Our findings indicate that denitrification operates within the pervasive context of nitrite reoxidation mechanisms, specifically highlighting the relative importance of nitrification in marine denitrifying systems and anammox in groundwater aquifers. (See pp. E6391–E6400.)

Genetic mapping of male pheromone response in the European corn borer identifies candidate genes regulating neurogenesis

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Most male moths find their mates by following species-specific pheromones released by females. Despite the importance of pheromone communication in reproductive isolation, much is still unknown about its genetic basis. We investigated male responses in the two pheromone races of the European corn borer. A reasonable hypothesis, that males of the two races differ in the genes encoding the receptor proteins that respond to the two pheromone components, was previously rejected without a convincing alternative. We found that instead male choice was correlated with genes affecting growth and differentiation of the nerve cells that may contain these receptors. This unexpected finding resolves the dilemma and points to another layer of complexity in the evolution of sexual pheromone communication systems. (See pp. E6401–E6408.)

Mutational landscape of *EGFR-*, *MYC-*, and *Kras*-driven genetically engineered mouse models of lung adenocarcinoma

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Knowledge of oncogenic alterations that drive lung adenocarcinoma formation has enabled the development of genetically engineered mouse models that are increasingly being used to study the biology and therapeutic vulnerabilities of this disease. Given the importance of genomic alterations in these processes in human lung cancer, information on the mutational landscape of the mouse tumors is valuable for the design and interpretation of these experiments. In this study, we compared whole-exome sequencing data from lung adenocarcinomas induced by different lung adenocarcinoma-associated drivers. In contrast to their human counterparts, oncogene-driven lung adenocarcinomas in genetically engineered mouse models harbor few somatic mutations. These results have important implications for the use of these models to study tumor progression and response and resistance to therapy. (See pp. E6409–E6417.)

Insulin resistance and diabetes caused by genetic or diet-induced KBTBD2 deficiency in mice

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We report an essential regulator of insulin sensitivity. Mutations affecting this protein, KBTBD2, cause severe insulin-resistant

diabetes, lipodystrophy, hepatic steatosis, and growth retardation. KBTBD2 is the substrate recognition subunit of a ubiquitin ligase, and its essential molecular target is p85α, the regulatory subunit of phosphoinositol-3-kinase. KBTBD2 is highly conserved among vertebrates and expressed in liver, brain, muscle, and adipocytes. In the absence of KBTBD2, p85α levels rise 30-fold in adipocytes, interrupting the insulin signal, which is fully restored by p85α knockout. Transfer of KBTBD2-sufficient adipose tissue to KBTBD2-deficient animals rescues insulin-resistant hyperglycemia. Within adipocytes, KBTBD2 expression is markedly down-regulated in response to a high-fat diet. This appears to be an important cause of the insulin resistance caused by obesity. (See pp. E6418–E6426.)

Regulated large-scale nucleosome density patterns and precise nucleosome positioning correlate with V(D)J recombination

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Immunoglobulin and T-cell receptor genes are assembled in lymphoid cells from gene fragments by the process known as V(D)J recombination, which is initiated by the recombination activating gene (RAG)1/RAG2 recombinase. To ensure that recombination occurs only in the correct cell type and at the right developmental stage, multiple layers of regulation are necessary, including specific modifications of chromatin. We show that nucleosome positioning is another important factor in this regulation. Developmentally regulated changes in nucleosome positioning help to guide RAG1/RAG2 to the correct sites in recombinationally active cells. These changes occur on the scale of hundreds of kilobases, a form of regulation not typically seen in the rest of the mammalian genome. (See pp. E6427–E6436.)

Superantigens hyperinduce inflammatory cytokines by enhancing the B7-2/CD28 costimulatory receptor interaction

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Superantigens—bacterial virulence factors—cause toxic shock by hyperinducing inflammatory cytokines. T-cell activation is mediated both by antigen and by interaction between principal costimulatory receptors B7-2 and CD28. Superantigens must bind CD28 to elicit cytokine overexpression through a hitherto unknown mechanism. We show that, by binding not only CD28 but also its coligand B7-2 directly, superantigens potently enhance the B7-2/CD28 interaction, thereby inducing T-cell hyperactivation. Superantigens engage B7-2 and CD28 at their homodimer interfaces, far from where the receptors interact, demonstrating the regulatory properties of these interfaces. B7-2 dimer interface peptides attenuate cytokine overexpression and prevent superantigen lethality by blocking costimulatory receptor engagement by superantigen. Thus, bacterial superantigens induce a pathogenic "cytokine storm" by strongly enhancing formation of the B7-2/CD28 costimulatory axis. (See pp. E6437–E6446.)

Cancer cells enter dormancy after cannibalizing mesenchymal stem/stromal cells (MSCs)

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In many patients with cancer, some tumor cells tolerate conventional treatments and persist for years in an undetectable/dormant state, after which these same cells can mysteriously resume their growth and seed, almost invariably fatal, recurrent cancerous lesions. The therapeutic challenges of tumor dormancy and need to decode the underlying mechanisms involved are apparent. Here, we revealed that mesenchymal stem/stromal cells (MSCs), recognized determinants of breast cancer cell (BCC) behavior, were readily cannibalized by the BCCs they mingled with in 3D cocultures, a process that distinctly altered cancer cell phenotype, suppressed tumor formation, and supported tumor dormancy. Our discoveries provide original insight into the interactions between MSCs and cancer cells, with the potential to identify novel molecular targets for cancer therapy. (See pp. E6447–E6456.)

Activation of Notch1 synergizes with multiple pathways in promoting castration-resistant prostate cancer

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A high nuclear Notch homolog 1, translocation-associated (Notch1) intracellular domain level distinguishes high-risk prostate cancer and castration-resistant prostate cancer from benign and low/intermediate-risk prostate cancer. Chronic activation of Notch1 cooperates with multiple oncogenic pathways altered in early prostate cancer, including AKT, Myc, and Ras/Raf/MAPK, to promote progression to androgen ablation-resistant prostate adenocarcinoma. (See pp. E6457–E6466.)

Epstein–Barr virus microRNAs reduce immune surveillance by virus-specific CD8$^+$ T cells

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Most humans are infected for their lifetime with Epstein–Barr virus (EBV), which can cause cancer and other EBV-associated diseases. Infected individuals develop strong immune responses to this virus, in particular cytotoxic CD8$^+$ T cells, but viral infection is never cleared nor is EBV eliminated from the body. This suggests that certain viral molecules might prevent effective elimination of EBV-infected cells by CD8$^+$ T cells. EBV is rich in genes coding for microRNAs, many with unknown function. We show that viral microRNAs interfere with recognition and killing of EBV-infected cells by CD8$^+$ T cells. Multiple mechanisms and molecules are targeted by microRNAs to achieve this immune evasion. Therefore, targeting of viral microRNAs may improve antiviral immunity and therapy. (See pp. E6467–E6475.)

The Architecture of *Trypanosoma brucei* editosomes

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Trypanosoma brucei is a deadly kinetoplastid parasite that causes the human and veterinarian diseases African sleeping sickness and nagana. We combine chemical cross-linking and mass spectrometry, structural modeling, genetics, and biochemistry to define the global architecture of RNA editing "editosome" complexes in these parasites. Editosomes are unique to kinetoplastids, which also include *Trypanosoma cruzi* and *Leishmania* parasites that cause Chagas disease and Leishmaniases, respectively. Editosomes are essential for parasite viability and are promising drug targets. This work creates a comprehensive, highly detailed map of editosome protein proximities and interactions, and furthers our understanding of editosome protein function in *T. brucei* and other kinetoplastids, thus aiding the potential development of new therapeutics for the treatment of several different parasitic diseases. (See pp. E6476–E6485.)

Allelic barley MLA immune receptors recognize sequence-unrelated avirulence effectors of the powdery mildew pathogen

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Gene-for-gene immunity is frequently found in interactions between plants and host-adapted pathogens and reflects population-level diversification of immune receptors detecting matching pathogen effectors. We identified effector genes of a pathogenic powdery mildew fungus that are recognized by allelic variants of barley intracellular nucleotide-binding domain and leucine-rich repeat protein-type receptors. These pathogen effectors are phylogenetically unrelated, demonstrating that allelic immune receptors can evolve to recognize sequence-unrelated proteins. Conserved effector recognition in distantly related *Arabidopsis* indicates that the underlying mechanism is not restricted to monocotyledonous plants. Furthermore, our study reveals that the expression of a fungal avirulence effector alone is necessary and sufficient for allele-specific mildew resistance locus A receptor activation in planta. (See pp. E6486–E6495.)

Oligodendrocytes contribute to motor neuron death in ALS via SOD1-dependent mechanism

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Oligodendrocytes have been implicated in disease pathology in amyotrophic lateral sclerosis (ALS) using transgenic mouse models. To date there is no human coculture system available to investigate oligodendrocyte involvement in motor neuron (MN) death in ALS. Our data highlight that oligodendrocytes derived from patients with familial and sporadic ALS from induced pluripotent stem cells and induced neural progenitor cells play an active role in MN death. Oligodendrocyte toxicity is mediated through soluble factors and cell-to-cell contact, thus identifying multiple mechanisms of action and therapeutic opportunities. Their pathogenic phenotype can be reversed by achieving superoxide dismutase 1 knockdown in early oligodendrocyte progenitors in both familial and sporadic cases, but not chromosome 9 ORF 72 samples. This study provides important insights for patient subgrouping and timelines for therapeutic approaches. (See pp. E6496–E6505.)

Environmental and genetic factors support the dissociation between α-synuclein aggregation and toxicity

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Many neurodegenerative diseases are characterized by the abnormal accumulation of aggregated proteins in the brain. In Parkinson's disease and related disorders, this process involves the accumulation of α-synuclein (aSyn). Thus, understanding the relationship between aSyn aggregation and pathological conditions is essential for the development of novel and efficient therapies against these disorders. Here, we studied the effects that different aSyn species have on neurons using a combination of neurodegeneration-associated factors: the H50Q aSyn mutant and the presence of copper. Importantly, we demonstrate that exogenous aSyn promotes toxicity and inclusion formation, and that these effects are inversely correlated. Our data shed light onto the pathological mechanisms associated with aSyn aggregation, forming the foundation for future therapeutic strategies. (See pp. E6506–E6515.)

Mushroom spine dynamics in medium spiny neurons of dorsal striatum associated with memory of moderate and intense training

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Interference with activity of cerebral structures, including the dorsal striatum, produces amnesia after moderate levels of training; such interference, nonetheless, becomes ineffective when animals are subjected to intense training procedures. The mechanisms that mediate the protective effect of intense training are unknown. This report shows an increase in relative density of mushroom spines in medium spiny neurons (MSNs) of the dorsal striatum, which depends upon the intensity of training; relative density of mushroom spines in MSNs of nucleus accumbens, on the other hand, is augmented after noncontingent administration of a foot-shock alone. These findings suggest that increased mushroom spinogenesis produced by intense training strengthens memory consolidation and facilitates transfer of information from the dorsal striatum to other cerebral structures. (See pp. E6516–E6525.)

Amyloid-β effects on synapses and memory require AMPA receptor subunit GluA3

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In Alzheimer's disease, soluble clusters of amyloid-β (Aβ) are believed to degrade synapses and impair memory formation. The removal of AMPA receptors from synapses was previously shown to be a critical step in Aβ-driven synapse loss. In this report, we establish that AMPA receptors that contain subunit GluA3 play a central role in Aβ-driven synaptic and memory deficits. Neurons that lack GluA3 are resistant to synaptic weakening and inhibition of synaptic plasticity, and mice that lack GluA3 were resistant to memory impairment and premature mortality. Our experiments suggest that Aβ initiates synaptic

and memory deficits by removing GluA3-containing AMPA receptors from synapses. (See pp. E6526–E6534.)

Bayesian model reveals latent atrophy factors with dissociable cognitive trajectories in Alzheimer's disease

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Alzheimer's disease (AD) affects 10% of the elderly population. The disease remains poorly understood with no cure. The main symptom is memory loss, but other symptoms might include impaired executive function (ability to plan and accomplish goals; e.g., grocery shopping). The severity of behavioral symptoms and brain atrophy (gray matter loss) can vary widely across patients. This variability complicates diagnosis, treatment, and prevention. A mathematical model reveals distinct brain atrophy patterns, explaining variation in gray matter loss among AD dementia patients. The atrophy patterns can also explain variation in memory and executive function decline among dementia patients and at-risk nondemented participants. This model can potentially be applied to understand brain disorders with varying symptoms, including autism and schizophrenia. (See pp. E6535–E6544.)