PNAS Plus Significance Statements

Self-interrupted synthesis of sterically hindered aliphatic polyamide dendrimers

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Hydrolytically and enzymatically stable nanoscale synthetic constructs, with well-defined structures that exhibit antimicrobial activity, offer exciting possibilities for diverse applications in the emerging field of nanomedicine. Herein, we demonstrate that it is the core conformation, rather than periodicity, that ultimately controls the synthesis of sterically hindered aliphatic polyamide dendrimers. The latter self-interrupt at a predictable low generation number due to backfolding of their peripheral groups, which in turn leads to well-defined nanoarchitectures. (See pp. E2275–E2284.)

Determining climate effects on US total agricultural productivity

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Projections of the economic consequences of climate change are valuable for policy making but generally rely on integrated assessments that cannot account for highly localized climate effects. Most agricultural climate impact studies focus on local effects or partial productivity measures insufficient to capture national economic outcomes. Here, we directly link climate variables in specific US regions to total factor productivity (TFP). We quantify the national economic consequences of past climate variations, identify critical agricultural regions with national significance, and project future changes in TFP under different climate scenarios. We provide a physical understanding of these climate–economic links, show that the agricultural economy is becoming increasingly sensitive to climate, and lay a more concrete foundation for informed decision-making. (See pp. E2285–E2292.)

Multisensor-integrated organs-on-chips platform for automated and continual in situ monitoring of organoid behaviors

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Monitoring human organ-on-a-chip systems presents a significant challenge, where the capability of in situ continual monitoring of organ behaviors and their responses to pharmaceutical compounds over extended periods of time is critical in understanding the dynamics of drug effects and therefore accurate prediction of human organ reactions. In this work, we report a fully integrated modular physical, biochemical, and optical sensing platform, interfaced through a fluidics-routing breadboard with a multi–organ-on-a-chip system to achieve in situ, continual, and automated sensing of microenvironment biophysical and biochemical parameters. It is anticipated that our platform technology that is conveniently compatible with existing organ-on-a-chip models will potentially enhance their performance in drug screening by providing a multitude of sensing data not previously available. (See pp. E2293–E2302.)

Entanglement of quantum clocks through gravity

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We find that there exist fundamental limitations to the joint measurability of time along neighboring space–time trajectories, arising from the interplay between quantum mechanics and general relativity. Because any quantum clock must be in a superposition of energy eigenstates, the mass–energy equivalence leads to a trade-off between the possibilities for an observer to define time intervals at the location of the clock and in its vicinity. This effect is fundamental, in the sense that it does not depend on the particular constitution of the clock, and is a necessary consequence

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of the superposition principle and the mass–energy equivalence. We show how the notion of time in general relativity emerges from this situation in the classical limit. (See pp. E2303–E2309.)

Deep-sea vent phage DNA polymerase specifically initiates DNA synthesis in the absence of primers

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Most DNA polymerases initiate DNA synthesis by extending a preexisting primer. Exceptions to this dogma are recently characterized bifunctional primase–polymerases (prim–pols) that resemble archaeal primases in their structure and initiate DNA synthesis de novo using only NTPs or dNTPs. We report here a DNA polymerase encoded by a phage NrS-1 from deep-sea vents. NrS-1 has a genome organization unlike any other known phage. Although this polymerase does not contain a zinc-binding motif typical for primases, it is nonetheless able to initiate DNA synthesis from a specific DNA sequence exclusively using dNTPs. Thus, it represents a unique de novo replicative DNA polymerase that possesses features found in DNA polymerases, primases, and RNA polymerases. (See pp. E2310–E2318.)

Arsenic trioxide targets MTHFD1 and SUMO-dependent nuclear de novo thymidylate biosynthesis

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We have identified de novo thymidylate biosynthesis as a target of arsenic at exposure levels observed in human populations. Arsenic enhances methylenetetrahydrofolate dehydrogenase 1 (MTHFD1) small ubiquitin-like modifier (SUMO)-ylation and subsequent proteolytic degradation of MTHFD1 and serine hydroxymethyltransferase (SHMT), resulting in depressed rates of de novo thymidylate synthesis, elevated uracil levels in nuclear DNA, and increased genome instability. These findings provide a molecular mechanism linking clastogenic and teratogenic effects of arsenic to impaired de novo thymidylate synthesis. (See pp. E2319–E2326.)

Rab5-regulated endocytosis plays a crucial role in apical extrusion of transformed cells

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At the initial stage of carcinogenesis, transformation occurs in a single cell within the epithelium. However, it is not clearly understood what happens at the interface between the newly emerging transformed cells and the surrounding normal epithelial cells. Here, using mammalian cell culture and zebrafish embryo systems, we demonstrate that Rab5, an important regulator of endocytosis, is accumulated and that endocytosis is enhanced in RasV12-transformed cells surrounded by normal cells. The elevation of endocytosis disrupts E-cadherin–based cell–cell adhesions with the surrounding normal cells and modulates signaling pathways, eventually leading to apical elimination of the transformed cells. This report demonstrates that endocytosis plays a crucial role in cell competition between normal and transformed epithelial cells in mammals. (See pp. E2327–E2336.)

Integrin-β4 identifies cancer stem cell-enriched populations of partially mesenchymal carcinoma cells

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It is widely appreciated that carcinoma cells exhibiting certain mesenchymal traits are enriched for cancer stem cells (CSCs) and can give rise to tumors with aggressive features. Whereas it has been proposed that mesenchymal carcinoma cell populations are internally heterogeneous, the field has made little progress in resolving the specific subtypes of mesenchymal carcinoma cells that pose the greatest risk for patients. We demonstrate the utility of integrin-β4 (ITGB4) in segregating these cells into distinct subpopulations with differing tumor-initiating abilities and pathological behaviors. In addition, we identified mechanistic links between ZEB1 (zinc finger E-box binding homeobox 1) and TAp63α (tumor protein 63 isoform 1) as regulators of ITGB4 expression and demonstrate that ITGB4 can be used as a marker to determine which patients are more likely to relapse after treatment. (See pp. E2337–E2346.)

SMN deficiency in severe models of spinal muscular atrophy causes widespread intron retention and DNA damage

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Spinal muscular atrophy is the leading monogenic cause of infant mortality and is caused by homozygous loss of the survival of motor neuron 1 (*SMN1*) gene. We investigated global transcriptome changes in the spinal cord of inducible SMA mice. SMN depletion caused widespread retention of introns with weak splice sites or belonging to the minor (U12) class. In addition, DNA double strand breaks accumulated in the spinal cord of SMA mice and in human SMA cell culture models. DNA damage was partially rescued by suppressing the formation of R-loops, which accumulated over retained introns. We propose that instead of single gene effects, pervasive splicing defects caused by severe SMN deficiency trigger a global DNA damage and stress response, thus compromising motor neuron survival. (See pp. E2347–E2356.)

Genetic dissection of colorectal cancer progression by orthotopic transplantation of engineered cancer organoids

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Metastasis is the main cause of cancer death, but the underlying mechanisms are largely unknown. Here, we developed an orthotopic organoid transplantation approach and used engineered human colon tumor organoids to study the contribution of common CRC mutations to metastasis. Using this approach, we show that the combination of oncogenic mutations in Wnt, EGFR, P53, and TGF-β signaling pathways facilitates efficient tumor cell migration and metastasis. These mutations allow growth independent of stem cell niche factors, enabling cells to grow at foreign distant sites that lack these factors. Our findings suggest that metastasis is a direct consequence of acquired niche independency. (See pp. E2357–E2364.)

miR-285–Yki/Mask double-negative feedback loop mediates blood–brain barrier integrity in *Drosophila*

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The blood–brain barrier (BBB) is evolutionarily conserved from invertebrates to vertebrates to ensure a well-balanced ionic environment for proper neuronal functions. The Hippo pathway is a highly conserved signaling pathway essential for organ size control and tissue homeostasis. Until now, whether Hippo pathway is required for BBB maintenance has been unknown. We show here that *miR-285* is an upstream regulator of the Hippo pathway, which can directly target Yorkie (Yki) cofactor Multiple Ankyrin repeats Single KH domain (Mask). *miR-285* and Yki/Mask form a double-negative feedback loop to finely tune endoreplication of subperineurial glial (SPG) cells to keep proper cell size and maintain a functional BBB. Our findings propose an exquisite microRNA-mediated regulatory circuit that regulates Hippo signaling activity and tissue homeostasis during development. (See pp. E2365–E2374.)

Massive increase in visual range preceded the origin of terrestrial vertebrates

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Starting 385 million years ago, certain fish slowly evolved into legged animals living on land. We show that eyes tripled in size and shifted from the sides to the top of the head long before fish modified their fins into limbs for land. Before permanent life on land, these animals probably hunted like crocodiles, looking at prey from just above the water line, where the vastly higher transparency of air enabled longdistance vision and selected for larger eyes. The "buena vista" hypothesis that our study forwards is that seeing opportunities far away provided an informational zip line to the bounty of invertebrate prey on land, aiding selection for limbs—first for brief forays onto land and eventually, for life there. (See pp. E2375–E2384.)

The presumed ginkgophyte *Umaltolepis* has seed-bearing structures resembling those of Peltaspermales and Umkomasiales

Fabiany Herrera, Gongle Shi, Niiden Ichinnorov, Masamichi Takahashi, Eugenia V. Bugdaeva, Patrick S. Herendeen, and Peter R. Crane

Understanding the origins of the five groups of living seed plants requires well-supported hypotheses of their relationships to extinct groups, many of which are poorly understood. New information from the Early Cretaceous of Mongolia on the enigmatic extinct plant *Umaltolepis* shows that its leaves are similar to those of *Ginkgo*, but its seed-bearing structures are unique, and more comparable to those of certain extinct Peltaspermales and Umkomasiales. *Umaltolepis* provides new data for understanding relationships among living and fossil seed plants and supports previous ideas that *Ginkgo biloba* may be the sole surviving relict of a once very diverse group of Mesozoic seed plants. (See pp. E2385–E2391.)

Unified reduction principle for the evolution of mutation, migration, and recombination

Lee Altenberg, Uri Liberman, and Marcus W. Feldman

Evolution by Darwinian natural selection can not only shape how organisms survive and reproduce, but also affect transmission of genetic and other information between generations. Modifier-gene models for the evolution of information transmission have revealed a universal tendency for more faithful transmission to evolve in populations at equilibrium where natural selection is balanced by errors in information transmission. This is shown to be a very general property of models that include mutation and migration under selection and recombination under selection on diploids. The breadth of this reduction principle focuses attention on the departures from its mathematical assumptions, which may explain those biological phenomena of information transmission between generations for which the reduction principle fails. (See pp. E2392–E2400.)

Multiple origins of viral capsid proteins from cellular ancestors

Mart Krupovic and Eugene V. Koonin

The entire history of life is the story of virus–host coevolution. Therefore the origins and evolution of viruses are an essential component of this process. A signature feature of the virus state is the capsid, the proteinaceous shell that encases the viral genome. Although homologous capsid proteins are encoded by highly diverse viruses, there are at least 20 unrelated varieties of these proteins. We show here that many, if not all, capsid proteins evolved from ancestral proteins of cellular organisms on multiple, independent occasions. These findings reveal a stronger connection between the virosphere and cellular life forms than previously suspected. (See pp. E2401–E2410.)

Conserved forkhead dimerization motif controls DNA replication timing and spatial organization of chromosomes in *S. cerevisiae*

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The spatial organization of chromatin within the nucleus regulates genomic functions including DNA repair, transcription, and replication. For example, replication origins cluster prior to initiating replication, likely to aggregate the many necessary factors, but the mechanism is poorly understood. We recently discovered yeast "Forkhead Box" (Fox) DNA binding proteins, Forkhead 1 (Fkh1) and Forkhead 2 (Fkh2), as required for this origin clustering and regulation of initiation timing. This study reveals that Fkh1 and Fkh2 share a structural motif that allows dimerization to bring distal DNA binding sites into close proximity. Mutation that disrupts dimerization ablates origin clustering and deregulates origin activation, suggesting causality between origin clustering and initiation control. We propose that Fkh1 and Fkh2 and related Fox proteins in metazoans establish chromatin architecture. (See pp. E2411–E2419.)

Myocardial aging as a T-cell–mediated phenomenon

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Aging is a risk factor for heart diseases, and it is also known to impact on several immunological processes. Nevertheless, most studies addressing the cardio-immune cross-talk have focused on juvenile rather than senescent animal models. In the present study, we addressed this gap and found that immunological activity contributes to myocardial aging. By using different lymphocyte-deficient animal models and heterochronic adoptive cell-transfer protocols, our study revealed a pivotal role for $CD4^+$ T cells in mediating spontaneous local inflammation and mild

organ dysfunction in aged hearts. These results might shed new light on the emerging field of "immunocardiology" because they reveal that spontaneous heart-directed immune responses arise even in the absence of previous myocardial tissue damage. (See pp. E2420–E2429.)

Immune protection against reinfection with nonprimate hepacivirus

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Hepatitis C virus (HCV) displays a narrow species tropism severely hampering development of small animal models that are required for vaccine and pathogenesis studies in vivo. The recent discoveries of HCV-related hepaciviruses in diverse hosts offer new opportunities with respect to the development of an immunocompetent animal model for HCV research. Among the hepaciviruses, the equine nonprimate hepacivirus (NPHV) represents the closest homolog of HCV discovered to date. We defined key aspects of natural immunity to NPHV challenge in the cognate host and provide evidence for natural protection from NPHV infection. Further characterization of the immune signatures that confer protection against NPHV could provide important information that may facilitate the development of new prophylactic strategies including protective vaccines against HCV. (See pp. E2430–E2439.)

Polyphosphate granule biogenesis is temporally and functionally tied to cell cycle exit during starvation in *Pseudomonas aeruginosa*

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Quiescent bacteria are intrinsically resistant to antibiotics and the host immune response. A conserved bacterial starvation survival response is the consumption of ATP to make an inorganic polymer, polyphosphate (polyP), which then forms granule superstructures. PolyP granules occur in all three domains of life, yet how and why cells form these structures is poorly understood. Through high-resolution spatiotemporal characterization of de novo granule genesis, we find that polyP granule synthesis is required for and coordinated with cell cycle exit in the opportunistic pathogen *Pseudomonas aeruginosa*. PolyP has also been functionally connected with the cell cycle in eukaryotes, suggesting that polyP may be a broadly conserved mediator between metabolic state and the cell cycle. (See pp. E2440–E2449.)

Simplified and representative bacterial community of maize roots

Ben Niu, Joseph Nathaniel Paulson, Xiaoqi Zheng, and Roberto Kolter

Many species of microbes colonize plants as members of complex communities. The high complexity of such plant microbial communities poses great difficulty for any experimental analyses aimed at understanding the principles underlying such microbe–plant interactions. In this work, we assembled a greatly simplified, yet representative, synthetic bacterial model community that allowed us to study the community assembly dynamics and function on axenic maize seedlings. This model community interfered with the growth of a plant pathogenic fungus, thus protecting the plant. This model system will prove to be a useful system for future research on plant–microbe interactions. (See pp. E2450–E2459.)

Modular electron-transport chains from eukaryotic organelles function to support nitrogenase activity

Jianguo Yang, Xiaqing Xie, Mingxuan Yang, Ray Dixon, and Yi-Ping Wang

Engineering nitrogenase into cereal crops requires detailed understanding of the components required for efficient nitrogen fixation. We have used a synthetic biology modular approach to evaluate components from chloroplast, root plastids, and mitochondria that function as electron donors to both conventional Mo nitrogenase and the alternative Fe nitrogenase systems. The knowledge obtained in this study not only identifies electron-transfer components from plant organelles that can be used to support nitrogenase activity, but also is likely to enable reduction of the number of target genes required to engineer nitrogen fixation in plants. (See pp. E2460–E2465.)

Amyotrophic lateral sclerosis-linked mutations increase the viscosity of liquid-like TDP-43 RNP granules in neurons

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Mutations in TAR-DNA binding protein 43 (TDP-43), an RNA-binding protein (RBP) with multiple functions in RNA metabolism, cause amyotrophic lateral sclerosis (ALS), but it is uncertain how defects in RNA biology cause disease. Purified RNA-binding protein FUS and heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) form liquid droplets in vitro through liquid–liquid phase separation. However, the biophysical properties of ribonucleoprotein (RNP) granules composed of wild-type (WT) or ALS-linked TDP-43 have not been studied in primary neurons. We show that TDP-43 WT RNP granules exhibit distinct biophysical properties depending on their axonal location, whereas granules formed by ALS-linked mutant TDP-43 are more viscous and show disrupted axonal transport dynamics. We propose the distinct biophysical properties of these neuronal RNP granules may reflect different maturational states and differential propensity for pathological transformation. (See pp. E2466–E2475.)

Synchronous circadian voltage rhythms with asynchronous calcium rhythms in the suprachiasmatic nucleus

Ryosuke Enoki, Yoshiaki Oda, Michihiro Mieda, Daisuke Ono, Sato Honma, and Ken-ichi Honma

The mammalian master circadian clock, the suprachiasmatic nucleus (SCN), contains a network composed of various neuron types. The SCN network plays critical roles in expressing robust circadian rhythms in physiology and behavior, such as sleep–wake cycles. The molecular clock in individual SCN neurons controls membrane excitability, and sends output signals to various organs. However, how the SCN neurons transmit output signals remains unknown. Using a genetically encoded voltage sensor, we directly measured the circadian rhythms of membrane voltage in the SCN network. Remarkably, the circadian voltage rhythms are synchronous across the entire SCN network, whereas simultaneously recorded Ca^{2+} rhythms are asynchronous in the dorsal and ventral SCN regions. These results indicate that the SCN network produces coherent output signals. (See pp. E2476–E2485.)

Novel combinatorial screening identifies neurotrophic factors for selective classes of motor neurons

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Neurotrophic factors are endogenous survival factors for developing neurons during their programmed cell death, and represent therapeutic candidates in several neurodegenerative

diseases. Studies in the developing spinal cord suggest that neurotrophic factors promote the survival of motor neurons in a combinatorial manner. To better understand this, we systematically assayed pairwise combinations of neurotrophic factors (NTFs) on highly standardized motor neuron cultures prepared by a unique FACS technique. Our data unravel potent additivity of three neurotrophic factors due to their specific survival effects on distinct classes of motor neurons innervating different targets. Further analyses are required to better understand combinatorial NTF effects in adulthood and to define optimized NTF combinations for degenerative motor neuron diseases. (See pp. E2486–E2493.)

Correlated variability modifies working memory fidelity in primate prefrontal neuronal ensembles

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The working memory (WM)-related activity in the primate prefrontal cortex (PFC) is hypothesized to arise from the structure of the network in which the neurons are embedded. Recent studies have also shown that it is difficult to predict the properties of neuronal ensembles from the properties of individually examined neurons. By recording the activity of neuronal ensembles in the macaque PFC, we found evidence supporting the network origins of WM activity and discovered features of WM coding in neuronal ensembles that were inaccessible in prior single neuron studies. Most notably, we found that correlated firing rate variability between neurons (i.e., noise correlations) can improve WM coding and that neurons not selective for WM can improve WM coding when part of an ensemble. (See pp. E2494–E2503.)

Deactivation kinetics of acid-sensing ion channel 1a are strongly pH-sensitive

David M. MacLean and Vasanthi Jayaraman

The response from every type of neurotransmitter-gated ion channel (NGIC) decays within a proscribed time when its agonist is removed. NGICs that decay faster are more involved in higher frequency signals, whereas slower channels promote cell excitability and plasticity. Here, we find that certain acid-sensing ion channels have the flexibility to convey either very rapid or quite slow signals, depending on the ambient pH over a very narrow range. This ability may allow these receptors to aid in synaptic plasticity, and hence learning and memory. (See pp. E2504–E2513.)

Mechanisms regulating angiogenesis underlie seasonal control of pituitary function

Jennifer Castle-Miller, David O. Bates, and Domingo J. Tortonese

Adaptation to seasonal changes in the environment is critical for survival in all species. In vertebrates, annual oscillations in pituitary hormones underlie the regulation of seasonal physiology. We found that, in sheep, the duration of pineal melatonin output at night controls the production of different forms of the protein vascular endothelial growth factor (VEGF) within a specific pituitary region, the pars tuberalis. Forms that block blood-vessel growth are made in winter, but those that stimulate it are made in summer. Further to the resulting remodelling of the vascular connection between the brain and pituitary, the temporally divergent VEGF-A variants operate as messenger signals on endocrine cells of a different part of the gland, the pars distalis, to regulate seasonal fertility. (See pp. E2514–E2523.)

Lys49 myotoxin from the Brazilian lancehead pit viper elicits pain through regulated ATP release

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Bites from venomous snakes can inflict substantial pain and inflammatory tissue damage. Snake phospholipase A2 (PLA2), and PLA2-like toxins that retain the PLA2 fold but lack enzymatic activity, are commonly found in snake venoms. In this study, we identify a PLA2-like toxin (BomoTx), from the Brazilian lancehead pit viper (*Bothrops moojeni*), that activates a subpopulation of somatosensory neurons that contribute to pain sensation. We show that BomoTx excites these neurons by stimulating the release of cellular ATP through a mechanism involving pannexin hemichannels. Consequent activation of purinergic receptors elicits acute pain, tissue inflammation, and pain hypersensitivity. Thus, we have elucidated the mechanism of action for a toxin from *Bothrops* snakes, which inflict a majority of bites in Latin America. (See pp. E2524–E2532.)

Auxin response cell-autonomously controls ground tissue initiation in the early *Arabidopsis* embryo

Barbara K. Möller, Colette A. ten Hove, Daoquan Xiang, Nerys Williams, Lorena González López, Saiko Yoshida, Margot Smit, Raju Datla, and Dolf Weijers

Higher plants are built from three major tissue types: epidermis, ground tissue, and vascular tissue. Each of these differentiates into several functionally distinct cell types. Although identity switches for the different cell types within the major three tissues have been identified, mechanisms that trigger the initiation of the three tissues themselves have remained obscure. Auxin response, in particular the auxin-dependent transcription factor MONOPTEROS (MP), plays a critical role in *Arabidopsis* embryonic root initiation. In our study, we identify a set of embryonic MP target genes and show that MP acts as a very first regulator of ground tissue initiation. Moreover, our data provide a framework for the simultaneous formation of multiple cell types by the same transcriptional regulator. (See pp. E2533–E2539.)