PNAS Plus Significance Statements

Requirement for transient metal ions revealed through computational analysis for DNA polymerase going in reverse
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DNA polymerases use a general two-metal ion mechanism for DNA synthesis. Recent time-lapse crystallographic studies identified additional adjunct metal ions in the polymerase active site. One of these ions correlates with appearance of pyrophosphate and was proposed to be involved in pyrophosphorolysis (reverse reaction of DNA synthesis). Because DNA polymerases can use pyrophosphorolysis to remove chain-terminating nucleotides during chemotherapy, a better understanding of this reaction is warranted. Through site-directed mutagenesis, pyrophosphorolysis measurements, and computational analysis, we examine the role of metal ions in the reverse reaction. The results indicate that the product-associated metal ion facilitates pyrophosphorolysis during the early stages of the reaction but deters the reaction at later stages, suggesting dynamic metal behavior that can modulate the chemical equilibrium. (See pp. E5228–E5236.)

RNA transcription modulates phase transition-driven nuclear body assembly
Joel Berry, Stephanie C. Weber, Nilesh Vaidya, Mikko Haataja, and Clifford P. Brangwynne

Living cells contain various membraneless organelles whose size and assembly appear to be governed by equilibrium thermodynamic phase separation. However, the dynamics of this process are poorly understood. Here, we quantify the assembly dynamics of liquid-phase nuclear bodies and find that they can be explained by classical models of phase separation and coarsening. In addition, active nonequilibrium processes, particularly rRNA transcription, can locally modulate thermodynamic parameters to stabilize nucleoli. Our findings demonstrate that the classical phase separation mechanisms long associated with nonliving condensed matter can mediate organelle assembly in living cells, whereas chemical activity may serve to regulate these processes in response to developmental or environmental conditions. (See pp. E5237–E5245.)

Caspase 3 cleavage of Pax7 inhibits self-renewal of satellite cells
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Satellite cells form the resident stem cell population in adult skeletal muscle, providing the foundation for postnatal growth and repair of this tissue. Satellite cell self-renewal is maintained by the paired-box transcription factor Pax7, suggesting that this protein is a key determinant in managing cell fate decisions for this niche. Here, we show activation of caspase 3 protease limits satellite cell self-renewal, through targeted cleavage and inactivation of Pax7 in a casein kinase (CK2)-dependent manner. Temporal regulation of caspase 3 activity may offer a robust mechanism to control the satellite cell compartment and enhance skeletal muscle regeneration. (See pp. E5246–E5252.)

Long-term litter decomposition controlled by manganese redox cycling
Marco Keiluweit, Peter Nico, Mark E. Harmon, Jingdong Mao, Jennifer Pett-Ridge, and Markus Kleber

The rate-controlling mechanisms of litter decomposition are of fundamental importance for ecosystem nutrient cycling, productivity, and net carbon (C) balance. Current C cycling models rely primarily on climatic factors and lignin content as the main predictors of litter decomposition rates. Here, we show how the ability of the integrated plant–soil system to promote active redox cycling of manganese (Mn) regulates litter decomposition. Our work suggests that incorporating the coupling of litter decomposition and other elemental cycles, such as the Mn cycle, into conceptual and numerical models may significantly improve our mechanistic understanding and predictions of C cycling in terrestrial ecosystems. (See pp. E5253–E5260.)

MINCR is a MYC-induced IncRNA able to modulate MYC’s transcriptional network in Burkitt lymphoma cells
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Gains of the MYC gene are the most common imbalances in cancer and are associated with poor prognosis, particularly in B-cell lymphoma. Recent advances in DNA sequencing have revealed the existence of thousands of long noncoding RNAs (lncRNAs) with unknown functional relevance. We have here identified a MYC-regulated lncRNA that we named MYC-induced long noncoding RNA (MINCR) that has a strong correlation with MYC expression in cancer. We show that MINCR is functional and controls cell cycle progression by influencing the expression of MYC-regulated cell cycle genes. MINCR is, therefore, a novel player in MYC’s transcriptional network, with the potential to open new therapeutic windows in the fight against malignant lymphoma and, possibly, all cancers that rely on MYC expression. (See pp. E5261–E5270.)
Caenorhabditis elegans ALG-1 antimorphic mutations uncover functions for Argonaute in microRNA guide strand selection and passenger strand disposal

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Loading of Argonautes with the correct strand of the pre-miRNA duplex and disposal of the other strand are essential steps in microRNA biogenesis. Here we report characterization of the protein and microRNA populations associated with mutant ALG-1 Argonautes that are defective in transitioning from microRNA processing to target repression. We show that mutant Argonaute erroneously associates with the normally discarded microRNA* strands, signifying a role for Argonaute ALG-1 in microRNA strand selection. Accumulation of microRNA* is dependent on the microRNA identity, suggesting that specific microRNA features allow wild-type Argonautes to distinguish among different microRNAs. These findings are relevant to understanding Argonaute roles in microRNA biogenesis and, more broadly, to the functions of microRNAs in development and disease. (See pp. E5271–E5280.)

Complexity of the human memory B-cell compartment is determined by the versatility of clonal diversification in germinal centers

Bettina Budeus, Stefanie Schweigle de Reynoso, Martina Przekopowitz, Daniel Hoffmann, Marc Seifert, and Ralf Küppers

The complexity of the human memory B-lymphocyte compartment is a key component to depict and understand adaptive immunity. Despite numerous prior investigations, the generation of certain memory B-cell subsets, the dependency on T-cell help, and the composition, size, and diversity of clonal expansions are either poorly understood or debated. Here we provide an extensive and tightly controlled immunoglobulin heavy chain variable (IGHV) gene repertoire analysis of four main human memory B-cell subpopulations, revealing that an ordered diversification in germinal centers determines a highly versatile memory B-cell compartment in humans with surprisingly many very large B-cell clones. (See pp. E5281–E5289.)

Unique potential of 4-1BB agonist antibody to promote durable regression of HPV+ tumors when combined with an E6/E7 peptide vaccine

Todd Bartkowiak, Shalibala Singh, Guojun Yang, Gloria Galvan, Dhwani Haria, Midan Ai, James P. Allison, K. Jagannadha Sastry, and Michael A. Curran

Nearly all cervical, anal, vulvar, and penile cancer and up to half of oropharyngeal cancers are driven by the E6 and E7 oncoproteins of human papilloma virus (HPV). Therapeutic vaccination against these HPV proteins can slow disease progression in animal models and in patients, but is rarely curative. We demonstrate that coadministration of agonist antibodies targeting the T-cell costimulatory receptor 4-1BB and an intranasal HPV E6/E7 peptide vaccine promoted durable regression in 100% of animals bearing HPV+ TC-1 tumors established in the female reproductive tract. The efficacy of 4-1BB in this system was unique among immune checkpoint antibodies and provides a paradigm for enhancement of therapeutic cancer vaccines with costimulatory agonist antibodies. (See pp. E5290–E5299.)

Hyperglycemia impairs left–right axis formation and thereby disturbs heart morphogenesis in mouse embryos

Masahiro Hachisuga, Shinya Oki, Keiko Kitajima, Satomi Ikuta, Tomoyuki Sumi, Kiyoko Kato, Norio Wake, and Chikara Meno

Epidemiological studies have revealed that pregestational diabetes mellitus increases the risk for congenital anomalies, including congenital heart defects (CHDs). Despite the importance of preventing diabetes-related congenital malformations, however, the underlying pathogenic mechanisms have remained largely unknown. Pregestational diabetes mellitus is associated specifically with CHDs accompanied by heterotaxia. We have now examined left–right (L–R) axis formation in embryos of diabetic female mice as well as in mouse embryos exposed to high-glucose concentrations in culture. We found that high-glucose levels prevent establishment of the L–R axis required for heart morphogenesis and the L–R asymmetry of visceral organs. Such a mechanism may thus explain, at least in part, the CHDs and accompanying heterotaxia in the offspring of diabetic mothers. (See pp. E5300–E5307.)

Evidence for α-synuclein prions causing multiple system atrophy in humans with parkinsonism

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Prions are proteins that assume alternate shapes that become self-propagating, and while some prions perform normal physiological functions, others cause disease. Prions were discovered while studying the cause of rare neurodegenerative diseases of animals and humans called scrapie and Creutzfeldt–Jakob disease, respectively. We report here the discovery of α-synuclein prions that cause a more common neurodegenerative disease in humans called multiple system atrophy (MSA). In contrast to MSA, brain extracts from Parkinson’s disease (PD) patients were not transmissible to genetically engineered cells or mice, although much evidence argues that PD is also caused by α-synuclein, suggesting that this strain (or variant) is different from those that cause MSA. (See pp. E5308–E5317.)

Provirophages in the Bigelowiella genome bear testimony to past encounters with giant viruses

Guillaume Blanc, Lucie Gallot-Lavallée, and Florian Maumus

Virophages are viruses that hijack the replication machinery of giant viruses for their own replication. Virophages negatively impact giant virus replication and improve the survival chances of eukaryotic cells infected by giant viruses. In this study, we identified segments of the Bigelowiella natans genome that originate from virophages and giant viruses, revealing genomic footprints of battles between these viral entities that occurred in this unicellular alga. Interestingly, genes of virophage origin are transcribed, suggesting that they are functional.
We hypothesize that virophage integration may be beneficial to both the virophage and *B. natans* by increasing the chances for the virophage to co-infect the cell with a giant virus prey and by defending the host cell against fatal giant virus infections. (See pp. E5318–E5326.)

**In-depth study of *Mollivirus sibericum*, a new 30,000-y-old giant virus infecting *Acanthamoeba***

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The saga of giant viruses (i.e. visible by light microscopy) started in 2003 with the discovery of Mimivirus. Two additional types of giant viruses infecting *Acanthamoeba* have been discovered since: the Pandoraviruses (2013) and *Pithovirus sibericum* (2014), the latter one revived from 30,000-y-old Siberian permafrost. We now describe *Mollivirus sibericum*, a fourth type of giant virus isolated from the same permafrost sample. These four types of giant virus exhibit different virion structures, sizes (0.6–1.5 m), genome length (0.6–2.8 Mb), and replication cycles. Their origin and mode of evolution are the subject of conflicting hypotheses. The fact that two different viruses could be easily revived from prehistoric permafrost should be of concern in a context of global warming. (See pp. E5327–E5335.)

**Candida albicans** adapts to host copper during infection by swapping metal cofactors for superoxide dismutase

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During infection, the host is known to elevate Cu to attack invading microbes with Cu toxicity. Because Cu is also a micronutrient, pathogens must capture Cu while defending against its toxicity. Here we describe an innovative method by which the fungal pathogen *Candida albicans* adapts to extremes in Cu. Specifically, *C. albicans* maintains its antioxidant defense over a spectrum of Cu conditions by expressing either Cu-dependent or Cu-independent forms of superoxide dismutase (SOD). This switching of fungal SODs becomes prevalent during a mouse model for disseminated candidiasis, where serum Cu rises and kidney Cu declines. The host both elevates and restricts Cu for invading pathogens and *C. albicans* adapts by modulating Cu uptake and metal cofactor selection for SODs. (See pp. E5336–E5342.)

**Population, genetic, and antigenic diversity of the apicomplexan *Eimeria tenella* and their relevance to vaccine development**

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Sixty billion chickens are produced worldwide each year, and all are at risk from *Eimeria*, parasites that cause coccidiosis. Control relies widely on chemophrophylaxis, but pressure to reduce drug use in farming urges development of cost-effective vaccines. Antigens such as apical membrane antigen 1 (AMA1) offer promise as anticoxidial vaccine candidates, but experience with related apicomplexans such as *Plasmodium*, in which pre-existing antigenic diversity and incompatible population structure have undermined vaccine development, tempers confidence. Parasite genotyping identified enormous region-specific variation in haplotype diversity for *Eimeria tenella* but a contrasting low level of polymorphism for *EtAMA1*. Although high levels of polyclonal *Eimeria* infection and hybridization indicate an ability to disseminate vaccine resistance rapidly, the low level of *EtAMA1* diversity promotes vaccine development. (See pp. E5343–E5350.)

**Automated measurement of mouse social behaviors using depth sensing, video tracking, and machine learning**

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Accurate, quantitative measurement of animal social behaviors is critical, not only for researchers in academic institutions studying social behavior and related mental disorders, but also for pharmaceutical companies developing drugs to treat disorders affecting social interactions, such as autism and schizophrenia. Here we describe an integrated hardware and software system that combines video tracking, depth-sensing technology, machine vision, and machine learning to automatically detect and score innate social behaviors, such as aggression, mating, and social investigation, between mice in a home-cage environment. This technology has the potential to have a transformative impact on the study of the neural mechanisms underlying social behavior and the development of new drug therapies for psychiatric disorders in humans. (See pp. E5351–E5360.)

**Ion channel degeneracy enables robust and tunable neuronal firing rates**

Guillaume Drion, Timothy O’Leary, and Eve Marder

Neurons need to be able to tune their firing rates to the input they receive. This requires a complex balance of different kinds of ion channels in the neuronal membrane, and most neurons express many more kinds of ion channels than are strictly necessary to produce spikes. We apply recently developed analysis techniques to uncover a hidden fragility in the spiking properties of neurons. Achieving a smooth relationship between input and output in a neuron is more difficult than previously thought, but reliable spiking rates can be achieved using multiple ion channel types with overlapping or degenerate properties. Our findings therefore suggest that biology exploits degeneracy to solve a difficult physiological tuning problem. (See pp. E5361–E5370.)