PNAS Plus Significance Statements

Patterns of coevolving amino acids unveil structural and dynamical domains

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Patterns of pairwise correlations in sequence alignments can be used to reconstruct the network of residue-residue contacts and thus the three-dimensional structure of proteins. Less explored, and yet extremely intriguing, is the functional relevance of such coevolving networks: Do they encode for the collective motions occurring in proteins at thermal equilibrium? Here, by combining coevolutionary coupling analysis with a state-of-the-art dimensionality reduction approach, we show that the network of pairwise evolutionary couplings can be analyzed to reveal communities of amino acids, which we term "evolutionary domains," that are in striking agreement with the quasi-rigid protein domains obtained from elastic network models and molecular dynamics simulations. (See pp. E10612–E10621.)

Direct measurements of meltwater runoff on the Greenland ice sheet surface

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Meltwater runoff is an important hydrological process operating on the Greenland ice sheet surface that is rarely studied directly. By combining satellite and drone remote sensing with continuous field measurements of discharge in a large supraglacial river, we obtained 72 h of runoff observations suitable for comparison with climate model predictions. The field observations quantify how a large, fluvial supraglacial catchment attenuates the magnitude and timing of runoff delivered to its terminal moulin and hence the bed. The data are used to calibrate classical fluvial hydrology equations to improve meltwater runoff models and to demonstrate that broad-scale surface water drainage patterns that form on the ice surface powerfully alter the timing, magnitude, and locations of meltwater penetrating into the ice sheet. (See pp. E10622–E10631.)

Coupled European and Greenland last glacial dust activity driven by North Atlantic climate

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Atmospheric dust is a major component of climate change. However, the relationship between glacial continental dust activity and abrupt centennial–millennial-scale climate changes of the North Atlantic is poorly known. Recent advances in high-precision radiocarbon dating of small gastropods in continental loess deposits provide an opportunity to gain unprecedented insights into dust variations and its major drivers at centennial–millennial scales from a near-source dust archive. Here, we show that Late Quaternary North Atlantic temperature and dustiness in Greenland and Europe were largely synchronous and suggest that this coupling was driven via precipitation changes and large-scale atmospheric circulation. (See pp. E10632–E10638.)

Storm, rogue wave, or tsunami origin for megaclast deposits in western Ireland and North Island, New Zealand?

John F. Dewey and Paul D. Ryan

The origin of boulderite deposits in the geological record is controversial. Many argue that boulders weighing over 30 tonnes characterize tsunamites. We compare and contrast two such deposits with boulders exceeding this weight: a tsunamite from the Miocene of New Zealand and a present-day boulderite at Annagh Head, western Ireland. Combining field, historical, and oceanographic data, we argue that the latter is a cliff-top storm deposit (CTSD). Numerical modeling shows that the weight of a boulder in such CTSDs does not just reflect storm wave height, which can be over 60 m in western Ireland, but is also a function of its shape. We review the characteristics that distinguish these two deposits. (See pp. E10639–E10647.)

Ideals, practices, and future prospects of stakeholder involvement in sustainability science

Jahel Mielke, Hannah Vermaßen, and Saskia Ellenbeck

Even though stakeholder involvement (SI) is increasingly relevant in scientific research processes, especially in interdisciplinary fields like sustainability science, there is limited academic literature investigating conceptual or methodological questions.

Through a survey among researchers from this field, this paper presents an overview of practices and ideals of SI as well as of their divergence. Furthermore, trade-offs between scientific ideals and SI, as well as necessary improvements concerning, for example, methods or funding, are described. To add to the conceptualization of SI, the survey data were related to a typology that differentiates democratic, technocratic, neoliberal-rational, and functionalist views of SI in science. The findings can form one possible basis for development of SI toward a more standardized research approach in sustainability science. (See pp. E10648–E10657.)

Prenatal stress accelerates offspring growth to compensate for reduced maternal investment across mammals

Andreas Berghänel, Michael Heistermann, Oliver Schülke, and Julia Ostner

Maternal stress during gestation causes numerous effects on infant physiology that extend well into adulthood. We contribute to the ongoing debate on whether these effects are adaptive outcomes or merely the product of energetic constraints by presenting an integrated hypothesis that predicts the diversity of observed maternal effects on offspring growth, incorporating both theoretical explanations into one coherent framework. Empirical tests of this hypothesis across mammals suggest that the timing of the stressor during gestation and a simultaneous consideration of maternal investment and adaptive growth plasticity effects are crucial for a full comprehension of prenatal stress effects on offspring growth. The results support an adaptive life history perspective on maternal effects that is relevant for evolutionary biology, medicine, and psychology. (See pp. E10658–E10666.)

Phosphorylation of CENP-C by Aurora B facilitates kinetochore attachment error correction in mitosis

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Kinetochores are large protein networks located on centromeres that mediate chromosome segregation during mitosis and maintain genomic stability. Mis12 complex (Mis12C) functions as a scaffold that targets Ndc80 and Knl1 complexes to the centromere by associating with CENP-C. Here, we provide insights into the molecular mechanism underlying the CENP-C–dependent kinetochore recruitment of Mis12C, which is negatively regulated by Aurora B-dependent CENP-C phosphorylation. Replacement of *Schizosaccharomyces pombe* Cnp3 with a phosphorylation-mimicking mutant, $Cnp3^{T28E}$, results in defective chromosome segregation caused by improper kinetochore assembly. These findings indicate that Aurora B-dependent phosphorylation of CENP-C plays a role in interrupting the connection between the inner and outer kinetochore and is thus involved in the error correction/spindle assembly checkpoint pathway to prevent chromosome missegregation during mitosis. (See pp. E10667–E10676.)

Interaction of intramembrane metalloprotease SpoIVFB with substrate Pro-σ^K

Sabyasachi Halder, Daniel Parrell, Douglas Whitten, Michael Feig, and Lee Kroos

Most proteases catalyze peptide bond hydrolysis of substrate proteins in aqueous environments. Intramembrane proteases (IPs) are unusual, cleaving substrates in hydrophobic cellular membranes. IPs regulate many processes that impact health, but potential benefits of manipulating IP activities remain elusive due to insufficient knowledge about how IPs interact with substrates. We report experimental and modeling results that illuminate how intramembrane metalloprotease SpoIVFB interacts with its substrate Pro-σ^K. A 26-residue linker between two domains of SpoIVFB is crucial, perhaps allowing an ATP-induced conformational change to position Pro-σ^K for cleavage. SpoIVFB and Pro-σ^K are broadly conserved in endospore-forming bacteria. Endospores are highly resistant cells that promote persistence of some important human pathogens. The work may lead to new strategies to control endospore formation. (See pp. E10677–E10686.)

EB1-binding–myomegalin protein complex promotes centrosomal microtubules functions

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Microtubule dynamics is tightly regulated during fundamental biological processes such as mitosis, thereby representing a major target for anticancer therapies. To better understand the molecular mechanisms underlying the organization of the microtubule network, we systematically investigated proteins interacting with EB1, a major regulator of microtubules dynamics. We identified a specific isoform of myomegalin, which we termed "SMYLE," that assembles a macromolecular complex associated with the centrosome, the major microtubule-organizing center in cells, and also connected to the microtubule nucleating complex. SMYLE promoted microtubule assembly from the centrosome and subsequent stabilization of microtubules at the cell periphery. This had consequences on cell motility, mitosis, and cell-cycle progression, suggesting that SMYLE might be an important player in tumor progression. (See pp. E10687–E10696.)

AP-4 mediates export of ATG9A from the *trans*-Golgi network to promote autophagosome formation

Rafael Mattera, Sang Yoon Park, Raffaella De Pace, Carlos M. Guardia, and Juan S. Bonifacino

A family of adaptor protein (AP) complexes functions to sort transmembrane cargos at different stages of the endomembrane system of eukaryotic cells. AP-4 is one of the most recently described and least well-understood members of this family. Interest in this complex has risen because mutations in any of its four subunits cause a form of hereditary spastic paraplegia (HSP) with intellectual disability. In this study, we demonstrate that AP-4 sorts ATG9A, the only transmembrane component of the core autophagy machinery, from the *trans*-Golgi network to peripheral compartments. This sorting is required to promote the early steps of autophagosome formation. Our observations implicate AP-4 as an autophagy regulator and altered autophagy as an underlying defect in AP-4–deficient HSP. (See pp. E10697–E10706.)

Bicaudal D2 facilitates the cytoplasmic trafficking and nuclear import of HIV-1 genomes during infection

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Following envelope-mediated fusion, the HIV-1 viral core, which houses the viral RNA and proteins required for virus reverse transcription and integration, must traffic toward the nucleus for subsequent nuclear import of the viral genome. In this study we examined the role of BICD2, a known dynein adaptor protein, for

its role during the postentry trafficking of HIV-1 virions. We show that BICD2 binds viral capsid and mediates the postentry trafficking of HIV-1. Moreover, we also show that depletion of BICD2 sensitizes the virus to detection by innate immune sensing, revealing this BICD2 is necessary for the virus to avoid detection by innate sensing mechanisms in macrophages. (See pp. E10707–E10716.)

Smek1/2 is a nuclear chaperone and cofactor for cleaved Wnt receptor Ryk, regulating cortical neurogenesis

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Receptor-like tyrosine kinase (Ryk) is a Wnt receptor and is important for many developmental processes, including cranial facial development, neurogenesis, and axon guidance. However, little is known about the role of the intracellular domain, Ryk-ICD, in signal transduction. Its downstream targets are also unknown. We have previously shown that Ryk-ICD is located in the cytoplasm of neural stem cells whereas it moves into the nucleus upon neuronal differentiation. In this study, we discovered that Smek1/2 function as a chaperone for Ryk-ICD during its nuclear localization and that both Smek and Ryk-ICD associate with chromatin to regulate the transcription of downstream target genes and neural differentiation. (See pp. E10717–E10725.)

FMRFamide-like peptides expand the behavioral repertoire of a densely connected nervous system

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Under environmental stress, animals can adopt different forms and behaviors through phenotypic plasticity. The roundworm *Caenorhabditis elegans* can exit reproductive growth and enter the stress-resistant dauer larval stage. We investigated phenotypic plasticity in a whole organism by comparing gene expression during dauer and reproductive development using RNA sequencing. As animals entered dauer, we observed striking up-regulation of neuronal signaling peptides, which promote the dauer-entry decision instead of reproductive growth. These neuropeptides also enable new behaviors in dauers for exploiting carrier animals for dispersal. Neuropeptides are similarly up-regulated in the infective, dauer-like stages of parasitic roundworms, indicating dauer as a strong model for studying parasitic behaviors. Our investigation reveals that neuropeptides can alter developmental decision-making and behavior in stressed *C. elegans*. (See pp. E10726–E10735.)

High-resolution mapping of *cis*-regulatory variation in budding yeast

Ryosuke Kita, Sandeep Venkataram, Yiqi Zhou, and Hunter B. Fraser

Genetic variants affecting gene-expression levels are a major source of phenotypic variation. Using 85 diverse isolates of *Saccharomyces cerevisiae*, we mapped genetic variants that affect gene expression with 50-fold higher resolution than previously possible. By doing so, we were able to pinpoint likely causal variants and investigate their molecular mechanisms. We found that these genetic variants are generally under negative selection, but also that clinical yeast isolates have undergone positive selection for up-regulation of genes involved in biofilm suppression. Altogether, our results demonstrate the power of high-resolution mapping of genetic variants that affect gene expression, particularly in understanding the molecular mechanisms of regulatory variation and the natural selection acting on this variation. (See pp. E10736–E10744.)

Precision genome editing using synthesis-dependent repair of Cas9-induced DNA breaks

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Genome editing, the introduction of precise changes in the genome, is revolutionizing our ability to decode the genome. Here we describe a simple method for genome editing in mammalian cells that takes advantage of an efficient mechanism for gene conversion that utilizes linear donors. We demonstrate that PCR fragments containing edits up to 1 kb require only 35-bp homology sequences to initiate repair of Cas9-induced double-stranded breaks in human cells and mouse embryos. We experimentally determine donor DNA design rules that maximize the recovery of edits without cloning or selection. (See pp. E10745–E10754.)

Improved detection of synthetic lethal interactions in *Drosophila* cells using variable dose analysis (VDA)

Benjamin E. Housden, Zhongchi Li, Colleen Kelley, Yuanli Wang, Yanhui Hu, Alexander J. Valvezan, Brendan D. Manning, and Norbert Perrimon

Synthetic sick or lethal (SS/L) interactions occur when disruption of two genes reduces cell viability to a greater extent than expected based on the individual gene disruptions. SS/L interactions involving tumor suppressors represent candidate drug targets for cancers because treatment is expected to kill tumor cells carrying the tumor suppressor mutation but leave healthy cells unaffected. Identification of SS/L interactions is of vital importance to develop new therapies for tumorigenic disease. We have developed an RNAi-based approach called variable dose analysis, which improves both sensitivity and robustness to noise compared with dsRNA-based methods for screening in *Drosophila*. Using this method, we identified four Food and Drug Administration-approved drugs with specific effects on cells deficient for the TSC1 and TSC2 tumor suppressor genes. (See pp. E10755–E10762.)

Transient receptor potential channel 6 regulates abnormal cardiac S-nitrosylation in Duchenne muscular dystrophy

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The pathological Duchenne muscular dystrophy (DMD) muscles show increased stretch-induced intracellular Ca^{2+} and nitrosative stress. Whether there is a link between the two, and how the former impacts the nitrosylated proteome, is unknown. Here, we report that transient receptor potential channel 6 (Trpc6) modulates increased nitrosative stress in dmd^{mdx}:$utrn^{+/-}$ mice, as reflected by an increase in protein S-nitrosylation, and provide a broad high-throughput analysis of S-nitrosylation in this model. We found that S-nitrosothiol targets are conserved in dmd^{mdx}:$utrn^{+/-}$ myocardium, but intensified in a Trpc6-dependent manner. Restoration of more normal S-nitrosylation profiles in dmd^{mdx}:$utrn^{+/-}$ mouse hearts lacking Trpc6 corresponds to improved cardiac function and reduced fibrosis. These findings link Trpc6-mediated Ca^{2+} signaling and nitrosative stress in the redox pathobiology of DMD. (See pp. E10763–E10771.)

WhiB6 regulation of ESX-1 gene expression is controlled by a negative feedback loop in *Mycobacterium marinum*

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Mycobacteria use ESX systems to transport protein substrates across the cytoplasmic membrane. The ESX-1 system is required for mycobacterial pathogenesis in *Mycobacterium tuberculosis* (*M. tb*), the cause of tuberculosis (TB). Differences in the expression of genes encoding ESX substrates directly impacts *M. tb* transmission and virulence. Deletion of genes encoding ESX exporters results in reduced levels of ESX substrates in mycobacteria. Here, we define a fundamental mechanism of regulation of ESX-1 substrates in *M. marinum*, a pathogenic mycobacterial species and a model for *M. tb*. We demonstrate that the transcriptional regulation of genes encoding ESX-1 substrates is linked to the presence or absence of the ESX-1 exporter. These findings provide insight into how substrate levels are intricately controlled in mycobacteria. (See pp. E10772–E10781.)

Characterization of SPP inhibitors suppressing propagation of HCV and protozoa

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Signal peptide peptidase (SPP) is an essential host factor for propagation of hepatitis C virus (HCV). Here, we show that dibenzoazepine-type γ-secretase inhibitors suppressed the maturation of all genotypes of HCV core proteins through a specific interaction with Val223 in SPP, and no drug-resistant virus emerged after several passages of HCV in the presence of the SPP inhibitors. In addition, SPP encoded by *Plasmodium falciparum* was functionally similar to human SPP, and treatment with the SPP inhibitors suppressed the propagation of protozoa, including *P. falciparum* and *Toxoplasma gondii*. Structural analysis in silico revealed that Phe258 of SPP participates in binding to the inhibitors. Compounds possessing a high affinity to Val223/Phe258 in SPP might be novel therapeutics for chronic hepatitis C and protozoiasis. (See pp. E10782–E10791.)

Osmosensing by the bacterial PhoQ/PhoP two-component system

Jing Yuan, Fan Jin, Timo Glatter, and Victor Sourjik

Whether residing in or invading the host, enterobacteria have to deal with host-related stress conditions. These stress factors also serve as sensory cues, informing bacteria that they are present inside the host. Here, we report that the PhoQ/PhoP two-component system, which was known to sense several host-related environmental changes, responds to osmotic upshift, another key stimulus associated with the host. This sensing is proposed to rely on a mechanism that detects changes in the physical properties of the membrane. Thus, a single enterobacterial kinase, PhoQ, senses a major part of host-associated stimuli. The PhoQ-mediated osmosensing increases bacterial fitness under hyperosmotic conditions found inside the host, and it is likely to play an important role in the regulation of virulence. (See pp. E10792–E10798.)

Expansion microscopy of zebrafish for neuroscience and developmental biology studies

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We explore the utility of expansion microscopy (ExM) in neuroscience and developmental biology using the zebrafish model. Regarding neuroscience studies, ExM enables the tracing of cellular processes in the zebrafish brain, as well as the imaging of synapses and their biomolecular content and organization. Regarding development, ExM enables the resolving of nuclear compartments, particularly nuclear invaginations and channels, and helps relate such cellular nanostructures to proteins of the cytoskeleton during embryogenesis. (See pp. E10799–E10808.)

Phosphorylation of huntingtin at residue T3 is decreased in Huntington's disease and modulates mutant huntingtin protein conformation

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The findings in this manuscript report on the identification of a posttranslational modification in the huntingtin protein (phosphorylation on residue T3 in the N17 region of the protein), which can revert the conformational effects of the Huntington's disease (HD) mutation itself on the huntingtin protein and inhibit its aggregation properties in vitro. Using the first ultrasensitive immunoassay for a posttranslational modification of huntingtin protein, we demonstrate that pT3 levels are decreased in mutant huntingtin in preclinical models as well as in clinically relevant samples from HD patients. These findings are of high significance to Huntington's disease biology, provide insights into mechanisms of Huntington's disease pathogenesis, and open new opportunities for the development of therapeutics and diagnostics for Huntington's disease. (See pp. E10809–E10818.)

Vasopressin excites interneurons to suppress hippocampal network activity across a broad span of brain maturity at birth

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The transition from placental to lung-based oxygen supply at mammalian birth involves an obligatory period of asphyxia, which is further aggravated by complications during delivery. This oxygen deprivation is a major threat to the fetal brain, and, under such conditions, hormonal and cardiovascular mechanisms are activated to enhance brain perfusion. Our work now demonstrates an intrinsic mechanism in the fetal brain whereby vasopressin activates hippocampal interneurons, leading to desynchronization and suppression of neuronal network activity in species (rat and guinea pig) that are born at widely different stages of brain maturation. Silencing of synchronous neuronal activity by vasopressin is expected to decrease neuronal energy demand and prevent maladaptive synaptic plasticity, thus acting as a pan-mammalian neuroprotective mechanism during birth. (See pp. E10819–E10828.)

Additive effects of climate and fisheries drive ongoing declines in multiple albatross species

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Three high-conservation priority populations were studied: the wandering, grey-headed, and black-browed albatrosses from Bird Island, South Georgia. They represent 12–50% of global numbers and have declined by 40–60% in 35 years. As temperatures and environmental stochasticity increase, polar species are particularly at risk, while fisheries accidentally kill hundreds of thousands individuals each year. Longitudinal monitoring of >40,000 individuals ringed since 1972 was used with detailed at-sea distributions, environmental data, and fisheries effort spanning the Southern Ocean to explore the factors driving population change and how they may combine. The powerful comparative framework used here is one of the most extensive to date and could be used to understand and better mitigate the fate of many threatened wild populations. (See pp. E10829–E10837.)

Proteomics of phosphorylation and protein dynamics during fertilization and meiotic exit in the *Xenopus* egg

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Protein phosphorylation and degradation drive critical events in early embryogenesis and the cell cycle; however, comprehensive and accurate analysis of these changes is currently difficult. Using a mass-spectrometric approach, we present a quantitative view of the protein and posttranslational economy of the fertilization response in the frog egg. Protein degradation affects a small but very important class of proteins, while regulatory phosphorylation and protein release occur on a far larger scale. We have developed broadly applicable analytical methods for phosphorylation that provide absolute quantification with confidence intervals for improved interpretability of posttranslational modification analysis. (See pp. E10838–E10847.)