PNAS Plus Significance Statements

Pore translocation of knotted DNA rings

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Pore translocation, the driven passage of molecules through narrow channels, has become an important tool for probing DNA properties. In a recent breakthrough experiment, this technique was used to detect knots that form spontaneously in DNA filaments and can hence impact their in vivo functionality. Here, by using an accurate model, we simulate the translocation of knotted DNA, expose its unexpectedly rich phenomenology, and clarify the implications for experiments. We show that knot translocation occurs in two possible modes, depending on the knot initial position and size. These properties also account for the typically late occurrence of the knot passage event. Finally, the passage duration is found to depend more on the translocation velocity of the knot than its size. (See pp. E2991–E2997.)

Statistical significance of seasonal warming/cooling trends

Josef Ludescher, Armin Bunde, and Hans Joachim Schellnhuber

The question whether a seasonal climatic trend (e.g., the increase of spring temperatures in Antarctica in the last decades) is of anthropogenic or natural origin is of great importance because seasonal climatic trends may considerably affect ecological systems, agricultural yields, and human societies. Previous studies assumed that the seasonal records can be treated as independent and are characterized by shortterm memory only. Here we show that both assumptions, which may lead to a considerable overestimation of the trend significance, do not apply to temperature data. Combining Monte Carlo simulations with the Holm–Bonferroni method, we demonstrate how to obtain reliable estimates of the statistical significance of seasonal climatic trends and apply our method to representative atmospheric temperature records of Antarctica. (See pp. E2998–E3003.)

Strategic siting and regional grid interconnections key to low-carbon futures in African countries

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This study identifies, characterizes, and values wind and solar electricity resources for 21 countries in the Eastern and Southern Africa Power Pools. We find that many countries possess potential many times their projected demand. However, because the most competitive wind and solar resources are spatially uneven, international transmission could allow the region as a whole to benefit from "no-regrets" or lowcost, low-impact, and highly accessible resources. International energy trade also lowers system costs by reducing the need for conventional power plants and allows lower impact, more accessible renewable energy sites to be cost competitive. Regional interconnections planned around strategic siting opportunities are crucial for realizing no-regrets wind and solar energy development that can be competitive with conventional generation in African countries. (See pp. E3004–E3012.)

Evolving polycentric governance of the Great Barrier Reef

Tiffany H. Morrison

Global sustainability depends on robust environmental governance regimes. An investigation of the Great Barrier Reef regime between 1975 and 2016 reveals how complex environmental regimes become increasingly structurally dense and eventually reach a point of stabilization. However, structural complexity and stability alone do not necessarily mean the system is robust. Instead, a complex but stable structure can mask exogenous change, which then can generate more endogenous change; this phenomenon has implications for the environmental outcomes of complex regimes. Therefore, it is vital to anticipate and account for change in designing, implementing, and evaluating sustainable environmental governance. (See pp. E3013–E3021.)

High-throughput identification of small molecules that affect human embryonic vascular development

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It is well recognized that several chemicals and/or drugs are potentially harmful if used during pregnancy. Unfortunately, systems capable of predicting which drugs affect embryonic development rely almost exclusively on prenatal animal testing, with all

the associated limitations. Using human pluripotent stem cells, we developed a fully humanized system capable of predicting which drugs affect, specifically, vascular embryonic development. The system was used to screen a library of chemicals (1,280 drugs), and two compounds were identified as specific inhibitors of human embryonic vasculature. The platform described here is a valid alternative to animal testing and can be used to screen existing and newly developed drugs. (See pp. E3022–E3031.)

Mechanism of transcription initiation and promoter escape by *E. coli* RNA polymerase

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The enzyme RNA polymerase (RNAP) transcribes DNA genetic information into RNA. Regulation of transcription occurs largely in initiation; these regulatory mechanisms must be understood. Lifetimes of transcription-capable RNAP-promoter open complexes (OCs) vary greatly, dictated largely by the DNA discriminator region, but the significance of OC lifetime for regulation was unknown. We observe that a significantly longer RNA:DNA hybrid is synthesized before RNAP escapes from long-lived λP_R-promoter OCs as compared with shorter-lived T7A1 promoter OCs. We quantify the free energy needed to overcome OC stability and allow escape from the promoter and elongation of the nascent RNA, and thereby predict escape points for ribosomal (rrnB P1) and lacUV5 promoters. Longer-lived OCs produce longer abortive RNAs, which likely have specific regulatory roles. (See pp. E3032–E3040.)

Structural basis of mitochondrial dysfunction in response to cytochrome *c* phosphorylation at tyrosine 48

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Cell response to physiological changes and oxidative stress involves the modulation of mitochondrial metabolism. Its dysfunction favors the development of hypoxia-dependent pathologies, including ischemia and cancer. A key modulator of mitochondrial activity is cytochrome *c*, whose cell function is regulated by tyrosine phosphorylation. However, how such modification affects cytochrome *c* structure and function is barely known. Here we report that a phosphomimetic mutant of cytochrome *c* exhibits enhanced dynamics, which could be responsible for the observed differences in cytochrome *c* functionality in oxidative stress and cell death. Thus, phosphorylation of cytochrome *c* becomes a target for further development of robust therapeutic approaches. (See pp. E3041–E3050.)

Structures of closed and open states of a voltage-gated sodium channel

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Bacterial voltage-gated sodium channels serve as models of their vertebrate counterparts because they have similar functional components in a simpler structure. We present high-resolution structures of tightly closed and open states. In the closed state, the activation gate fully occludes the conduction pathway, and the intracellular C-terminal domain is revealed as a long four-helix bundle. In the open state, the activation gate has an orifice of ~10 Å. Molecular dynamics simulations confirm that this conformation would allow permeation of hydrated Na^+. These structures are significant advances because they provide a complete closed–open–inactivated conformational cycle in a single voltage-gated sodium channel and give insight into the structural basis for state-dependent binding of sodium channel-blocking drugs. (See pp. E3051–E3060.)

Form and function of topologically associating genomic domains in budding yeast

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In metazoans, topological domains are regions in the genome that more frequently associate with themselves than with neighboring regions. These domains are important for regulating transcription and replication. However, topological domains were thought to be absent in budding yeast. Thus, we did not know the degree of conservation of topological organization and its associated functions. Herein, we describe the existence of topologically associating domains in budding yeast and show that these domains regulate replication timing so that origins within a domain fire synchronously. Our work showing conservation in budding yeast sets the stage to use yeast genetics to interrogate the molecular basis of the topological domains defining genome architecture. (See pp. E3061–E3070.)

Deltex2 represses MyoD expression and inhibits myogenic differentiation by acting as a negative regulator of Jmjd1c

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The data presented address a fundamental mechanism controlling the expression of a master regulator of cellular differentiation, MyoD, by a member of the Deltex family of proteins. We show that MyoD expression is regulated by modulation of histone methylation in its promoter region by the histone demethylase, Jjmd1c. These data provide insight into the epigenetic control of gene expression in the regulation of myogenic differentiation. (See pp. E3071–E3080.)

Spemann organizer transcriptome induction by early beta-catenin, Wnt, Nodal, and Siamois signals in *Xenopus laevis*

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We present a genome-wide study of the signals responsible for the early induction of the body axis in the following experimental conditions: β-catenin morpholino; *Wnt*, *Siamois*, and *Cerberus* mRNAs; LiCl treatment; and dorsal-ventral regenerating half-embryos bisected at gastrula. Comparing 46 RNA-seq libraries, we uncovered the genetic networks that initiate dorsal-ventral patterning and Spemann's organizer formation. We defined an early β-catenin signature that has only minor overlap with recently published late zygotic Wnt signatures. The relation of these early steps of development to endomesodermal germ layer induction was studied by overexpressing the growth factor antagonist Cerberus. This study offers a rich resource for understanding the earliest inductive events in the body plan of a model vertebrate embryo. (See pp. E3081–E3090.)

Metabolic evolution and the self-organization of ecosystems

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Understanding what drives self-organization in complex systems and how it arises is a major challenge. We addressed this challenge using dominant oceanic photosynthetic and heterotrophic microbes as a model system. Reconstructing the metabolic evolution of this system suggests that its self-organization and self-amplification were coupled and driven by an increasing cellular energy flux. Specifically, the evolution of cells steadily increased their metabolic rate and excretion of organic carbon. We describe how this increases cellular nutrient uptake and thereby ecosystem biomass. The release of organic carbon, in turn, promotes positive feedbacks among species that reinforce this evolutionary drive at the ecosystem level. We propose the evolutionary self-organization of oceanic microbial ecosystems contributed to the oxygenation of Earth. (See pp. E3091–E3100.)

Mutational spectra of aflatoxin B_1 in vivo establish biomarkers of exposure for human hepatocellular carcinoma

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Several decades elapse between liver cancer initiation and the appearance of tumors, and there are rarely overt clues that presage the appearance of disease. There is an acute need for biomarkers of incipient carcinogenesis when the disease is clinically addressable. This work used high-fidelity DNA sequencing and a mouse model to reveal high-resolution mutational spectra of the liver carcinogen aflatoxin B_1 in histopathologically normal liver as early as 10 wk after exposure. The spectrum, which is mirrored in human liver tumors, persisted through carcinoma development more than a year later. Identification of tumor mutational spectra in a manipulable animal model affords opportunities for the efficient testing of strategies relevant to early detection, prevention, and management of human cancer. (See pp. E3101–E3109.)

Efficacy, long-term toxicity, and mechanistic studies of gold nanorods photothermal therapy of cancer in xenograft mice

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This is a systematic in vivo study of gold nanorods (AuNRs)-assisted plasmonic photothermal therapy (AuNRs-PPTT) for cancer. We have optimized the properties of our AuNRs and the conditions of PPTT to achieve maximal induction of tumor apoptosis. To examine the molecular mechanisms of action of AuNRs-PPTT, we used quantitative proteomics to study protein expression levels in mouse tumor tissues and found the apoptosis pathway to be significantly perturbed. We report a long-term toxicity study (up to 15 months in the mouse model) that showed no toxicity of the AuNRs. Together, these data suggest that our AuNRs-PPTT has potential as an approach to cancer therapy. (See pp. E3110–E3118.)

Mouse-adapted MERS coronavirus causes lethal lung disease in human DPP4 knockin mice

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Middle East respiratory syndrome, caused by a zoonotically transmitted coronavirus (MERS-CoV), has a high mortality (~36%). Because of limited autopsy data on tissues from MERS fatalities, a small animal model can provide an important tool to better understand the disease. We humanized the mouse locus of the virus receptor DPP4, preserving native DPP4 expression. After inoculating hDPP4 knockin mice with MERS-CoV, there was virus replication without disease. We then generated a mouse-adapted MERS-CoV by serial passage in hDPP4 knockin mice. The resultant virus causes fatal lung disease that includes diffuse alveolar damage and immune dysregulation. Here, we characterize the pathologic features of the model and elucidate key aspects of the immunopathology and factors contributing to virulence. (See pp. E3119–E3128.)

ErbB2 regulates autophagic flux to modulate the proteostasis of APP-CTFs in Alzheimer's disease

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We demonstrate that ErbB2 can regulate autophagic flux through its direct interaction with Beclin-1, which effectively blocks autophagy initiation. Although the expression of ErbB2 becomes dormant during adulthood, it becomes reactivated during the pathogenesis of Alzheimer's disease (AD), blocking the autophagy-mediated clearance of amyloid precursor protein (APP) C-terminal fragments (CTFs) [99-residue CTF (C99)]. Consequently, the accumulated APP-C99 can be further processed by γ-secretase, resulting in augmented production of amyloid-β and the APP intracellular domain. The chemical inhibition of ErbB2 by CL-387,785 effectively rescues the cognitive impairment of APP/presenilin-1 (PS1) transgenic AD mice. The present study thus defines a molecular basis by which the aberrant expression of ErbB2 could instigate the pathogenesis of AD. These findings provide the proof-of-principle evidence for rational design of ErbB2-targeted therapeutics for AD. (See pp. E3129–E3138.)

Transfer of pathogenic and nonpathogenic cytosolic proteins between spinal cord motor neurons in vivo in chimeric mice

Eleanor V. Thomas, Wayne A. Fenton, James McGrath, and Arthur L. Horwich

This report contains observations of transfer of both pathogenic and nonpathogenic cytosolic proteins between spinal cord motor neurons in intact (noninjected) mice that are genetic chimeras formed by aggregating eight-cell embryos of two strains with different fluorescently marked proteins. Transfer was also observed in cranial nerve motor nuclei known to be affected in amyotrophic lateral sclerosis but not in extraocular cranial nerve nuclei that are spared in amyotrophic lateral sclerosis. Transfer from motor neurons to neighboring mature gray matter oligodendrocytes implicates oligodendrocytes as the potential mediator of protein transfer. (See pp. E3139–E3148.)

Long noncoding miRNA gene represses wheat β-diketone waxes

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Higher plants have waxy surface layers that prevent uncontrolled water loss. Many wheat cultivars accumulate diketone epicuticular waxes in reproductive-age plants that produce a glaucous appearance. We identify *INHIBITOR of WAX1* (*Iw1*), a dominant glaucous repressor, as a young miRNA gene (MIRNA) that produces an miRNA, miRW1, which targets the transcript of the biosynthetic gene *WAX1-CARBOXYLESTERASE* (*W1-COE*) for degradation. The high sequence similarity between the *Iw1* hairpin sequence and *W1-COE* suggests that this MIRNA gene arose from an inverted duplication of its target. The cleavage specificity of miRW1 for its target gene defines the unique role of a young MIRNA gene in the regulation of an important agricultural trait related to stress tolerance. (See pp. E3149–E3158.)