PNAS Plus Significance Statements

Micromechanics of cellularized biopolymer networks

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Mechanical interactions between cells, mediated by the elastic response of the extracellular matrix to active applied forces, play a critical role in developmental biology, wound healing, and cancer progression. This work applies sophisticated technical means, both in experiment and computational modeling, to investigate the micron-scale mechanics of a popular model of this medium, a collagen gel. The results obtained show clearly that on the cellular scale, there are significant spatial variations in the micromechanics due to network heterogeneities. (See pp. E5117–E5122.)

Natural variation in *ARF18* gene simultaneously affects seed weight and silique length in polyploid rapeseed

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Seed weight is a complex trait controlled by polygenes, and its underlying regulatory mechanisms, especially those involving polyploidy crops, remain elusive. *Brassica napus* L., which is the second leading crop source of vegetable oil around the world, is an important tetraploid (4×) crop. Our results have generated three significant findings. (*i*) By combining the linkage and associated analysis, this study revealed the first (to our knowledge) quantitative trait locus (QTL) in rapeseed, which will provide insights for QTL cloning in polyploidy crops. (*ii*) The functional gene and marker could be useful in rapeseed breeding. (*iii*) We revealed a maternal regulatory pathway affecting seed weight that differs from the mechanisms described in previous reports. (See pp. E5123–E5132.)

Assembly, translocation, and activation of XerCD-*dif* recombination by FtsK translocase analyzed in real-time by FRET and two-color tethered fluorophore motion

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This study develops and exploits an expanded single-molecule fluorescence technique to understand the molecular mechanism of assembly, translocation, and disassembly of a hexameric DNA motor, FtsK, which functions ubiquitously in bacterial chromosome segregation. Assembly of single hexamers on DNA and their subsequent rapid translocation were directly assayed. FtsK hexamers dissociated soon after encountering and activating XerCD-*dif* recombination complexes. This work contrasts with previously published reports, which suggested that FtsK can reverse spontaneously during translocation, or upon encountering XerCD-*dif*. Furthermore, in some previous assays, the readout was DNA looping; here, we show that looping does not occur with single hexamer translocation. The technique used provides a blueprint for mechanistic real-time studies of individual protein–nucleic acid complexes. (See pp. E5133–E5141.)

Balancing between affinity and speed in target DNA search by zinc-finger proteins via modulation of dynamic conformational ensemble

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Many transcription factors and DNA repair/modifying enzymes must first locate the target sites through stochastic scanning of DNA in the vast presence of nonspecific sites. In this work, we demonstrate that target search by these proteins can be accelerated via engineering based on structural dynamic knowledge of the DNA-scanning process. Our biophysical data for the Egr-1 zinc-finger protein and its nuclease derivatives reveal kinetic and thermodynamic roles of the conformational equilibrium between two modes in the DNA-scanning process: one suitable for search and the other for recognition. We found that optimizing the balance between the search and recognition modes improves efficiency in zinc-finger proteins' target search. This finding can help advance zinc-finger technology for artificial gene regulation and genome editing. (See pp. E5142–E5149.)

Chronophin coordinates cell leading edge dynamics by controlling active cofilin levels

Violaine Delorme-Walker, Ji-Yeon Seo, Antje Gohla, Bruce Fowler, Ben Bohl, and Céline DerMardirossian

Cell motility plays important roles in normal physiology and numerous disease states, including cancers. Cofilin, a key player in cell locomotion, controls the direction and the force of cell protrusion. Our study establishes the cofilin phosphatase chronophin (CIN) as a major component of a PI3-kinase–mediated, Rac1-dependent signaling mechanism that activates cofilin downstream of EGF receptor in mammary carcinoma cells. During EGF stimulation, CIN redistributes to the cell edge, where it regulates cofilin-dependent actin turnover and coordinates cell protrusion and retraction dynamics. Our data uncover previously unidentified molecular mechanisms regulating cofilin in time and space in tumor cells and expand our understanding of cancer cell movement, a critical step in the process of metastasis. (See pp. E5150–E5159.)

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Receptor sequestration in response to β-arrestin-2 phosphorylation by ERK1/2 governs steady-state levels of GPCR cell-surface expression

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ERK1/2 are important G protein-coupled receptor (GPCR) signaling effectors, but their role as possible GPCR regulators remains largely uncharted. We report that ERK1/2 activation leads to the phosphorylation of β-arrestin-2 on Ser14 and Thr276, promoting the intracellular sequestration of unliganded GPCRs. This subcellular redistribution results in the dampening of cell responsiveness to GPCRs' ligand-mediated activation, positioning ERK1/2 as both a downstream effector and a negative regulator of GPCRs. Because ERK1/2 also is stimulated by receptor tyrosine kinases and is deregulated in many diseases, and because GPCRs respond to a large number of hormones and neurotransmitters, this newly uncovered regulatory process is poised to play a central role in controlling cell responsiveness in health and disease. (See pp. E5160–E5168.)

Differential control of Yorkie activity by LKB1/AMPK and the Hippo/Warts cascade in the central nervous system

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The correct regulation of tissue growth in developing organisms is essential for functional organ formation. The evolutionarily conserved transcriptional coactivator Yorkie (Yki)/Yes-activated protein (YAP) responds to a variety of upstream inputs to promote tissue growth. Yki/YAP is known to regulate stem cell proliferation, thus affecting final organ size. The Hippo (Hpo)/Warts kinase cascade is a key inhibitor of Yki activity in many epithelial tissues. Here, we show that Yki is inhibited by the nutrient-sensing liver kinase B1 (LKB1)/AMP-activated protein kinase (AMPK) cascade independent of Hpo/Warts in a population of neural progenitors in the developing *Drosophila* larval brain. Our results suggest a tissue-specific nutrient-dependent mode of Yki activity regulation. Furthermore, a tissue-specific differential wiring of Hpo signaling could represent an adjustment to the proliferation requirements of different tissue types. (See pp. E5169–E5178.)

Insect's intestinal organ for symbiont sorting

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In general, animals have a mouth for feeding, an anus for defecation, and a gut connecting them for digestion and absorption. However, we discovered that the stinkbug's gut is functionally disconnected in the middle by a previously unrecognized organ for symbiont sorting, which blocks food fluid and nonsymbiotic bacteria but selectively allows passing of a specific bacterial symbiont. Though very tiny and inconspicuous, the organ governs the configuration and specificity of stinkbug gut symbiosis, wherein the posterior gut region is devoid of food flow, populated by a specific bacterial symbiont, and transformed into an isolated organ for symbiosis. Mutant analyses showed that the symbiont's flagellar motility is needed for passing the host organ, highlighting intricate host–symbiont interactions underpinning the symbiont sorting process. (See pp. E5179–E5188.)

Comparison of predicted and actual consequences of missense mutations

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Computational tools applied to any human genome sequence identify hundreds of genetic variants predicted to disrupt the function of individual proteins as the result of a single codon change. These tools have been trained on disease mutations and common polymorphisms but have yet to be tested against an unbiased spectrum of random mutations arising de novo. Here we perform such a test comparing the predicted and actual effects of de novo mutations in 23 genes with essential functions for normal immunity and all possible mutations in the *TP53* tumor suppressor gene. These results highlight an important gap in our ability to relate genotype to phenotype in clinical genome sequencing: the inability to differentiate immediately clinically relevant mutations from nearly neutral mutations. (See pp. E5189–E5198.)

Epigenetic silencing of tumor suppressor genes during in vitro Epstein–Barr virus infection

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Aberrant promoter methylation of tumor suppressor genes (TSGs) has been found in a large number of human cancers, including Epstein–Barr virus (EBV)-associated cancers. However, how epigenetic modification controls TSG expression in primary B lymphocytes in response to EBV infection has not been fully investigated. In vitro EBV readily transforms quiescent B-cells into lymphoblastoid cell lines, providing a working model for understanding the underlying molecular mechanisms of B-cell lymphoma development. To our knowledge, our work represents the first report to demonstrate that EBV-infection of naïve B lymphocytes resulted in global transcriptional repression of TSGs through recruitment of hypermethylation activities at CpG islands. These results can be further used as potential prognostic-markers and for current therapeutic enhancements against acute-infection and EBV-associated B-cell lymphomas. (See pp. E5199–E5207.)

MavN is a *Legionella pneumophila* vacuole-associated protein required for efficient iron acquisition during intracellular growth

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Limiting access of intracellular iron to intracellular microbes is a critical arm of the nutritional immune response. Little is known about the mechanisms used by intravacuolar pathogens to overcome this attempted starvation. We show here that *Legionella pneumophila* employs its major virulence-associated secretion system to deliver a unique transmembrane protein into host cells during infection. Utilizing microscopic, biochemical, and transcriptional analysis of intracellular bacteria, we demonstrate that the MavN protein integrates into the host-derived vacuolar membrane and facilitates transport of essential iron into the lumen of the vacuole to promote bacterial growth. These findings shed light on what is likely to be a myriad of strategies used by bacteria to overcome potent host nutrient restrictions. (See pp. E5208–E5217.)