PNAS Plus Significance Statements

Intrinsic evolutionary constraints on protease structure, enzyme acylation, and the identity of the catalytic triad

Andrew R. Buller and Craig A. Townsend

The structure–function relationship of proteases is central to our understanding of biochemistry. Nature has evolved at least 23 independent solutions to this problem, using an acylation mechanism. We examined (pp. E653–E661) the structures of these proteases, using a new framework to characterize the geometric relationships within each active site. This analysis revealed the orientation of the base determines the stereochemistry of catalysis and elucidated why threonine does not substitute for serine in the catalytic triad. These observations explain how the absolute stereostructures of natural protease inhibitors prevent off-target inhibition and serve as boundary conditions to enzyme design.

A generalized G-SFED continuum solvation free energy calculation model

Sehan Lee, Kwang-Hwi Cho, Young-Mook Kang, Harold A. Scheraga, and Kyoung Tai No

This paper deals with a long-standing problem in biophysics, which is resolved here. The model has a strong physical background and will have a wide range of applications to physical and biological problems. The model proposed in this article (pp. E662–E667), GSFED model, can be used for the solvation free energy calculation of most organic solutes in most organic solvents. Since the computing time depends linearly on the size of the molecule, the model can be applied easily to large molecules, for example proteins. The model can provide reliable salvation free energies of experimentally unavailable solute-solvent pairs.

Arl1p regulates spatial membrane organization at the *trans*-Golgi network through interaction with Arf-GEF Gea2p and flippase Drs2p

Pei-Chin Tsai, Jia-Wei Hsu, Ya-Wen Liu, Kuan-Yu Chen, and Fang-Jen S. Lee

Membrane asymmetry, curvature, and dynamics have major roles in cellular processes, including vesicle transport. The GTPase ADP ribosylation factor (Arf) and a lipid translocase (flippase) are critical for membrane reorganization during vesicle formation. Direct evidence that Arf and flippase work in concert on membrane transformation/architecture is, however, lacking. We demonstrate (pp. E668–E677) that activated Arf-like protein Arl1 interacts with the Arf-activating guanine nucleotide-exchange factor Gea2 and flippase Drs2, forming a ternary complex that is required for lipid asymmetry and Arl1 function at the Golgi. These findings represent a previously missing piece of the puzzle that is our understanding of Arf-mediated membrane remodeling.

Differential requirements for mRNA folding partially explain why highly expressed proteins evolve slowly

Chungoo Park, Xiaoshu Chen, Jian-Rong Yang, and Jianzhi Zhang

The expression level of a gene is a leading determinant of its rate of protein sequence evolution, but the underlying mechanisms are unclear. We show (pp. E678–E686) that as the mRNA concentration increases, natural selection for mRNA folding intensifies, resulting in larger fractions of mutations deleterious to mRNA folding and lower rates of protein evolution. Counterintuitively, selection for mRNA folding also impacts the nonsynonymous-to-synonymous nucleotide substitution rate ratio, requiring a revision of the current interpretation of this ratio as a measure of protein-level selection. These findings demonstrate a prominent role of selection at the mRNA level in molecular evolution.

Dual role for mammalian DNA polymerase ζ in maintaining genome stability and proliferative responses

Sabine S. Lange, Ella Bedford, Shelley Reh, John P. Wittschieben, Steve Carbajal, Donna F. Kusewitt, John DiGiovanni, and Richard D. Wood

In mammalian cells DNA polymerase ζ (polζ) appears critical for bypass of DNA damage and was expected to be important for UV-induced skin carcinogenesis. To investigate the response to UV radiation, we engineered mice lacking polζ in the epidermis, circumventing a requirement for embryonic development. These mice (pp. E687–E696) were much more sensitive to UVB radiation than predicted, failed to mount skin-regenerative responses, and did not develop UV-induced skin tumors. Even unirradiated polζ-deficient keratinocytes had a marked proliferation defect and increased chromosomal breaks. Thus in rapidly proliferating cells, polζ maintains levels of DNA breaks below a lethal threshold.

Modulation of AgRP-neuronal function by SOCS3 as an initiating event in diet-induced hypothalamic leptin resistance

Louise E. Olofsson, Elizabeth K. Unger, Clement C. Cheung, and Allison W. Xu

Multiple neuronal subtypes are involved in metabolic regulation, but little is known about the temporal dysregulation of neuronal functions upon acute consumption of fat-rich diets. We show (pp. E697–E706) that AgRP neurons are the predominant cell type situated outside the blood-brain barrier in the mediobasal hypothalamus. AgRP neurons are able to sense slight changes in plasma metabolic signals, such as leptin, but they also more quickly develop cellular leptin resistance compared with other hypothalamic neurons. We further show that modulation of SOCS3 expression in AgRP neurons plays a dynamic role in metabolic fine tuning in response to acute change of nutritional status.

In vivo quantitative proteomics of somatosensory cortical synapses shows which protein levels are modulated by sensory deprivation

Margaret T. Butko, Jeffrey N. Savas, Beth Friedman, Claire Delahunty, Ford Ebner, John R. Yates III, and Roger Y. Tsien

We applied quantitative mass spectrometry to define how sensory experience alters the synaptic proteome in primary sensory cortex. Our results (pp. E726–E735) demonstrate that sensory deprivation reduced proteins implicated in spine enlargement and synaptic strength and increased protein-degradation machinery at synapses. Importantly, we identified novel synaptic proteins whose levels were affected by sensory deprivation but whose synaptic roles have not yet been characterized in mammalian neurons. Thus, this study provides a crucial starting point for numerous investigations of the molecular basis for synaptic modulation and demonstrates the feasibility of using this method to define synaptic proteomes under different sensory rearing conditions.

ALS-linked TDP-43 mutations produce aberrant RNA splicing and adult-onset motor neuron disease without aggregation or loss of nuclear TDP-43

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Mutations in the RNA binding protein TDP-43 cause amyotrophic lateral sclerosis and frontotemporal dementia. Through expressing disease-causing mutants in mice and genome-wide RNA splicing analyses, mutant TDP-43 is shown to retain normal or enhanced activity for facilitating splicing of some RNA targets, but "loss-of-function" for others. These splicing changes (pp. E736–E745), as well as age-dependent, mutant-dependent lower motor neuron disease, occur without loss of nuclear TDP-43 or accumulation of insoluble aggregates of TDP-43.

Alleviation of chronic pain following rat spinal cord compression injury with multimodal actions of huperzine A

Dou Yu, Devang K. Thakor, Inbo Han, Alexander E. Ropper, Hariprakash Haragopal, Richard L. Sidman, Ross Zafonte, Steven C. Schachter, and Yang D. Teng

Neuropathic pain, one of the most debilitating sequelae of neurotrauma, is an unmet clinical need for at least 40% of patients with spinal cord injury (SCI). We demonstrate (pp. E746–E755) that [-]-huperzine A (HUP-A), a naturally occurring *Lycopodium* alkaloid isolated from the Chinese club moss, *Huperzia serrata*, with potent reversible inhibitory action on acetylcholinesterase and *N*-methyl-D-aspartate glutamate receptors, offers an exceptional prospect for multimodal treatment of SCI-induced neuropathic pain in rats. HUP-A restores homeostasis of central sensory neurocircuitry without invoking drug tolerance and dependence or respiratory suppression. We therefore conclude that multimodal actions provide a fresh translational approach to reduce chronic pain.

Intricate interplay between astrocytes and motor neurons in ALS

Hemali P. Phatnani, Paolo Guarnieri, Brad A. Friedman, Monica A. Carrasco, Michael Muratet, Sean O'Keeffe, Chiamaka Nwakeze, Florencia Pauli-Behn, Kimberly M. Newberry, Sarah K. Meadows, Juan Carlos Tapia, Richard M. Myers, and Tom Maniatis

Although ALS is a motor neuron disease, processes within glial cells contribute significantly to motor neuron-specific degeneration. Using a mouse model of ALS (pp. E756–E765), we identified cell autonomous and nonautonomous changes in gene expression in motor neurons cocultured with glia. We also found a remarkable concordance between the cell culture data and expression profiles of whole spinal cords and acutely isolated spinal cord cells during disease progression in this model. We identified changes in the expression of specific genes and signaling pathways that may contribute to motor neuron degeneration in ALS, among which are TGF-β signaling pathways.

Genome-wide analysis of thyroid hormone receptors shared and specific functions in neural cells

Fabrice Chatonnet, Romain Guyot, Gérard Benoît, and Frederic Flamant

This article (pp. E766–E775) presents a unique genome-wide transcriptome and cistrome analysis for thyroid hormone receptors. It defines 3,3′,5-triiodo-L-thyronine (T3) target genes in a neural cell line expressing either TRα1 or TRβ1. A substantial fraction of the T3 target genes display a marked preference for one of the two receptors. However, receptor-selective regulation of T3 target genes does not result from receptor-selective chromatin occupancy of their promoter regions. We conclude that modification of TRα1 and TRβ1 intrinsic properties contributes to the divergent evolution of the receptors' function.

Growth differentiation factor 9:bone morphogenetic protein 15 heterodimers are potent regulators of ovarian functions

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Although genetic studies have uncovered critical functions of GDF9 and BMP15 in female reproduction, many genetic and physiologic data for these ligands remain perplexing. Here (pp. E776–E785) we establish that mouse and human GDF9:BMP15 heterodimers are the most biopotent regulators of ovarian granulosa cell functions. Moreover, GDF9:BMP15 heterodimers require a unique signaling complex that includes the type 2 receptor BMPR2, an ALK4/5/7 type 1 kinase receptor, and an ALK6 type 1 co-receptor. GDF9:BMP15 binding to this complex stimulates phosphorylation of SMAD2/3. Our findings explain intraspecies and interspecies functions of these oocyte-synthesized proteins and have key implications for the regulation of female fertility.