PNAS Plus Significance Statements

Generic, phenomenological, on-the-fly renormalized repulsion model for self-limited organization of terminal supraparticle assemblies

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Engineering nanoassemblies with uniform characteristic dimensions is of great interest given the unique properties of nanoscale building blocks and their collective response in superstructures. We present (pp. E3161–E3168) a generic, particle-based model that predicts the formation of self-limited, or "terminal," supraparticle assemblies observed in many inorganic, colloidal, and biological systems. The key factor that leads to the self-limiting behavior is shown to be that the repulsion between particles becomes renormalized "on-the-fly" as the particles aggregate. The model explains the monodispersity of terminal assemblies formed from polydisperse nanoparticles, as observed in recent experiments. Our findings not only deepen the understanding of how self-limited, or terminal assemblies form, but also offer versatile approaches to control the dimension and shape of synthetic nanoassemblies.

Regulation by a chaperone improves substrate selectivity during cotranslational protein targeting

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Correct protein biogenesis is crucial for all cells. Numerous factors including molecular chaperones, modification enzymes, and protein-targeting machineries bind near the ribosome exit site and can access the nascent protein. How nascent proteins are accurately selected into the correct biogenesis pathway in such a crowded environment is an emerging question central to accurate protein biogenesis. Using chemical biology and biochemical and biophysical tools, we show (pp. E3169–E3178) that the major cotranslational chaperone, trigger factor, and cotranslational targeting machinery, signal recognition particle, regulate each other at multiple stages, including initial binding, ribosome delivery to the membrane, and enforcement of a timer for cotranslational protein targeting. Together, these mechanisms enhance accurate substrate selection into both cotranslational and posttranslational protein targeting pathways.

Conserved SMP domains of the ERMES complex bind phospholipids and mediate tether assembly

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Phospholipid exchange between the endoplasmic reticulum (ER) and mitochondria is essential for membrane biogenesis and, ultimately, cell survival. It remains unclear, however, how this exchange is facilitated. Our study (pp. E3179–E3188) investigates a putative involvement of the ER-mitochondrial encounter structure (ERMES), a tethering complex that bridges the ER and mitochondria, in phospholipid transport in yeast. We show that a conserved ERMES domain called the "synaptotagmin-like mitochondrial lipid-binding protein" (SMP) domain preferentially binds phosphatidylcholines and mediates the hierarchical assembly of the tether. The 17-Å-resolution EM structure of the complex formed between the SMP domains present in two ERMES subunits, Mdm12 and Mmm1, reveals an elongated, tubular-shaped heterotetramer traversed by a hydrophobic channel, suggesting a mechanism for lipid transport between the two organelles.

Conformational processing of oncogenic v-Src kinase by the molecular chaperone Hsp90

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Hsp90 is a molecular chaperone involved in the activation of numerous client proteins, including 60% of the human kinases. Previous studies on the Hsp90–kinase interaction were limited due to the particular instability of client kinases. Here (pp. E3189–E3198), we reconstituted v-Src kinase chaperoning in vitro and used this to mechanistically elucidate how Hsp90 supports kinases. We show that its activation is ATP-dependent and requires the phosphorylated form of the cochaperone Cdc37. Hsp90 does not influence the almost identical c-Src kinase. The structural analysis of Src kinase chimeras that gradually transformed c-Src into v-Src unveiled that Hsp90 dependence correlates with client compactness, folding cooperativity, and lowered energy barriers between different states. These findings establish a new concept for the client specificity of Hsp90.

Phosphoregulatory protein 14-3-3 facilitates SAC1 transport from the endoplasmic reticulum

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Eukaryotes have evolved a multitrack transport process: the secretory pathway to route proteins to different destinations in a cell. Most cargo proteins originate on ribosomes bound to the endoplasmic reticulum (ER) and exit from the ER in membrane vesicles. The cytosolic coat protein complex II (COPII) coat is responsible for cargo protein sorting and vesicle budding from the ER. For some cargo proteins, additional cytosolic proteins serve to assist the core COPII machinery. One such cargo protein is the Sac1 lipid phosphatase, whose packaging into COPII vesicles depends upon the phosphopeptide binding protein, 14-3-3. Sac1 is essential to regulate cell signaling, division, and transport processes. We suggest

(pp. E3199–E3206) that the 14-3-3 protein serves a regulatory role in the packaging of Sac1 into COPII vesicles.

BMP9 and BMP10 are necessary for proper closure of the ductus arteriosus

Sandrine Levet, Marie Ouarné, Delphine Ciais, Charles Coutton, Mariela Subileau, Christine Mallet, Nicolas Ricard, Marie Bidart, Thierry Debillon, Francesca Faravelli, Caroline Rooryck, Jean-Jacques Feige, Emmanuelle Tillet, and Sabine Bailly

At birth, newborns must switch from the fetal aquatic life to the aerial one, by closure of a vessel named the ductus arteriosus. During fetal life, it allows blood to bypass the lungs, and a failure of its closure at birth is a major cause of mortality, particularly in preterm neonates. This pathological condition is known as patent ductus arteriosus and occurs in approximately 60% of preterm infants born before 28 wk of gestation. Herein (pp. E3207–E3215), we show, for the first time to our knowledge, the involvement of two circulating growth factors, bone morphogenetic proteins BMP9 and BMP10, in the anatomical closure of this vessel. This finding will have potential clinical applications in the management of this pathology.

TLR4 has a TP53-dependent dual role in regulating breast cancer cell growth

Svasti Haricharan and Powel Brown

This study fundamentally alters our understanding of how TLR4 drives breast cancer. Although TLR4 was previously considered a tumor promoter, we demonstrate a complex, TP53-dependent role for TLR4 in regulating tumor growth. TP53 is a tumor suppressor commonly inactivated across cancer types. In *TP53* wild-type cancer cells, TLR4 activation causes secretion of IFN-γ into the microenvironment, resulting in induction of p21 and inhibition of cell growth. Conversely, TLR4 activation in *TP53* mutant cells promotes cancer cell growth by regulating CXCL1 and CD154 secretion. In this paper (pp. E3216–E3225), we identify a previously unidentified role for TLR4 in modulating tumor cell growth and microenvironment. The TLR4–TP53 association likely extends across cancer types, suggesting the need to determine the *TP53* status of any tumor before implementing anti-TLR4 therapy.

Contingency and entrenchment in protein evolution under purifying selection

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How large a role does history play in evolution? Do later events depend critically on specific earlier events, or do all events occur more or less independently? If a change occurs early in evolution, does it become easier or harder to revert the change as time proceeds? Here (pp. E3226–E3235), we explore these ideas in the context of protein evolution, by simulating sequence evolution under purifying selection and then systematically permuting the order of amino acid substitutions. Our results suggest that the amino acid substitutions that occur in evolution are typically contingent on the presence of prior substitutions, and that substitutions that occur early in evolution become entrenched and difficult to modify as subsequent substitutions accrue.

MiR-204 is responsible for inherited retinal dystrophy associated with ocular coloboma

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MicroRNAs are key players in the regulation of gene expression. An understanding of human conditions caused by microRNA mutations provides insight into mechanisms of gene regulation and into the interplay between development and maintenance in tissue homeostasis. The eye represents a notable target tissue of genetic diseases. Inherited retinal degenerations and developmental eye disorders are two separate groups that represent leading causes of blindness worldwide. Identifying underlying genetic causes of such conditions is important for diagnosis, counseling, and potential therapy development. We identified (pp. E3236–E3245) a dominant mutation in microRNA-204 as the genetic cause of a unique phenotype of retinal degeneration and coloboma and thus highlight the importance of microRNA-204 as a master regulator of ocular development and normal maintenance.

Inflammation negatively regulates FOXP3 and regulatory T-cell function via DBC1

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Treg cells suppress excessive and aberrant immune responses. Impaired function or homeostasis of Treg cells would induce severe autoimmune and inflammatory diseases. Forkhead box P3 (FOXP3), as a master regulator of Treg cells, forms a large complex with other binding factors to modulate Treg-cell function subtly in pathological and physiological conditions. We identified (pp. E3246–E3254) that Deleted in breast cancer 1 (DBC1) is an essential subunit of the FOXP3 complex in human $CD4^+$ Treg cells. Our results show that the inflammatory cytokines TNF-α or IL-6 trigger FOXP3 degradation, whereas downregulation of DBC1 expression prevents FOXP3 degradation and maintains Treg-cell function under inflammatory stimuli in vitro and in vivo. These findings unveil a previously unidentified pathway for therapeutically modulating $FOXP3^+$ Treg-cell stability under inflammation.

Molecular transitions from papillomavirus infection to cervical precancer and cancer: Role of stromal estrogen receptor signaling

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Cervical cancer (CxCa) is the second most frequent cancer in women and the third leading cause of cancer death in women worldwide. Our global analysis of gene expression in normal, precancerous, and

cancerous cervical tissue shows increased DNA replication/repair and cell proliferation followed by substantial metabolic shifts. We observed a dramatic, progressive decrease in estrogen receptor alpha (ERα) in tumor progression, and ranking specimens by estrogen-responsive gene expression correlated remarkably with histopathology. Whereas ERα expression shuts off in tumor epithelium, stromal fibroblasts in the microenvironment retain ERα, and the data indicate estrogen-related alteration of several candidate stroma–tumor signaling pathways. Our findings (pp. E3255–E3264) strongly support a role of stromal estrogen signaling in CxCa development with implications for CxCa management and control.

Extracellular ATP induces the rapid release of HIV-1 from virus containing compartments of human macrophages

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A major obstacle to the eradication of HIV-1 by combination antiretroviral therapy (cART) is the formation of cellular reservoirs in $CD4^+$ T lymphocytes (carrying latently integrated provirus) and tissue macrophages. Infected macrophages assemble new virions in subcellular vacuoles known as virus-containing compartments (VCC), hiding them from the immune system and, in part, from antiretroviral agents. Here (pp. E3265–E3273) we report that extracellular ATP is capable of inducing the rapid release of virions accumulated in VCC via interaction with the P2X7 receptor and without inducing cell death, whereas the antidepressant agent Imipramine blocks the release. Thus, our study identifies two "druggable" targets affecting the release of stored virions from infected human macrophages that could bear relevance for purging HIV-1 reservoirs in individuals receiving cART.

Protein synthesis during cellular quiescence is inhibited by phosphorylation of a translational elongation factor

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In nature, cells sense and fine-tune their metabolism in response to nutrient availability. Protein synthesis is one of the most energy-demanding metabolic processes and as such is subject to a tight regulation. A key open question, however, is how the components of the translation machinery, which are among the most abundant cellular proteins, can be regulated quickly and robustly in response to acute nutrient deprivation. We show (pp. E3274–E3281) that starved cells down-regulate protein synthesis by phosphorylation of essential and universally conserved translational GTPase Elongation factor tu (EF-Tu). Importantly, phosphorylated EF-Tu has a dominant-negative effect in elongation, resulting in the overall inhibition of protein synthesis. Thus, this novel regulatory mechanism allows for the quick and efficient regulation of protein synthesis.

Bioimage analysis of *Shigella* infection reveals targeting of colonic crypts

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Shigella spp. are responsible for devastating diarrheal diseases, primarily in children, within underdeveloped countries. *Shigella* invades the mucosa of the large intestine, causing inflammation and damage to the epithelium. Here (pp. E3282–E3290), we have measured the progression of *Shigella* infection in a small animal model of the disease to better understand the mechanism of invasion of the colonic mucosa. The novelty of our approach relies on the tracking of fluorescent bacteria inside the infected tissue at various time points using confocal microscopy and subsequent quantitative bioimage analyses. Our approach is readily applicable to other host–pathogen systems to quantify host–pathogen interactions.

$GABA_B$ receptor deficiency causes failure of neuronal homeostasis in hippocampal networks

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How neuronal circuits maintain stable activity despite continuous environmental changes is one of the most intriguing questions in neuroscience. Previous studies proposed that deficits in homeostatic control systems may underlie common neurological symptoms in a variety of brain disorders. However, the key regulatory molecules that control homeostasis of central neural circuits remain obscure. We show here that basal activity of $GABA_B$ receptors is required for firing rate homeostasis in hippocampal networks. We identified the principal mechanisms by which $GABA_B$ receptors control homeostatic augmentation of synaptic strength to chronic neuronal silencing. We propose (pp. E3291–E3299) that deficits in $GABA_B$ receptor signaling, associated with epilepsy and psychiatric disorders, may lead to aberrant brain activity by erasing homeostatic plasticity.

Impaired mitochondrial fat oxidation induces adaptive remodeling of muscle metabolism

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Many theories regarding the causes of insulin resistance in skeletal muscle center on the ability of muscle to oxidize fat, with evidence supporting either decreased or increased fatty acid oxidation (FAO) as causal to insulin resistance. Inhibition of fatty acid transport into mitochondria specifically in mouse muscle results in a rather remarkable phenotype. Despite an accumulation of lipids in muscle, insulin sensitivity is maintained. The muscle responds to decreased FAO by adapting muscle metabolism to use other fuel sources, and by an increased reliance upon peroxisomal fat oxidation. There is also an increase in mitochondrial biogenesis. At the whole-body level, the mice seem to enter an energy conservation mode with reduced activity, energy expenditure, and resistance to diet-induced obesity (pp. E3300–E3309).