PNAS Plus Significance Statements

Kinetically coupled folding of a single HIV-1 glycoprotein 41 complex in viral membrane fusion and inhibition

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Enveloped viruses infect cells via fusion of viral and host cell membranes mediated by highly conserved fusion proteins. HIV-1 glycoprotein 41 (gp41) represents a family of fusion proteins with similar structures and fusion mechanisms. They couple their energetic folding to draw two membranes close for fusion, forming trimers of helical hairpins. Yet, the energy release, force generation, and kinetics associated with folding of these proteins are poorly quantified. We found (pp. E2855–E2864) that gp41 hairpins fold sequentially but in a kinetically coupled manner and that an anti-HIV drug blocked gp41 folding by a new mechanism. As major proteins on viral surfaces, fusion proteins are primary targets for vaccine development and fusion inhibitors to intervene in major infectious diseases such as AIDS, Ebola, and influenza.

Substrate-binding domain conformational dynamics mediate Hsp70 allostery

Anastasia Zhuravleva and Lila M. Gierasch

Heat shock protein 70 (Hsp70) molecular chaperones help maintain protein homeostasis. Hsp70 functions require regulated promiscuous binding and release of a wide range of protein substrates. ATP binding to the Hsp70 nucleotide-binding domain (NBD) regulates the affinity and kinetics of substrate binding to their substrate-binding domain (SBD). Our work (pp. E2865–E2873) sought deeper understanding of the role of conformational dynamics for allosteric signaling in Hsp70s: The SBD undergoes a seesaw-like conformational change from a high substrate affinity state to one with lower substrate affinity, and we show that this conformational change results in drastic changes in conformational flexibility for the SBD that are essential for efficient substrate binding and release. These insights will help efforts to use Hsp70s as therapeutic targets.

Imperfect drug penetration leads to spatial monotherapy and rapid evolution of multidrug resistance

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The evolution of drug resistance is a major health threat. In chronic infections with rapidly mutating pathogens—including HIV, tuberculosis, and hepatitis B and C viruses—multidrug resistance can cause even aggressive combination drug treatment to fail. Oftentimes, individual drugs within a combination do not penetrate equally to all infected regions of the body. Here (pp. E2874–E2883) we present a mathematical model suggesting that this imperfect penetration can dramatically increase the chance of treatment failure by creating regions where only one drug from a combination reaches a therapeutic concentration. The resulting single-drug compartments allow the pathogen to evolve resistance to each drug sequentially, rapidly causing multidrug resistance. More broadly, our model provides a quantitative framework for reasoning about trade-offs between aggressive and moderate drug therapies.

Entamoeba mitosomes play an important role in encystation by association with cholesteryl sulfate synthesis

Fumika Mi-ichi, Tomofumi Miyamoto, Shouko Takao, Ghulam Jeelani, Tetsuo Hashimoto, Hiromitsu Hara, Tomoyoshi Nozaki, and Hiroki Yoshida

Evolution and diversification of organelles is a central topic in biology. Mitochondrion-related organelles (MROs) are highly modified forms of mitochondria found in anaerobic eukaryotes. MROs show a spectrum of functions that are either reduced or modified from those of canonical mitochondria by environmental constraints and evolutionary selection. Hence, elucidation of MRO functions will improve our understanding of organelle evolution and the speciation of eukaryotes. Here (pp. E2884–E2890), we substantiate a role of the *Entamoeba* mitosome, a type of MRO, by showing that cholesteryl sulfate synthesized through a mitosomal pathway regulates differentiation that is essential for the parasite's life cycle. These findings support the contribution of an endosymbiont-derived organelle to parasitism, a previously unrecognized concept that casts new light on organelle evolution.

AMCase is a crucial regulator of type 2 immune responses to inhaled house dust mites

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Millions of people are affected by asthma and that number is growing. A clear understanding of how the disease develops is lacking. The immune responses to inhaled allergens like house dust mite (HDM) provide much of the basis of asthma. Acidic mammalian chitinase (AMCase) is an enzyme that degrades chitin, a major structural polymer in the exoskeleton of HDM. By the use of a newly generated enzymatically dead AMCase knockin mouse, we found that AMCase enzymatic activity is of critical importance in the control of type 2 immune responses to inhaled HDM. This discovery (pp. E2891–E2899) may help the understanding of the mechanisms that govern the development of chitin-related asthma and allergy and may lead to new therapeutic strategies in these disorders.

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VEGF-B promotes cancer metastasis through a VEGF-A–independent mechanism and serves as a marker of poor prognosis for cancer patients

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Cancer metastasis is responsible for a majority of the mortality in cancer patients and involves complex interactions, modulated by various factors and cytokines, between malignant and host cells. Vascular structures in solid tumors are crucial for cancer cell intravasation into the circulation. Our present work (pp. E2900–E2909) shows that VEGF-B produced by tumor cells significantly remodels tumor microvasculature, leading to leaky vascular networks that are highly permissive for tumor cell invasion. VEGF-B–promoted cancer metastasis occurs through a VEGF-A–independent mechanism. Thus, inhibition of VEGF-B should be considered an independent approach for the development of new drugs for the treatment of cancer invasion and metastasis. VEGF-B also may be considered as an independent prognostic marker for cancer metastasis.

Reversal of mitochondrial defects with CSB-dependent serine protease inhibitors in patient cells of the progeroid Cockayne syndrome

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Ageing is dramatically accelerated in Cockayne syndrome (CS), but the impairments that lead to this phenotype have not been elucidated. The DNA repair proteins CSA or CSB are mutated in CS, but premature ageing is not caused by the DNA repair defect. CSB also affects mitochondrial turnover. Our data reveal a novel pathway that is affected in CS cells. We show (pp. E2910–E2919) that CSB deregulates the expression of a serine protease, which degrades mitochondrial DNA polymerase gamma and impairs mitochondrial function. We rescue this defect, by two independent strategies, in primary cells from patients. Our findings open novel possibilities for developing treatments, which are presently missing for CS patients. Abnormalities revealed here might occur at a slower rate during normal physiological ageing.

Virus-induced translational arrest through 4EBP1/2-dependent decay of 5′-TOP mRNAs restricts viral infection

Kaycie C. Hopkins, Michael A. Tartell, Christin Herrmann, Brent A. Hackett, Frances Taschuk, Debasis Panda, Sanjay V. Menghani, Leah R. Sabin, and Sara Cherry

Rift Valley fever virus (RVFV), a mosquito-transmitted bunyavirus, blocks the two common methods of antiviral translational shutdown, PKR and type I interferon. However, it has previously been shown that RVFV infection halts protein production in infected human cells. Here (pp. E2920–E2929), we demonstrate that RVFV is restricted by a previously unknown mechanism of antiviral translational shutdown, wherein 5′-terminal oligopyrimidine (5′-TOP) mRNAs encoding the core translational machinery are selectively degraded by the RNA decapping enzyme NUDT16 during RVFV infection, and that this decay is triggered in response to mTOR attenuation via the translational repressor 4EBP1/2 axis. We present a previously unknown mechanism for translational shutdown in response to viral infection and identify mTOR attenuation as a potential therapeutic target against bunyaviral infection.

Identifying personal microbiomes using metagenomic codes

Eric A. Franzosa, Katherine Huang, James F. Meadow, Dirk Gevers, Katherine P. Lemon, Brendan J. M. Bohannan, and Curtis Huttenhower

Recent surveys of the microbial communities living on and in the human body—the human microbiome—have revealed strong variation in community membership between individuals. Some of this variation is stable over time, leading to speculation that individuals might possess unique microbial "fingerprints" that distinguish them from the population. We rigorously evaluated this idea by combining concepts from microbial ecology and computer science. Our results (pp. E2930–E2938) demonstrated that individuals could be uniquely identified among populations of 100s based on their microbiomes alone. In the case of the gut microbiome, >80% of individuals could still be uniquely identified up to a year later—a result that raises potential privacy concerns for subjects enrolled in human microbiome research projects.

Cell rejuvenation and social behaviors promoted by LPS exchange in myxobacteria

Christopher Vassallo, Darshankumar T. Pathak, Pengbo Cao, David M. Zuckerman, Egbert Hoiczyk, and Daniel Wall

Social organisms benefit from group behaviors that endow favorable fitness consequences among kin. We describe such a behavior in the bacterium *Myxococcus xanthus* in which damaged members of a population are repaired by their kin by exchange of outer membrane material (pp. E2939–E2946). This behavior rescues lethal cellular damage, restores antibiotic resistance to a compromised cell membrane, and increases the overall fitness of a heterogeneous population. To our knowledge, we provide the first evidence that a social bacterium can use cell-content sharing to repair damaged siblings, leading to beneficial fitness outcomes for both the donor and recipient.

Avian sarcoma leukosis virus receptor-envelope system for simultaneous dissection of multiple neural circuits in mammalian brain

Makoto Matsuyama, Yohei Ohashi, Tadashi Tsubota, Masae Yaguchi, Shigeki Kato, Kazuto Kobayashi, and Yasushi Miyashita

Genetic dissection of multiple neural pathways remains challenging because of the limited number of genetic methods that can be used simultaneously. To overcome this limitation, we used modified avian sarcoma and leukosis virus envelopes and receptors to develop highly orthogonal genetic tools that can achieve expression of different genes in different target cells. From in vitro and in vivo

screens, we identified tools that can specifically transfer genes of interest into mammalian neurons via engineered receptors, with minimal unintended interactions. Using this approach (pp. E2947–E2956), we achieved pathway-specific, differential fluorescent labeling of three thalamic neuronal populations, each projecting into different cortical regions. Thus, our approach provides independent, simultaneous, and specific genetic tools for manipulating intermingled neural pathways in vivo.

Retinal waves regulate afferent terminal targeting in the early visual pathway

Samuel Failor, Barbara Chapman, and Hwai-Jong Cheng

Spontaneous neural activity is known to play a role in the maturation of nascent neural circuitry. Here (pp. E2957–E2966) we show for the first time a role for early spontaneous correlated retinal activity (i.e., retinal waves) in regulating the laminar targeting and functional development of the retinogeniculate pathway in the monocular condition—a condition where intereye competition and eye-specific segregation are not present. These findings demonstrate the importance of intraeye competition in early visual pathway circuit development. Our results provide a revision to the model of retinogeniculate development and to our general understanding of how neural activity guides the establishment of proper connectivity in the developing brain.

Optimized tools for multicolor stochastic labeling reveal diverse stereotyped cell arrangements in the fly visual system

Aljoscha Nern, Barret D. Pfeiffer, and Gerald M. Rubin

Nervous systems contain vast numbers of neurons with diverse shapes and complex spatial relationships. We describe (pp. E2967–E2976) new genetic tools for the efficient visualization by light microscopy of individual neurons and their relative positions in *Drosophila*. The application of these methods to the visual system revealed an unexpected diversity of cell-type–specific arrangements of neuronal processes within a single brain region. This wide range of stereotyped cell arrangements provides distinct circuit elements for processing visual information and implies the existence of a surprisingly large number of genetic programs that produce these arrangements during development.