PNAS Plus Significance Statements

Global models underestimate large decadal declining and rising water storage trends relative to GRACE satellite data

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We increasingly rely on global models to project impacts of humans and climate on water resources. How reliable are these models? While past model intercomparison projects focused on water fluxes, we provide here the first comprehensive comparison of land total water storage trends from seven global models to trends from Gravity Recovery and Climate Experiment (GRACE) satellites, which have been likened to giant weighing scales in the sky. The models underestimate the large decadal (2002–2014) trends in water storage relative to GRACE satellites, both decreasing trends related to human intervention and climate and increasing trends related primarily to climate variations. The poor agreement between models and GRACE underscores the challenges remaining for global models to capture human or climate impacts on global water storage trends. (See pp. E1080–E1089.)

Shape-directed dynamics of active colloids powered by induced-charge electrophoresis

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Despite recent advances in the ability to "program" the selfassembly of colloidal components, the resulting structures are often static and therefore incapable of performing dynamic functions such as the ability to actuate, heal, replicate, and compute. The realization of colloidal machines that organize in space and time to perform such functions requires new strategies for encoding the dynamic behaviors of colloidal components. Focusing on active colloids powered by inducedcharge electrophoresis, we use theory and simulation to show how the shape of a colloidal particle can be rationally tailored to specify complex motions powered by simple energy inputs. (See pp. E1090–E1099.)

Surface structure evolution in a homologous series of ionic liquids

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This high-resolution X-ray study resolves the liquid–air interface structure for a long homologous series of room temperature ionic liquids (RTILs). RTILs are intensely studied for many potential "green" applications and for their intriguingly complex and rare combination of intermolecular interactions. Varying their cation's alkyl chain length provides, therefore, an opportunity to tune the main interaction from mostly long-range electrostatic to mostly short-range van der Waals. This variation is found here to drive the interface structure from simple, to layered, to liquid crystalline. The quantitative results obtained constitute an accurate yardstick for testing simulations and theory, impact the bulk–surface structure relations in general, and provide currently scarce data for many RTIL applications, like batteries and supercapacitors. (See pp. E1100–E1107.)

A neurochemical hypothesis for the origin of hominids

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Two factors vital to the human clade are our unique demographic success and our social facilities including language, empathy, and altruism. These have always been difficult to reconcile with individual reproductive success. However, the striatum, a region of the basal ganglia, modulates social behavior and exhibits a unique neurochemical profile in humans. The human signature amplifies sensitivity to social cues that encourage social conformity and affiliative behavior and could have favored provisioning and monogamy in emergent hominids, consilient with the simultaneous origin of upright walking and elimination of the sectorial canine. Such exceptional neurochemistry would have favored individuals especially sensitive to social cues throughout later human evolution and may account for cerebral cortical expansion and the emergence of language. (See pp. E1108–E1116.)

Hsp90 chaperones hemoglobin maturation in erythroid and nonerythroid cells

Arnab Ghosh, Greer Garee, Elizabeth A. Sweeny, Yukio Nakamura, and Dennis J. Stuehr

Maturation of functional adult ($\alpha 2\beta 2$) or fetal ($\alpha 2\gamma 2$) hemoglobin (Hb) tetramers requires that a heme cofactor be incorporated into each globin. During erythropoiesis, Hb-α maturation is aided by the alpha Hb-stabilizing protein (AHSP), but what enables the maturation and heme insertion of the other globins is unknown. We found that chaperone hsp90 stabilizes

the immature, heme-free forms of Hb-β and Hb-γ and then drives their heme insertion reactions in an ATP-dependent process. This finding fills an important gap in our understanding of hemoglobin maturation during erythropoiesis and also helps to explain how functional mature Hb can be expressed outside the circulation in nonerythroid cells and tissues that do not express AHSP. (See pp. E1117–E1126.)

Extracellular vesicle budding is inhibited by redundant regulators of TAT-5 flippase localization and phospholipid asymmetry

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Cells must interact with their environment to survive. The lipids and proteins of the plasma membrane send and receive signals at the cell surface to respond to stimuli. When the lipid bilayer of the plasma membrane is damaged, cells release membrane-bound extracellular vesicles to repair the membrane. Cells also release signals on extracellular vesicles to communicate at a distance. Here, we identify proteins that regulate the formation of extracellular vesicles from the plasma membrane, providing additional tools to control their release that can be used to test potential functions of extracellular vesicles. Furthermore, we reveal that proteins regulating the asymmetric localization of the lipid phosphatidylethanolamine are critical for extracellular vesicle release, implicating this abundant but understudied lipid. (See pp. E1127–E1136.)

Cytocapsular tubes conduct cell translocation

Tingfang Yi and Gerhard Wagner

Cell migration in multicellular organisms is crucial for embryonic development, immune responses, organ homeostasis, tissue regeneration, and tumor metastasis. However, mechanisms of cell locomotion in tissues are elusive. Here we report that single mammalian cells generate extracellular and membranous cytocapsular tubes as highways for directed cell transportation. Cytocapsular tubes interconnect and form tubular networks for directed cell transportation in multiple directions. Increased eukaryotic translation initiation factor eIF4E-mediated cap-dependent translation initiation promotes cytocapsular tube development. This study reveals that cytocapsular tubes provide membranous tubes for directed cell translocation. (See pp. E1137–E1146.)

Saa3 is a key mediator of the protumorigenic properties of cancer-associated fibroblasts in pancreatic tumors

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Pancreatic ductal adenocarcinoma is one of the most malignant human tumors for which there are no efficacious therapeutic strategies. This tumor type is characterized by an abundant desmoplastic stroma that promotes tumor progression. Yet recent studies have shown that physical or genetic elimination of the stroma leads to more aggressive tumor development. Here, we decided to reprogram the stromal tissue by identifying and subsequently targeting genes responsible for their protumorigenic properties. Comparative transcriptome analysis revealed several genes overexpressed in cancer-associated fibroblasts compared with those present in normal pancreata. We provide genetic evidence that one of these genes, *Saa3*, plays a key role on the protumorigenic properties of the stroma, opening the door to the design of future therapeutic strategies. (See pp. E1147–E1156.)

Assembly and ecological function of the root microbiome across angiosperm plant species

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Microbial communities living on and within plants and animals contribute to host function. How host evolution shapes associated microbial communities, and in turn, how these microbes affect the ecology of their hosts is relatively unknown. Here, we demonstrate that evolution occurring across plant species affects root microbial diversity and composition. Greater similarity in root microbiota among host plant species leads to reduced plant performance through negative soil feedbacks. Additionally, drought shifts the composition of root microbiomes, where changes in the relative abundance of specific bacterial taxa are associated with increased drought tolerance of plants. Our work highlights the potential role of host-associated microbial communities in mediating interactions between hosts and their biotic and abiotic environment. (See pp. E1157–E1165.)

Evolutionary history of carbon monoxide dehydrogenase/acetyl-CoA synthase, one of the oldest enzymatic complexes

Panagiotis S. Adam, Guillaume Borrel, and Simonetta Gribaldo

Before the emergence of oxygenic photosynthesis and the accumulation of oxygen on Earth, life was essentially composed of anaerobic microorganisms. However, very little is known about which metabolisms were present at the time. Anaerobic carbon fixation through the Wood–Ljungdahl pathway is believed to be among the most ancient, and still plays a pivotal role in modern ecosystems. However, its origin and evolutionary history has been disputed. We analysed the distribution and phylogeny of carbon monoxide dehydrogenase/acetyl-CoA synthase, the main enzymatic complex of the pathway in thousands of bacterial and archaeal genomes. We show that this complex was already at work in the last universal common ancestor and has been remarkably conserved in microorganisms over more than 3.5 billion years. (See pp. E1166–E1173.)

Evolution of vertical and oblique transmission under fluctuating selection

Yoav Ram, Uri Liberman, and Marcus W. Feldman

Evolutionary dynamics of phenotypes in populations depend on how the traits are transmitted across generations and how the environments that cause selection on the traits fluctuate over time. We show that, under periodically fluctuating selection, a gene that increases the rate of vertical transmission is disfavored when the periods are short but approaches an intermediate stable rate for longer periods. This stable rate differs markedly from the rate that maximizes the geometric mean fitness. The evolution of learning rules thus differs qualitatively from the evolution of genetically modified rules of genetic transmission. (See pp. E1174–E1183.)

Nonsense-mediated mRNA decay factors cure most [PSI+] prion variants

Moonil Son and Reed B. Wickner

The [PSI+] prion (infectious protein) is an amyloid (filamentous polymer) of the Sup35 protein, producing detrimental effects on yeast. We find that at their normal expression levels the core components of nonsense-mediated mRNA decay for mRNA quality control, Upf1p, Upf2p, and Upf3p, block the propagation of most new [PSI+] prion variants. The curing mechanism relies upon both Sup35p-binding by each Upf protein and by the trimeric Upf complex. Our results support the notion that normal protein–protein interactions prevent abnormal interactions. (See pp. E1184–E1193.)

IL-1β enables CNS access to CCR2^{hi} monocytes and the generation of pathogenic cells through GM-CSF released by CNS endothelial cells

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Multiple sclerosis (MS) is a neuroinflammatory disease characterized by demyelinating plaques in the brain and spinal cord, causing progressive loss of functions. While the causes of MS remain undefined, treatments that target immune cells or their functions greatly improve the clinical outcome. Our laboratory has previously shown that production of the inflammatory cytokine interleukin (IL)-1β by myeloid cells is required for the development of experimental autoimmune encephalomyelitis (EAE), an MS model. Here, we show that activation of central nervous system (CNS) endothelial cells by IL-1β enables inflammatory monocytes to enter the CNS and differentiate into antigen-presenting cells. Additionally, we demonstrate that factors released from the interaction between IL-1β–producing myeloid cells and autoreactive CD4$^+$ T cells are toxic to neurons. (See pp. E1194–E1203.)

Differing roles of CD1d2 and CD1d1 proteins in type I natural killer T cell development and function

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Natural killer T (NKT) cells are selected by CD1d molecules in the thymus. Two homologous genes in the mouse genome encode for CD1d proteins. The two CD1d isoforms are not equivalent in their lipid antigen presentation capabilities, affecting the development and the T cell antigen receptor repertoire of NKT cells. (See pp. E1204–E1213.)

Regulation of inflammatory responses by dynamic subcellular localization of RNA-binding protein Arid5a

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Immune cell activation is accompanied by dynamic changes in expression of genes related to inflammation. Concomitantly, immune reactions are tightly controlled to prevent harmful pathologies due to sustained inflammation. Gene expression is controlled at multiple checkpoints. Among these, the post-transcriptional regulation of the balance between Arid5a and Regnase-1 is important for the induction of inflammation. Our findings provide important insight into the role of the regulation of nucleocytoplasmic localization of Arid5a in the development of inflammation through the induction of a change in the ratio of Arid5a to Regnase-1. (See pp. E1214–E1220.)

Establishment of the early cilia preassembly protein complex during motile ciliogenesis

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Motile cilia preassembly proteins processing dynein motor proteins have been identified in patients with motile cilia dysfunction. However, the exact function and interaction of these proteins are not understood. Using primary airway cell culture and regulated induced pluripotent stem cell culture, we provide a roadmap for preassembly protein expression, showing that HEATR2 is the first preassembly protein to appear, emerging early in ciliogenesis and preceding known regulatory factors. HEATR2, SPAG1, and DNAAF2 colocalize within previously unrecognized cytoplasmic foci to form an early preassembly complex. The findings suggest that a lead scaffold structure essential for motile cilia function is destroyed by proteostasis in HEATR2 mutants, leading to degradation of the whole preassembly complex and identifying the cellular mechanism for disease. (See pp. E1221–E1228.)

MYCN-amplified neuroblastoma maintains an aggressive and undifferentiated phenotype by deregulation of estrogen and NGF signaling

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High-risk neuroblastoma (NB), a cancer of the sympathetic nervous system, is challenging to treat. *MYCN* is frequently amplified in high-risk NB and is linked to an undifferentiated phenotype and poor prognosis. Estrogen and nerve growth factor (NGF) are inducers of neural differentiation, a process associated with a favorable disease. We show that MYCN suppresses estrogen receptor alpha (ERα) and thereby NGF signaling and neural differentiation. ERα overexpression is sufficient to interfere with different tumorigenic processes and tumor growth. In patients with NB, *ERα* expression correlates with several clinical markers for good prognosis. Importantly, not only ERα but also the majority of other nuclear hormone receptors are linked to favorable NB, suggesting a potential prognostic and therapeutic value for these proteins. (See pp. E1229–E1238.)

Chemotherapy induces enrichment of CD47$^+$/CD73$^+$/PDL1$^+$ immune evasive triple-negative breast cancer cells

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Cytotoxic chemotherapy is frequently used in patients with triple-negative breast cancer (TNBC). Although patients initially respond to the treatment, the cancer often comes back and kills the patient. Recent studies have demonstrated that cancer cells express genes that protect them from killing by immune cells, but the stimulus that prompts this response is unknown. We show that when TNBC cells are treated with chemotherapy, the surviving cells turn on genes that enable them to escape killing by the immune system. We identify hypoxia-inducible factors (HIFs), which are known to promote metastasis of TNBC, as responsible for this countertherapeutic effect. We show that coadministration of an HIF inhibitor with chemotherapy blocks the ability of surviving TNBC cells to evade the immune system. (See pp. E1239–E1248.)

Increased thermogenesis by a noncanonical pathway in ANGPTL3/8-deficient mice

Serena Banfi, Viktoria Gusarova, Jesper Gromada, Jonathan C. Cohen, and Helen H. Hobbs

White adipose tissue (WAT) serves as an energy reservoir during fasting and is replenished with fatty acids from circulating triglycerides upon refeeding. We showed previously that postprandial partitioning of fatty acids between oxidative and storage tissues is mediated by angiopoietin-like proteins 3 (A3) and 8 (A8). Here, we show that disruption of both *Angptl3* and *Angptl8* in mice causes striking alterations in energy metabolism: reduced fat mass, hyperthermia, increased metabolic rate, and beiging of subcutaneous WAT. The hypermetabolic features of the $A3^{-/-}A8^{-/-}$ mice were most pronounced in fed animals, and attenuated with fasting and β3-adrenergic receptor blockade. These data indicate that A3 and A8 promote efficient energy utilization by tissues and limit the increase in energy expenditure associated with food intake. (See pp. E1249–E1258.)

Structure and function of the archaeal response regulator CheY

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Motility is a key feature for the success of microorganisms, as it allows the movement to optimal growth environments. Bacteria and archaea possess filamentous motility structures capable of rotation. However, both molecular machines consist of fundamentally different proteins and lack structural similarity. Intriguingly, some archaea possess the chemotaxis system. This system allows bacteria to travel along chemical gradients and is dependent on interaction of the response regulator CheY with the motor of the motility structure. In this study, we map the changes of the CheY protein structure required for its interaction with components of the archaeal motility machinery. (See pp. E1259–E1268.)

Sporadic on/off switching of HTLV-1 Tax expression is crucial to maintain the whole population of virus-induced leukemic cells

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The oncogenic retrovirus human T-cell leukemia virus type 1 (HTLV-1) encodes Tax, an activator of both viral replication and cellular oncogenic pathways. Despite the potent activities of Tax, its precise roles in pathogenesis remain unclear, since it is faintly expressed in vivo. This study shows that sporadic and transient Tax expression is observed in a small subpopulation of HTLV-1–induced leukemic cells. This limited Tax expression is critical for survival of the whole population through ignition of antiapoptotic signals. Tax is induced by various stresses, suggesting that Tax efficiently protects cells from apoptosis and reactivates virus from reservoirs under conditions of cellular stress. It is an elaborated strategy of HTLV-1 to evade host immunity and enable persistence in vivo. (See pp. E1269–E1278.)

Intracellular Ca^{2+} stores control in vivo neuronal hyperactivity in a mouse model of Alzheimer's disease

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Although both patients with Alzheimer's disease (AD) and mouse models of the disease suffer from a profound neuronal hyperactivity mediating an impairment of memory and cognition, the underlying mechanisms remain elusive. Here, we show that neuronal hyperactivity is a hallmark of healthy aging but is further aggravated by AD-related mutations in presenilin 1 protein. AD-mediated enhancement of neuronal hyperactivity occurred even in the absence of amyloid plaques and neuroinflammation, mainly due to a presenilin-mediated dysfunction of intracellular Ca^{2+} stores in presynaptic boutons, likely causing more frequent activation of synaptic NMDA receptors. Emptying the stores normalized neuronal network activity in mutant mice, thus identifying presynaptic Ca^{2+} stores as a key element controlling AD-related neuronal hyperactivity and as a target for disease-modifying treatments. (See pp. E1279–E1288.)

Tau induces blood vessel abnormalities and angiogenesis-related gene expression in P301L transgenic mice and human Alzheimer's disease

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This work provides evidence that the protein tau induces changes in blood vessels distinct from the effects of amyloid beta on vasculature and indicates a previously unknown pathway by which pathological tau may accelerate cognitive decline in Alzheimer's disease. (See pp. E1289–E1298.)

Focal versus distributed temporal cortex activity for speech sound category assignment

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When listening to speech, phonemes are represented in a distributed fashion in our temporal and prefrontal cortices. How these representations are selected in a phonemic decision context, and in particular whether distributed or focal neural information is required for explicit phoneme recognition, is unclear. We hypothesized that focal and early neural encoding of acoustic signals is sufficiently informative to access speech sound representations and permit phoneme recognition. We tested this hypothesis by combining a simple speech-phoneme categorization task with univariate and multivariate analyses of fMRI, magnetoencephalography, intracortical, and clinical data. We show that neural information available focally in the temporal cortex prior to decision-related neural activity is specific enough to account for human phonemic identification. (See pp. E1299–E1308.)

The eardrums move when the eyes move: A multisensory effect on the mechanics of hearing

Kurtis G. Gruters, David L. K. Murphy, Cole D. Jenson, David W. Smith, Christopher A. Shera, and Jennifer M. Groh

The peripheral hearing system contains several motor mechanisms that allow the brain to modify the auditory transduction process. Movements or tensioning of either the middle ear muscles or the outer hair cells modifies eardrum motion, producing sounds that can be detected by a microphone placed in the ear canal (e.g., as otoacoustic emissions). Here, we report a form of eardrum motion produced by the brain via these systems: oscillations synchronized with and covarying with the direction and amplitude of saccades. These observations suggest that a vision-related process modulates the first stage of hearing. In particular, these eye movement-related eardrum oscillations may help the brain connect sights and sounds despite changes in the spatial relationship between the eyes and the ears. (See pp. E1309–E1318.)

Involvement of Aryl hydrocarbon receptor in myelination and in human nerve sheath tumorigenesis

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Aryl hydrocarbon receptor (AHR) is well known to mediate xenobiotic metabolism in vertebrates. Growing evidence reveals that AHR seems to have endogenous roles in the development and functioning of different organs. In our current study, we describe a role of AHR in peripheral myelination and in nerve sheath tumors. We show that the AHR pathway is dysregulated in human biopsies of nerve tumors. The blockade of AHR provokes cell death in nerve tumors, suggesting a therapeutic avenue in the treatment of this invasive cancer. Furthermore, the inhibition of *Ahr* in mice provokes locomotor defects and alteration of myelin structure. This work unravels an endogenous role of *Ahr* in peripheral myelination and a potential treatment of nerve tumours. (See pp. E1319–E1328.)