PNAS Plus Significance Statements

Tissue-based metabolic labeling of polysialic acids in living primary hippocampal neurons

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The roles of cell-surface glycans remain elusive compared with those of proteins or lipids because of their diverse and dynamic nature. Metabolic incorporation of unnatural monosaccharides in the biochemical synthesis of glycans as a chemical reporter has been a successful method to investigate the functions of cell-surface glycans but has also left an issue of cytotoxicity for certain cells. In this work (pp. E241–E248), we developed a tissue-based strategy for metabolic incorporation of a chemical reporter to primary neurons. We let an unnatural monosaccharide be metabolized by hippocampal tissues before dissociation into individual cells, and thereby, we could eliminate cytotoxicity. We used this method to describe, for the first time to our knowledge, the real-time distribution of polysialic acids on the membranes of neurons.

Cdc45 (cell division cycle protein 45) guards the gate of the Eukaryote Replisome helicase stabilizing leading strand engagement

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Cell division control protein 45 (Cdc45), a RecJ homologue, is essential in all eukaryotes. Cdc45 functions with the replisome CMG helicase where minichromosome maintenance (Mcm2–7) proteins provide motor activity for unwinding duplex during replication. We report (pp. E249–E258) that the dynamic gate between Mcm subunits 2 and 5, which is essential for the initial loading of the motor, may be an Achilles heel because the leading strand may slip from its central channel in an open gate state. Studies show that the side channel formed by the Cdc45 and GINS works as a trap and guards this gate; the Recombination protein J fold is key for this activity. We propose that this new function for Cdc45 will be important for fork integrity during the S-phase in response to double-strand breaks or replication stress.

Modulation of folding energy landscape by charge–charge interactions: Linking experiments with computational modeling

Franco O. Tzul, Katrina L. Schweiker, and George I. Makhatadze

Quantitative understanding of how individual interactions contribute to the kinetics and thermodynamics of protein folding is critical for deciphering the underlying molecular mechanisms that define the energy folding landscape. We applied (pp. E259–E266) a structure-based model that explicitly accounts for the interactions between charges, to folding–unfolding of four different protein pairs: rationally stabilized, via optimization of surface charge–charge interactions, variants, and respective wild types. First, we established that the models predict both thermodynamic and kinetic differences observed experimentally for all four studied protein pairs. Second, we used the results of the computational modeling to provide a molecular level explanation of how optimization of charge–charge interactions leads to an increase in the folding rates of designed variants, without changes in the unfolding rates.

Single-molecule tracking of small GTPase Rac1 uncovers spatial regulation of membrane translocation and mechanism for polarized signaling

Sulagna Das, Taofei Yin, Qingfen Yang, Jingqiao Zhang, Yi I. Wu, and Ji Yu

Rac1 activation involves two steps: translocation to plasma membrane and nucleotide exchange. Most previous studies focused on the nucleotide exchange cycle. Here (pp. E267–E276) we sought to understand membrane translocation dynamics by developing a single-particle tracking-based method. The labeled Rac1 molecules were further adapted for simultaneous FRET sensing of Rac1 nucleotide state, enabling a simultaneous comparison between Rac1 translocation dynamics and its nucleotide exchange dynamics. Elevated membrane recruitment can contribute significantly to polarized Rac1-signaling. This finding draws attention to the importance of spatial regulation of the Rac1 translocation process in the regulation of RhoGTPase signaling. Rac1 recruitment to membrane precedes its interaction with protein factors (e.g., GEFs) and is governed by phospholipid distributions. This finding resolves a long-standing question of the mechanism of Rac1 activation.

Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants

Ankita Bansal, Lihua J. Zhu, Kelvin Yen, and Heidi A. Tissenbaum

Genetic and environmental manipulations have been identified that result in lifespan extension. The underlying assumption that lifespan extension would also result in an increase in healthspan is seemingly valid but infrequently examined. Here (pp. E277–E286), we examined multiple pathways that modulate lifespan to investigate the relationship between lifespan extension and health. We analyzed wild-type and four long-lived mutants in an unbiased cross-sectional study with multiple assays until animals reached 80% maximum lifespan. We show lifespan and healthspan can be separated and all of the long-lived mutants extend the period of frailty as a consequence. If applied to humans, this would likely lead to unsustainable healthcare costs and demonstrates the importance of examining healthspan as opposed to lifespan for future research.

Control of stem cell self-renewal and differentiation by the heterochronic genes and the cellular asymmetry machinery in *Caenorhabditis elegans*

Omid F. Harandi and Victor R. Ambros

Transitions between asymmetric (self-renewal) and symmetric (proliferative) divisions for stem cells are precisely regulated during development and tissue regeneration. *Caenorhabditis elegans* heterochronic genes encode evolutionarily conserved developmental regulators, including *lin-4* (lineage defect) and *let-7* (lethal defects) microRNAs and the stem cell factor LIN-28 that control patterns of stem cell self-renewal and proliferation. It is not known how these developmental regulators interface with the machinery of division asymmetry. We report (pp. E287–E296) that, in *C. elegans*, the timing of transitions between asymmetric and symmetric stem cell divisions reflects developmental modulation of the LIT-1/POP-1/APR-1 (loss of intestine/posterior pharynx defect/APC related) asymmetry machinery by the heterochronic genes. These findings illuminate how evolutionarily conserved cellular asymmetry machinery can be coupled to microRNA-regulated developmental pathways for robust stem cell maintenance and proliferation.

Inflammation-sensitive super enhancers form domains of coordinately regulated enhancer RNAs

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Super enhancers (SEs) are enhancer-dense regions found near genes that play key roles in determining cellular identity. Using global nuclear run-on sequencing (GRO-Seq), we find (pp. E297–E302) extensive regulation of enhancer RNAs (eRNAs) within SEs in response to lipopolysaccharide (LPS) treatment in macrophages. Both activation and repression of gene expression are associated with SEs and eRNA transcription dynamics. Furthermore, we find that each SE acts as a single regulatory unit within which eRNA and genic transcripts are coordinately regulated. We also find that transcription factor (TF) composition within an SE determines regulatory properties of each SE and associated eRNAs. We propose that signal-dependent SEs and their eRNAs function as molecular rheostats integrating the binding profiles of key regulators to produce dynamic profiles of gene expression.

Single-cell, real-time detection of oxidative stress induced in *Escherichia coli* by the antimicrobial peptide CM15

Heejun Choi, Zhilin Yang, and James C. Weisshaar

Antimicrobial peptides (AMPs) help to kill invading bacteria on skin and lung surfaces. We are developing fluorescence microscopy assays that reveal the mechanisms of action of AMPs in real time. It is increasingly clear AMP damage to bacterial cells goes far beyond permeabilization of membranes. Here (pp. E303–E310) we demonstrate that for *Escherichia coli* in aerobic conditions, the peptide CM15 [combining residues 1–7 of cecropin A (from moth) with residues 2–9 of melittin (bee venom)], induces a burst of biochemically harmful reactive oxygen species within 30 s of membrane permeabilization. In anaerobic conditions, CM15 is 20-fold less potent. AMP efficacy in vivo may be tuned to the local level of oxygenation.

Genetic dissection of pheromone processing reveals main olfactory system-mediated social behaviors in mice

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It is now widely accepted that the range of pheromones that control social behaviors are processed by both the vomeronasal system (VNS) and the main olfactory system (MOS). However, the functional contributions of each subsystem in social behavior remain unclear. Here (pp. E311–E320), we showed that mice with loss-of-function confined to the dorsal MOS maintained innate odor recognition and VNS activity, but failed to demonstrate multiple male and female social behaviors. Functional dissociation of the MOS and VNS enabled the identification of an MOS-mediated processing of semiochemical information, independent of the VNS.

Acceleration of conduction velocity linked to clustering of nodal components precedes myelination

Sean A. Freeman, Anne Desmazières, Jean Simonnet, Marie Gatta, Friederike Pfeiffer, Marie Stéphane Aigrot, Quentin Rappeneau, Serge Guerreiro, Patrick Pierre Michel, Yuchio Yanagawa, Gilles Barbin, Peter J. Brophy, Desdemona Fricker, Catherine Lubetzki, and Nathalie Sol-Foulon

Cellular and molecular mechanisms underlying the assembly of nodes of Ranvier of myelinated axons in the CNS are still only partly understood. Our study (pp. E321–E328) shows the influence of intrinsic cues and glial extrinsic factors for nodal protein clustering before myelination on specific hippocampal neuronal subpopulations and extends to electrophysiological understandings and in vivo relevance. Although conduction velocity along axons has long been thought to mostly rely on the insulating properties of myelin, we here show that nodal protein aggregation can increase this speed in the absence of such insulation. These results highlight the role of nodal clusters per se on conduction velocity by uncoupling it from myelination.

Intracellular Cl^- as a signaling ion that potently regulates $Na^+/HCO3^-$ transporters

Nikolay Shcheynikov, Aran Son, Jeong Hee Hong, Osamu Yamazaki, Ehud Ohana, Ira Kurtz, Dong Min Shin, and Shmuel Muallem

Cl^- is the major cellular anion that controls the intracellular activity of many ions, the membrane potential, and transepithelial fluid and electrolyte secretion. How cells sense intracellular Cl^- (Cl^-_{in}) to coordinate all Cl^--dependent activities is not known. We report (pp. E329–E337) a molecular mechanism for Cl^-_{in} sensing that involves interaction of Cl^- with GXXXP-containing sites and show how these sites are used to regulate the activity of several Na^+-HCO_3^- cotransporters. Although these transporters do not transport Cl^-, they sense Cl^-_{in} in a manner specific for each transporter that is suitable for the transporter physiological activity. Our data has fundamental implications for the role of Cl^- in cellular ion homeostasis and fluid and electrolyte secretion.