PNAS Plus Significance Statements

Phosphoproteomic analysis identifies the tumor suppressor PDCD4 as a RSK substrate negatively regulated by 14-3-3

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The RSK family is a group of Ser/Thr kinases that promotes cell growth and proliferation in response to the Ras/MAPK pathway. Deregulated RSK activity has been associated with different disorders and diseases, such as cancer, but relatively little is known regarding the contribution of RSK to tumorigenesis. In this study (pp. E2918–E2927), we describe, to our knowledge, the first global quantitative phosphoproteomic screen to characterize RSK-dependent signaling events in melanoma. Our results show that RSK negatively regulates the tumor suppressor PDCD4 by promoting its association to 14-3-3 proteins and subsequent proteasomal degradation. These findings further implicate RSK as a promising therapeutic target for the treatment of melanoma and suggest that RSK plays widespread biological functions downstream of the Ras/MAPK pathway.

DEAD-box protein CYT-19 is activated by exposed helices in a group I intron RNA

Inga Jarmoskaite, Hari Bhaskaran, Soenke Seifert, and Rick Russell

DEAD-box proteins are ubiquitous in RNA metabolism and can act as RNA chaperones by promoting folding and rearrangements of structured RNAs. However, the mechanistic basis for these chaperone activities is not well understood. Here we show (pp. E2928–E2936) that the DEAD-box protein CYT-19 unfolds a large, structured RNA molecule in a stability-dependent fashion and is more active toward less compact conformations of the RNA. Our results indicate that CYT-19 functions predominantly by disrupting accessible RNA secondary structure and depends on spontaneous openings in tightly packed RNAs to gain access to RNA helices. Unwinding of exposed secondary structure promotes global RNA unfolding and facilitates formation of new structures. Analogous mechanisms are observed in other molecular chaperones and are likely widespread among DEAD-box helicases.

Molecular mechanisms for the regulation of histone mRNA stem-loop–binding protein by phosphorylation

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As DNA is replicated during cell division, it must be packaged by histones. To match the level of available histones to DNA replication, histone mRNA expression is controlled by a 3′-end stem-loop structure unique to replication-dependent histone mRNAs. In *Drosophila*, this regulation is mediated by histone mRNA stem-loop–binding protein (dSLBP), which has minimal tertiary structure when not bound to RNA. We show here (pp. E2937–E2946) that phosphorylation of dSLBP dramatically increases binding affinity for stem-loop RNA. The phosphorylated C-terminal tail of dSLBP does not contact RNA. Instead, increased negative charge on the C-terminal tail and stabilization of structural elements by a phosphorylation site within the RNA-binding domain promote more compact conformations that should reduce the entropic barrier to binding histone mRNA.

Catalytic strategy used by the myosin motor to hydrolyze ATP

Farooq Ahmad Kiani and Stefan Fischer

Biomolecular motor proteins like myosin generate mechanical force from the chemical energy of ATP. Like gas engines, they have different parts (protein domains) that run through a well-defined cycle of motions, consuming one ATP per cycle. Because ATP is very stable, motor proteins catalyze its breakdown (hydrolysis). The catalytic mechanism is at the core of understanding how these motors work (pp. E2947–E2956), because the activation of the catalytic ATPase-function coordinates the motion of the different domains. Identifying which protein groups are essential for catalysis allows one to understand how the precise coupling between ATPase activation and mechanical motion is achieved. Moreover, ATPases are involved in most biochemical processes and are expected to have a catalytic trategy very similar to the one reported here.

The fossilized birth–death process for coherent calibration of divergence-time estimates

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Divergence time estimation on an absolute timescale requires external calibration information, which typically is derived from the fossil record. The common practice in Bayesian divergence time estimation involves applying calibration densities to individual nodes. Often, these priors are arbitrarily chosen and specified yet have an excessive impact on estimates of absolute time. We introduce (pp. E2957–E2966) the fossilized birth–death process—a fossil calibration method that unifies extinct and extant species with a single macroevolutionary model, eliminating the need for ad hoc calibration priors. Compared with common calibration density approaches, Bayesian inference under this mechanistic model yields more accurate node age estimates while providing a coherent measure of statistical uncertainty. Furthermore, unlike calibration densities, our model accommodates all the reliable fossils for a given phylogenetic dataset.

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Optimized CRISPR/Cas tools for efficient germline and somatic genome engineering in *Drosophila*

Fillip Port, Hui-Min Chen, Tzumin Lee, and Simon L. Bullock

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas)-mediated genome engineering promises to revolutionize genetic studies in a variety of systems. Here we describe (pp. E2967–E2976) an optimized set of tools for CRISPR/Cas experiments in the model organism *Drosophila melanogaster*. These tools can be used for remarkably efficient germline transmission of (*i*) loss-of-function insertion and deletion mutations in essential genes or (*ii*) precise changes in the genome sequence introduced by homology-directed repair. These tools also permit efficient biallelic targeting of genes in somatic cells, thereby demonstrating a novel application of CRISPR/Cas in rapidly revealing mutant phenotypes within the organism. Our work also paves the way for high-throughput genetic screens in *Drosophila* with CRISPR/Cas.

Adaptive immunity to murine skin commensals

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Barrier function of the skin in blocking microbial invasion has been attributed to the structural integrity of the epithelium, augmented by innate immune mechanisms. T cells and antigen-presenting cells have long been observed in the skin, but what is their role? Here we report, for the first time, that commensal skin bacteria are recognized by major populations of T cells in skin-draining lymph nodes of mice. We report (pp. E2977–E2986) a previously unrecognized role for T cells in preventing breach of the skin epithelial barrier by certain species of commensal bacteria, especially mycobacteria, and we examine the mechanism. Patients deficient in T cells frequently show infectious cutaneous manifestations and mycobacterial susceptibility, reflecting features of our study in mice.

Astrocyte activation is suppressed in both normal and injured brain by FGF signaling

Wenfei Kang, Francesca Balordi, Nan Su, Lin Chen, Gordon Fishell, and Jean M. Hébert

Most if not all types of insults to the brain, including trauma, stroke, tumor growth, and neurodegeneration, for example, are believed to elicit a complex response involving several cell types. Central to this response is the activation of astrocytes. Although many proinflammatory molecules activate astrocytes, few factors are known to suppress their activation. Here we show (pp. E2987–E2995) that disrupting one particular signal specifically in adult astrocytes in the normal or injured neocortex leads to an increase in astrocyte activation. Conversely, increasing this signal after injury suppresses their activation. Therefore, at least one suppressor of astrocyte activation exists. Of potential therapeutic interest, disrupting this suppressor in astrocytes after injury results in smaller scars without affecting neuron survival.

Genetic evidence that *Celsr3* and *Celsr2*, together with *Fzd3*, regulate forebrain wiring in a *Vangl*-independent manner

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Connections are crucial to brain function and a variety of molecular systems direct axonal growth during development and regeneration. An important system involves Celsr2, Celsr3, and Fzd3, membrane proteins that also regulate epithelial planar cell polarity (PCP). Here, we show genetically that Celsr2 and Celsr3 guide axons redundantly, in collaboration with Fzd3 in the same cell populations. However, unlike in epithelial PCP, their action is Vangl1 and Vangl2 independent. Furthermore, expression of Celsr2-3 and Fzd3 in thalamocortical axons and cortical cells is required for the fine mapping of cortical areas. Our findings (pp. E2996–E3004) that Celsr2, Celsr3, and Fzd3 regulate axonal guidance using mechanisms different than epithelial PCP have implications for brain wiring during normal development and regeneration.

Frizzled3 is required for the development of multiple axon tracts in the mouse central nervous system

Zhong L. Hua, Sangmin Jeon, Michael J. Caterina, and Jeremy Nathans

Axons within the mammalian central nervous system must navigate with high accuracy over long distances. Tissue polarity (also called planar cell polarity) signaling is emerging as a major axon guidance system. *Frizzled3* (*Fz3*), a core polarity gene, is shown here to be essential for a wide variety of axon guidance decisions in the mouse brain and spinal cord beginning with the earliest axon trajectories in the day 12 embryo. In particular, this work (pp. E3005–E3014) shows that axon guidance defects in *Fz3* mutant embryos involve erroneous directions of growth rather than axon elongation per se. In adult mice in which the spinal cord is missing Fz3, anatomic and behavioral analyses show that sensory information from the limbs does not reach the brain.

Auxin inhibits stomatal development through MONOPTEROS repression of a mobile peptide gene *STOMAGEN* in mesophyll

Jing-Yi Zhang, Sheng-Bo He, Ling Li, and Hong-Quan Yang

Stomata are widespread in aerial part of plants as passages exchanging gas and water with environment. Therefore, stomata are crucial for photosynthesis as well as global carbon and water circulation. Auxin, as the first identified phytohormone, participates in many aspects of plant growth and development, but whether auxin regulates stomatal development is unknown. This study (pp. E3015–E3023) establishes that auxin negatively regulates stomatal development through MONOPTEROS (MP) repression of mobile peptide gene *STOMAGEN* expression in mesophyll cells, which is mediated by direct binding of MP to auxin response elements in the *STOMAGEN* promoter. This study advances our knowledge about the roles of auxin and the versatile regulator MP in plant growth and development.