PNAS Plus Significance Statements

Laterally confined growth of cells induces nuclear reprogramming in the absence of exogenous biochemical factors

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In this study, we demonstrate a platform for reprogramming somatic cells with high efficiency in the absence of exogenous reprogramming factors. Sustained laterally confined growth of cells on micropatterned substrates results in sequential changes to the nucleus and chromatin with each cell division, leading to the progressive erasure of lineage specific characteristics and incorporation of pluripotency. After 10 days of confined growth, the cells exhibit stemness and have multilineage differentiation potential. Our observation highlights a previously unknown role of mechanical constraints in nuclear reprogramming. Our method provides a unique approach to greatly improve stem cell technologies for developing patient specific disease models and regenerative medicine. (See pp. E4741–E4750.)

Physics of lumen growth

Sabyasachi Dasgupta, Kapish Gupta, Yue Zhang, Virgile Viasnoff, and Jacques Prost

The development of intercellular cavities (lumens) is a ubiquitous mechanism to form complex tissue structures in organisms. The generation of Ciona Notochord, the formation of Zebrafish vasculature, or the formation of bile canaliculi between hepatic cells constitute a few examples. Lumen growth is governed by water intake that usually results from the creation of a salt concentration difference (osmotic gradients) between the inside and the outside of the lumen. During morphogenesis or in diseases, lumens can also leak due to improper maturation of the cell junctions that seal them. In this paper, we theoretically describe different conditions and dynamical regimes of lumen growth based on the balance of osmotic pressure, fluid intake, and paracellular leak. (See pp. E4751–E4757.)

Developing a molecular dynamics force field for both folded and disordered protein states

Paul Robustelli, Stefano Piana, and David E. Shaw

Many proteins that perform important biological functions are completely or partially disordered under physiological conditions. Molecular dynamics simulations could be a powerful tool for the structural characterization of such proteins, but it has been unclear whether the physical models (force fields) used in simulations are sufficiently accurate. Here, we systematically compare the accuracy of a number of different force fields in simulations of both ordered and disordered proteins, finding that each force field has strengths and limitations. We then describe a force field that substantially improves on the state-of-the-art accuracy for simulations of disordered proteins without sacrificing accuracy for folded proteins, thus broadening the range of biological systems amenable to molecular dynamics simulations. (See pp. E4758–E4766.)

IonStar enables high-precision, low-missing-data proteomics quantification in large biological cohorts

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Reliable proteome-wide quantification in large biological cohorts is highly valuable for clinical and pharmaceutical research yet remains extremely challenging despite recent technical advancements. Specifically, elevated missing data levels and compromised quantitative quality are common issues for prevalent methods. Here, we describe an IonStar technique taking advantage of sensitive and selective MS1 ion current-base quantification via innovations in effective and reproducible quantitative feature generation. Compared with several label-free strategies, IonStar showed superior performance in large-cohort analysis, manifested by excellent accuracy/precision, extremely low missing data, and confident discovery of subtle protein changes. In a proof-of-concept study, we demonstrated that IonStar quantified >7,000 unique proteins in 100 brain samples with no missing data and excellent quantitative quality, which has not been achievable by existing methods. (See pp. E4767–E4776.)

Cisplatin-DNA adduct repair of transcribed genes is controlled by two circadian programs in mouse tissues

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Cisplatin is a front-line drug in treatment of most solid tissue cancers. It kills cancer cells by damaging their DNA. Although it is quite effective it has two major drawbacks. First, it has serious side effects, including nephrotoxicity, hepatotoxicity, and neurotoxicity. Secondly, some cancers exhibit primary or acquired

resistance to the drug which limit its usefulness. Attempts have been made to administer the drug at certain times of the day (chronochemotherapy) to overcome these limitations but these attempts have had very limited success. Here, we generate genome-wide and at single-nucleotide-resolution circadian DNA repair maps for mouse kidney and liver with the ultimate goal of developing a rational cisplatin chronochemotherapy regimen. (See pp. E4777–E4785.)

Cotranslocational processing of the protein substrate calmodulin by an AAA+ unfoldase occurs via unfolding and refolding intermediates

Rafal Augustyniak and Lewis E. Kay

In the cell, proteins are continuously synthesized and degraded. Degradation as well as unraveling of aggregated proteins depends on the activity of a family of ATP-driven ring-shaped unfoldases that catalyze unfolding of protein substrates by threading them through central ring channels. Here we follow the unfolding, translocation, and refolding of a substrate as it passes through the central pore of VAT, an archetypal unfoldase, using a method based on chemical cross-linking and methyl transverse relaxation-optimized NMR spectroscopy. The approach yields insights about how molecular machines are able to unfold protein targets, showing that for multidomain proteins unfolding can proceed in a series of steps that vary depending on whether the substrate is unfolded from its N or C terminus. (See pp. E4786–E4795.)

Systematic approach for dissecting the molecular mechanisms of transcriptional regulation in bacteria

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Organisms must constantly make regulatory decisions in response to a change in cellular state or environment. However, while the catalog of genomes expands rapidly, we remain ignorant about how the genes in these genomes are regulated. Here, we show how a massively parallel reporter assay, Sort-Seq, and information-theoretic modeling can be used to identify regulatory sequences. We then use chromatography and mass spectrometry to identify the regulatory proteins that bind these sequences. The approach results in quantitative base pair-resolution models of promoter mechanism and was shown in both well-characterized and unannotated promoters in *Escherichia coli*. Given the generality of the approach, it opens up the possibility of quantitatively dissecting the mechanisms of promoter function in a wide range of bacteria. (See pp. E4796–E4805.)

STXBP4 regulates APC/C-mediated p63 turnover and drives squamous cell carcinogenesis

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The N-terminally truncated isoform of p63 (ΔNp63) is overexpressed in some forms of squamous cell carcinoma (SCC). Here we show that the anaphase-promoting complex/cyclosome (APC/C) degradation machinery plays an essential role in regulating the proteolysis of ΔNp63. We report as well that syntaxin-binding protein 4 (Stxbp4) suppresses APC/C-mediated ubiquitination and proteolysis of ΔNp63 and thereby drives the oncogenic potential of SCC. Aberrancies in this newly defined mechanism could account for ΔNp63 overexpression in SCC. These findings suggest that Stxbp4 could be a relevant therapeutic target for SCC detection and treatment. (See pp. E4806–E4814.)

Embryonic regeneration by relocalization of the Spemann organizer during twinning in *Xenopus*

Yuki Moriyama and Edward M. De Robertis

Many animals, including humans, can generate identical twins from a single egg. We perfected a method by which a frog (*Xenopus*) egg cut in half along the dorsal–ventral (back to belly) axis at the 4,000-cell stage produced twins at high frequency. The large wound generated by bisection healed within an hour, juxtaposing cells that would normally form the most dorsal and ventral tissues in the intact embryo. Tracing the fate of microinjected cells showed that the dorsal Spemann organizer was formed 90° away from its original location in bisected embryos. A new gradient of dorsal–ventral signaling was generated by this displacement, explaining the regeneration of the missing half. The experiments help explain twinning in a classic model system. (See pp. E4815–E4822.)

Strategic investment explains patterns of cooperation and cheating in a microbe

Philip G. Madgwick, Balint Stewart, Laurence J. Belcher, Christopher R. L. Thompson, and Jason B. Wolf

Contributing to cooperation is costly, while its rewards are often available to all members of a social group. Therefore, cooperation is vulnerable to exploitation by individuals that do not contribute but nevertheless share the benefits. So why contribute to cooperation? This dilemma can be resolved if individuals modulate their "investment" into cooperation dependent on whether benefits go to relatives or nonrelatives, which maximizes the return on investment to their genes. To evaluate this idea, we derived a model for cooperative investment and tested its predictions using a social microbe that cooperatively builds a stalk to facilitate spore dispersal. We find that cooperative investment into stalk closely matches predictions, with strains strategically adjusting investment according to their relatedness to their group. (See pp. E4823–E4832.)

Suppressor mutation analysis combined with 3D modeling explains cohesin's capacity to hold and release DNA

Xingya Xu, Ryuta Kanai, Norihiko Nakazawa, Li Wang, Chikashi Toyoshima, and Mitsuhiro Yanagida

The heterodimeric cohesin SMC complex embraces duplex DNA and is associated with Rad21, which is cleaved in mitotic anaphase by a protease called separase/Cut1. Upon Rad21 cleavage, chromosomal DNAs are released from cohesin and segregated. We identified extragenic suppressors for separase and cohesin temperature-sensitive (ts) mutants using whole-genome sequencing and made the surprising discovery that cleavage of Rad21 is largely dispensable if suppressor causes physical disorders of cohesin interfaces among essential subunits. The predicted disorders provide insights into a DNA "hold-and-release" model in which hinge and head of SMC subunits are proximal to form arched coiled coils that close or open by their orientation. The model is distinct from the "ring" model and may promote further study. (See pp. E4833–E4842.)

Identification of cytokine-specific sensory neural signals by decoding murine vagus nerve activity

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Evolution conferred animals with molecular sensors that monitor cellular and organ function to detect changes in the

environment. These activate sensory neural responses that drive the action of reflexes that maintain cellular and physiological homeostasis. Recent advances reveal that neural reflexes modulate the immune system, but it was previously unknown whether cytokine mediators of immunity mediate specific neural signals. Here we develop methods to isolate and decode specific neural signals recorded from the vagus nerve to discriminate between the cytokines IL-1β and TNF. This methodological waveform successfully detects and discriminates between specific cytokine exposures using neural signals. (See pp. E4843–E4852.)

Osmotic stabilization prevents cochlear synaptopathy after blast trauma

Jinkyung Kim, Anping Xia, Nicolas Grillet, Brian E. Applegate, and John S. Oghalai

Trauma due to roadside bombs is an unfortunate consequence of modern warfare and terrorist attacks. Hearing loss often occurs because the cochlea is the body's most sensitive pressure transducer. Here, we used in vivo imaging of the mouse cochlea using optical coherence tomography to show that increased endolymph volume correlates with damage to the auditory synapse. Reducing endolymph volume by increasing perilymph tonicity treated the synaptic loss. Therefore, this study identifies a treatment for noise-induced hearing loss. Furthermore, it suggests that this treatment may help patients with Meniere's disease, a disabling syndrome of vertigo and hearing loss due to increased endolymph volume. (See pp. E4853–E4860.)

P_{II}-like signaling protein SbtB links cAMP sensing with cyanobacterial inorganic carbon response

Khaled A. Selim, Florian Haase, Marcus D. Hartmann, Martin Hagemann, and Karl Forchhammer

Life on Earth depends on photosynthetic CO_2 fixation to form organic carbon. This process evolved in cyanobacteria and was later conveyed to eukaryotes, giving rise to plastids in algae and plants. To cope with low atmospheric CO_2 concentrations that developed over the course of evolution, cyanobacteria evolved a CO_2-concentrating mechanism (CCM), which elevates CO_2 levels in the vicinity of RubisCO, the key enzyme of CO_2 fixation. Here we describe a conserved cyclic AMP receptor protein, SbtB, which participates in the sensing of fluctuating C_i levels to regulate the cyanobacterial CCM system. SbtB represents a new principle of C_i sensing, which is important for acclimation to varying C_i regimes in the ecological niches of cyanobacteria. (See pp. E4861–E4869.)

Periplasmic depolymerase provides insight into ABC transporter-dependent secretion of bacterial capsular polysaccharides

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Capsules are critical virulence determinants for bacterial pathogens. They are composed of capsular polysaccharides (CPSs) with diverse structures, whose assembly on the cell surface is often powered by a conserved ABC transporter. Current capsule-assembly models include a contiguous trans-envelope channel directing nascent CPSs from the transporter to the cell surface. This conserved apparatus is an attractive target for antivirulence antimicrobial development. This work describes a CPS depolymerizing lyase enzyme found in the *Burkholderiales* and unique structural features that define its mechanism, CPS specificity, and evolution to function in the periplasm in a noncatabolic role. The activity of this enzyme provides evidence that CPS assembled in an ABC transporter-dependent system is exposed to periplasm during translocation to the cell surface. (See pp. E4870–E4879.)

Specificity and robustness of long-distance connections in weighted, interareal connectomes

Richard F. Betzel and Danielle S. Bassett

Interareal communication occurs along physical pathways. The prevailing hypothesis is that long-distance connections reduce the processing length between brain areas, facilitating efficient communication. We show, in five weighted interareal network datasets, that the correlation of connection weight with distance implies that long-distance connections play only a minor role in reducing path length. Instead, long-distance connections add diversity to brain area inputs and outputs, leading to increasingly complex brain dynamics. These findings help to clarify our understanding of how brain structure contributes to interareal communication. (See pp. E4880–E4889.)

Dopamine receptors mediate strategy abandoning via modulation of a specific prelimbic cortex–nucleus accumbens pathway in mice

Qiaoling Cui, Qian Li, Hongyan Geng, Lei Chen, Nancy Y. Ip, Ya Ke, and Wing-Ho Yung

Strategy-switching flexibility is a critical executive function necessary for living in an ever-evolving environment, and this ability is often impaired in attentional deficit and hyperactivity disorder, schizophrenia, and early Parkinson's disease. To date, the underlying brain circuitry and receptor mechanisms are not entirely clear. The results of the present study suggest the essential role of a specific projection from prelimbic cortex to nucleus accumbens (NAc) D2 medium spiny neurons as well as NAc dopamine and presynaptic dopamine receptors of this projection in controlling the strategy-switching flexibility. These findings promote a better understanding of circuitry and neurobiology of strategy-switching flexibility and could contribute to identifying novel therapeutic targets for patients suffering from strategy-switching inflexibility. (See pp. E4890–E4899.)

A selective class of inhibitors for the CLC-Ka chloride ion channel

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Chloride ion channels and transporters (CLCs) are critical to cardiovascular, neurological, and musculoskeletal function. Small molecules capable of selectively inhibiting CLCs would serve as valuable tools for investigating CLC function and would have potential applications for treating CLC-related disorders. The lack of such agents has impeded efforts to study this family of proteins. This work introduces a class of inhibitors with unprecedented selectivity for a single CLC homolog, CLC-Ka. Insights gained through experiments to validate a predicted ligand binding site and to evaluate structure–activity relationships rationalize inhibitor potency and CLC-Ka selectivity. Our findings provide tools for studies of CLC-Ka function and will assist subsequent efforts to advance specific molecular probes for different CLC homologs. (See pp. E4900–E4909.)

Genomic integration of ERRγ-HNF1β regulates renal bioenergetics and prevents chronic kidney disease

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Renal epithelial cells (RECs) contain abundant mitochondria that are essential to support renal reabsorption of electrolytes, glucose, and amino acids. However, it remains poorly understood how mitochondrial metabolism is coordinated with kidney reabsorptive functions. Here we show that deletion of estrogen-related receptor gamma (ERRγ) in RECs results in severe renal mitochondrial and reabsorptive dysfunction with fluid-filled cysts. ERRγ directly regulates mitochondrial metabolism and cooperates in regulating renal reabsorption genes with hepatic nuclear factor 1 beta (HNF1β), mutations of which cause strikingly similar renal dysfunction and cysts in animals and humans. These findings reveal a role for ERRγ in simultaneously coordinating a transcriptional program of renal energy-generating mitochondrial and energy-consuming reabsorptive functions relevant to kidney disease. (See pp. E4910–E4919.)

N-hydroxy-pipecolic acid is a mobile metabolite that induces systemic disease resistance in *Arabidopsis*

Yun-Chu Chen, Eric C. Holmes, Jakub Rajniak, Jung-Gun Kim, Sandy Tang, Curt R. Fischer, Mary Beth Mudgett, and Elizabeth S. Sattely

Plants lack circulating immune cells and instead rely on small molecule chemistry for local and long-distance defense signaling. Following pathogen attack, plants activate innate immune pathways at the site of infection to limit pathogen growth. Plants also possess the ability to prime similar immune responses in uninfected tissues to prevent the spread of pathogens or protect against new infections. Despite the importance of systemic immunity, the mechanism for signaling is not clear. In this study, we show that *N*-hydroxy-pipecolic acid metabolites are mobile defense signals produced at the site of bacterial infection and establish and amplify defense in uninfected, distal tissues. Our study illuminates the chemical nature of a mobile bioactive metabolite that confers pathogen resistance throughout the plant. (See pp. E4920–E4929.)

Time-evolving genetic networks reveal a NAC troika that negatively regulates leaf senescence in *Arabidopsis*

Hyo Jung Kim, Ji-Hwan Park, Jingil Kim, Jung Ju Kim, Sunghyun Hong, Jeongsik Kim, Jin Hee Kim, Hye Ryun Woo, Changbong Hyeon, Pyung Ok Lim, Hong Gil Nam, and Daehee Hwang

Leaf senescence is regulated in a complex manner, involving time-dependent interactions with developmental and environmental signals. Genetic screens have identified key regulators of senescence, particularly late-stage senescence regulators. Recently, time-course gene-expression and network analyses, mostly analyses of static networks, have predicted many senescence regulators. However, senescence is defined by time-evolving networks, involving the temporal transition of interactions among senescence regulators. Here, we present time-evolving networks of NAM/ATAF/CUC (NAC) transcription factors, central regulators of leaf senescence in *Arabidopsis*, via time-course gene-expression analysis of NACs in their mutants. These time-evolving networks revealed a unique regulatory module of NACs that controls the timely induction of senescence-promoting processes at a presenescent stage of leaf aging. (See pp. E4930–E4939.)

Codon usage of highly expressed genes affects proteome-wide translation efficiency

Idan Frumkin, Marc J. Lajoie, Christopher J. Gregg, Gil Hornung, George M. Church, and Yitzhak Pilpel

Highly expressed genes are encoded by codons that correspond to abundant tRNAs, a phenomenon thought to ensure high expression levels. An alternative interpretation is that highly expressed genes are codon-biased to support efficient translation of the rest of the proteome. Until recently, it was impossible to examine these alternatives, since statistical analyses provided correlations but not causal mechanistic explanations. Massive genome engineering now allows recoding genes and examining effects on cellular physiology and protein translation. We engineered the *Escherichia coli* genome by changing the codon bias of highly expressed genes. The perturbation affected the translation of other genes, depending on their codon demand, suggesting that codon bias of highly expressed genes ensures translation integrity of the rest of the proteome. (See pp. E4940–E4949.)