PNAS Plus Significance Statements

Functional evolution of IGF2:IGF2R domain 11 binding generates novel structural interactions and a specific IGF2 antagonist

Susana Frago, Ryan D. Nicholls, Madeleine Strickland, Jennifer Hughes, Christopher Williams, Lee Garner, Mirvat Surakhy, Rory Maclean, Dellel Rezgui, Stuart N. Prince, Oliver J. Zaccheo, Daniel Ebner, Sabina Sanegre, Sheng Yu, Francesca M. Buffa, Matthew P. Crump, and Andrew Bassim Hassan

During 150 million years of mammalian evolution, the membrane-bound mannose 6-phosphate receptor evolved high-affinity binding loops for insulin-like growth factor 2. It remains unknown whether this highly evolved ligand interaction is optimal, and whether it can be further evolved. We addressed these questions using a mutation and selection strategy that incorporated surface display and protein structure. Multiple mutations of all the binding loops were identified and improved affinity by 100-fold when combined, yet retained IGF2 specificity. Structurally, IGF2 surface interactions with binding loops were reshaped, indicating that binding site evolution could not be predicted. High IGF2 affinity binding domains could selectively inhibit IGF2-dependent cell signaling, and may be applied in therapeutic IGF2 targeting in cancer. (See pp. E2766–E2775.)

Control of serotonin transporter phosphorylation by conformational state

Yuan-Wei Zhang, Benjamin E. Turk, and Gary Rudnick

Mutations in serotonin transporter (SERT) affecting regulation of a cGMP-dependent signaling pathway have been associated with psychiatric disorders. Earlier studies provided preliminary evidence that cGMP stimulates phosphorylation of SERT in a transmembrane helix. Transporters like SERT function by undergoing a cycle of conformational changes as they move their substrate (in this case the neurotransmitter 5-HT) across the membrane. This work shows that SERT conformation dramatically affects phosphorylation, which is stimulated when the transporter is in a conformation known to increase during 5-HT transport. The results suggest a novel mechanism of regulation in which transport of 5-HT increases the level of an inward-open SERT conformation that provides accessibility of the phosphorylation site to a kinase. (See pp. E2776–E2783.)

Coordinated beating of algal flagella is mediated by basal coupling

Kirsty Y. Wan and Raymond E. Goldstein

In areas as diverse as developmental biology, physiology, and biomimetics, there is great interest in understanding the mechanisms by which active hair-like cellular appendages known as flagella or cilia are brought into coordinated motion. The prevailing theoretical hypothesis over many years is that fluid flows driven by beating flagella provide the coupling that leads to synchronization, but this is surprisingly inconsistent with certain experimentally observed phenomena. Here we demonstrate the insufficiency of hydrodynamic coupling in an evolutionarily significant range of unicellular algal species bearing multiple flagella, and suggest that the key additional ingredient for precise coordination of flagellar beating is provided by contractile fibers of the basal apparatus. (See pp. E2784–E2793.)

Hsp70 biases the folding pathways of client proteins

Ashok Sekhar, Rina Rosenzweig, Guillaume Bouvignies, and Lewis E. Kay

Hsp70 (70-kDa heat shock protein) chaperones bind cognate substrates to prevent their aggregation and guide them toward their correctly folded, functional states. Here we use NMR spectroscopy to understand how this is achieved by studying a complex of Hsp70 with a folding competent substrate. Using an NMR experiment presented here, we show that long-range transient contacts are established in the unfolded, unbound state of the substrate. These contacts are greatly attenuated in the bound form of the substrate that also exists as an unfolded ensemble. Our results establish that Hsp70 binding can significantly bias the folding mechanism of client substrate molecules toward pathways where secondary structure is first generated, followed by the establishment of longer-range interactions in a distance-dependent fashion. (See pp. E2794–E2801.)

Living biofouling-resistant membranes as a model for the beneficial use of engineered biofilms

Thammajun L. Wood, Rajarshi Guha, Li Tang, Michael Geitner, Manish Kumar, and Thomas K. Wood

Biofouling is a significant problem for membrane-based systems because it reduces flow and increases energy consumption. This work shows a previously unreported approach to prevent membrane biofouling by using a beneficial biofilm. The beneficial strain was engineered to have a dispersal "feedback circuit," based on secretion and uptake of a communication signal, limiting its own biofilm formation by self-monitoring and selective dispersal. The beneficial strain was also engineered to

produce nitric oxide, which prevents biofilm formation by harmful bacteria; biofouling by the two most prevalent organisms was shown to be controlled by the beneficial strain. Moreover, the beneficial biofilm was engineered to produce an evolved epoxide hydrolase to enable it to remove the environmental pollutant epichlorohydrin. (See pp. E2802–E2811.)

Predicting the evolutionary dynamics of seasonal adaptation to novel climates in *Arabidopsis thaliana*

Alexandre Fournier-Level, Emily O. Perry, Jonathan A. Wang, Peter T. Braun, Andrew Migneault, Martha D. Cooper, C. Jessica E. Metcalf, and Johanna Schmitt

Anticipating the effect of climate change on plants requires understanding its evolutionary consequence on traits and genes in complex realistic environments. How seasonal variation has an impact on the dynamics of adaptation in natural populations remains unclear. We simulated adaptation to different climate change scenarios, grounding our analysis in experimental data and explicitly exploring seasonal variation. Seasonal variation dramatically affected the dynamics of adaptation: Marked seasonality led to genetic differentiation within the population to different seasonal periods, whereas low seasonality led to a single population with fast-evolving fitness. Our results suggest the prevalence of phenotypic plasticity across environmental conditions in determining how climate change will shift selection on traits and loci. In this unpredictable context, maintaining broad genomic diversity is critical. (See pp. E2812–E2821.)

Riches of phenotype computationally extracted from microbial colonies

Tzu-Yu Liu, Anne E. Dodson, Jonathan Terhorst, Yun S. Song, and Jasper Rine

The genome and physiology of a cell can undergo complex changes among the many cells that make up a growing microbial colony. Genetic and physiological dynamics can be revealed by measuring reporter-gene expression, but rigorous quantitative analysis of colony-wide patterns has been underexplored. Here, we developed a suite of automated image processing, feature extraction, visualization, and classification algorithms to facilitate the analysis of sectoring patterns in *Saccharomyces* colonies. Classification results for various mutants and for colonies grown under different environmental conditions revealed significant differences in sectoring that were not apparent by visual inspection. (See pp. E2822–E2831.)

Roles for ROS and hydrogen sulfide in the longevity response to germline loss in *Caenorhabditis elegans*

Yuehua Wei and Cynthia Kenyon

Signals from reproductive tissues and germ cells influence the lifespans of many organisms, including mammals. How germ cells, which give rise to the next generation, control the aging of the animal in which they reside is poorly understood. Counterintuitively, we found that removing germ cells in *Caenorhabditis elegans* triggers the generation of two potentially toxic substances, reactive oxygen species and hydrogen sulfide, in nonreproductive somatic tissues. These substances, in turn, induce protective responses that slow aging. (See pp. E2832–E2841.)

Thy1$^+$IL-7$^+$ lymphatic endothelial cells in iBALT provide a survival niche for memory T-helper cells in allergic airway inflammation

Kenta Shinoda, Kiyoshi Hirahara, Tomohisa Iinuma, Tomomi Ichikawa, Akane S. Suzuki, Kaoru Sugaya, Damon J. Tumes, Heizaburo Yamamoto, Takahiro Hara, Shizue Tani-ichi, Koichi Ikuta, Yoshitaka Okamoto, and Toshinori Nakayama

A substantial proportion of people have intractable chronic allergic diseases for which no curative treatment exists. A clear understanding of how these allergic diseases develop and persist is lacking. Here, unique ectopic lymphoid-like structures called inducible bronchus-associated lymphoid tissue were found to be formed during chronic airway inflammation, and were critical in persistent inflammation. In addition, we identified a Thy1$^+$IL-7$^+$IL-33$^+$ subset of lymphatic endothelial cells (LECs) that support the maintenance of memory-type pathogenic T helper 2 (Tpath2) cells. A similar population of IL-7$^+$IL-33$^+$ LECs was found in nasal polyps of patients with eosinophilic chronic rhinosinusitis. Thus, we revealed that Thy1$^+$IL-7–producing LECs control chronic allergic airway inflammation by supporting memory-type Tpath2 cells in human and mouse systems. (See pp. E2842–E2851.)

Novel DLK-independent neuronal regeneration in *Caenorhabditis elegans* shares links with activity-dependent ectopic outgrowth

Samuel H. Chung, Mehraj R. Awal, James Shay, Melissa M. McLoed, Eric Mazur, and Christopher V. Gabel

By laser surgery, genetics, and pharmacology, we demonstrate that neurons of the nematode *Caenorhabditis elegans* undergo a novel form of regeneration that is largely independent of defined regeneration pathways, including DLK, which underlies axon regeneration from *C. elegans* to mammals. Our results indicate genetic and molecular connections between DLK-independent regeneration and a previously studied activity-dependent ectopic axon outgrowth in *C. elegans*. We also note numerous similarities with lesion-conditioned regeneration, in which reduction of sensory activity triggers robust axon regeneration in the mammalian CNS. Our study unites disparate forms of neurite outgrowth to uncover the molecular mechanisms that modulate regeneration in the adult CNS and suggests that ectopic outgrowth might represent a powerful gene discovery platform for regeneration. (See pp. E2852–E2860.)

Genetic background and epigenetic modifications in the core of the nucleus accumbens predict addiction-like behavior in a rat model

Shelly B. Flagel, Sraboni Chaudhury, Maria Waselus, Rebeca Kelly, Salima Sewani, Sarah M. Clinton, Robert C. Thompson, Stanley J. Watson Jr., and Huda Akil

Determining the factors that render an individual more susceptible or resilient to cocaine addiction has great implications for treatment. We exploit a unique model to demonstrate that genetic differences in vulnerability to cocaine addiction exist in the rat. We examined gene expression and the epigenetic regulation of two genes—fibroblast growth factor (FGF2) and the dopamine D2 receptor (D2)—in the nucleus accumbens core. Low levels of D2 mRNA, via epigenetic modifications, may play a role in susceptibility to cocaine addiction. Specifically, binding of a repressive mark on histones (H3K9me3) at the D2 promoter is associated with the propensity to relapse. In contrast, low levels of FGF2, which persist even following prolonged self-administration, may protect individuals from cocaine addiction. (See pp. E2861–E2870.)