PNAS Plus Significance Statements

Ctr2 regulates biogenesis of a cleaved form of mammalian Ctr1 metal transporter lacking the copper- and cisplatin-binding ecto-domain

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Copper is essential for normal growth and development because it serves roles in catalysis, signaling, and structure. Cells acquire copper through the copper transporter 1 (Ctr1) protein, a copper transporter that localizes to the cell membrane and intracellular vesicles. Both copper and the anticancer drug cisplatin are imported by Ctr1 by virtue of an extracellular domain rich in metal-binding amino acids. In this report (pp. E4279–E4288) we demonstrate that a protein structurally related to Ctr1, called Ctr2, plays a role in the generation or stability of a truncated form of Ctr1 lacking a large portion of the extracellular domain. Retention of this domain in mice or cells lacking Ctr2 enhances copper and cisplatin uptake, thereby establishing Ctr2 as a regulator of Ctr1 function.

Substrate protein switches GroE chaperonins from asymmetric to symmetric cycling by catalyzing nucleotide exchange

Xiang Ye and George H. Lorimer

Kinetic analyses of the GroEL/GroES chaperonin nanomachine using multiple spectroscopic probes shows that without substrate protein, the two GroEL rings function alternately, 180° out of phase with one another, via an asymmetric cycle. The rate-determining step is the dissociation of the product ADP and the predominant species is the asymmetric "bullet" GroEL-$GroES_1$. With substrate protein present, a change in the kinetic mechanism occurs. The two rings now function simultaneously (pp. E4289–E4297) via a symmetric cycle in which ATP hydrolysis becomes the rate-limiting step, ADP/ATP exchange is catalyzed by substrate protein and the predominant species is the symmetric "football" GroEL-$GroES_2$.

Symmetric GroEL:$GroES_2$ complexes are the protein-folding functional form of the chaperonin nanomachine

Dong Yang, Xiang Ye, and George H. Lorimer

The canonical mechanism of GroEL/GroES protein folding rests on at least three untested assumptions: (*i*) symmetric GroEL-$GroES_2$ "football" particles have no role; (*ii*) because of negative cooperativity, ATP binds to GroEL, one ring at a time; and (*iii*) the turnover of the chaperonin system occurs at the same rate in the presence of substrate protein (SP) as in its absence. Each of these assumptions is shown to be incorrect. (*i*) Because of an SP-induced change in the kinetic mechanism (pp. E4298–E4305), the predominant species under protein folding conditions is the symmetric football; (*ii*) simultaneous occupancy of both GroEL rings by ATP and GroES occurs; and (*iii*) the residence time of encapsulated SP is much shorter than believed.

Conformational selection and adaptation to ligand binding in T4 lysozyme cavity mutants

Carlos J. López, Zhongyu Yang, Christian Altenbach, and Wayne L. Hubbell

Analysis of protein packing reveals the prevalence of cavities, some of which are evolutionarily conserved. Despite the fact that these packing defects are generally destabilizing, they can play important roles in facilitating functionally important protein motions and in the evolution of protein function. In this study (pp. E4306–E4315), we used site-directed spin-labeling with electron paramagnetic resonance spectroscopy to investigate the structural and dynamical response of an enzyme to engineered ligand-binding cavities. The results show that the engineered cavities introduce conformational fluctuations between a native-like state and a new state. Ligand binding shifts the equilibrium toward the native-like state, suggesting binding via conformational selection, although additional structural adaptation is observed for one mutant. The results underscore the potential role of cavities in modulating molecular flexibility.

Transcript processing and export kinetics are rate-limiting steps in expressing vertebrate segmentation clock genes

Nathaniel P. Hoyle and David Ish-Horowicz

This paper (pp. E4316–E4324) describes in vivo measurements of the kinetics of transcript processing and export for endogenous genes in mouse and chick embryos. It shows that transcript export is unexpectedly slow, even slower than splicing, and relates its finding to rate-limiting steps that would contribute to the molecular oscillator that drives segmentation in vertebrate embryos. It also relates them to interspecies differences in clock period.

Sleeping Beauty mutagenesis in a mouse medulloblastoma model defines networks that discriminate between human molecular subgroups

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Medulloblastoma is a common malignant pediatric brain tumor. Gene expression data have indicated that the tumors fall into four molecular subgroups, "Wnt," "Hedgehog," "group 3," and "group 4." With the exception of the Hedgehog subgroup, few functional data exist defining key molecular pathways driving tumor growth. Using a transposon mutagenesis approach, we identified genes that functionally cooperate with Hedgehog signalling to promote tumorigenesis in a Ptch1 mouse model of medulloblastoma. Surprisingly, the genes we identified (pp. E4325–E4334) were able to accurately define all four human molecular subtypes, not just Hedgehog, when used to interrogate published expression data. Thus, we have functionally defined key regulatory networks that illustrate both the differences and commonalities between tumor subgroups indicating a number of therapeutic strategies.

Fibrinogen-specific antibody induces abdominal aortic aneurysm in mice through complement lectin pathway activation

Hui-fang Zhou, Huimin Yan, Paula Bertram, Ying Hu, Luke E. Springer, Robert W. Thompson, John A. Curci, Dennis E. Hourcade, and Christine T. N. Pham

Abdominal aortic aneurysm (AAA) is a common and potentially fatal vascular disease. Although surgery is recommended for large aneurysms, surgical repair offers no clear survival advantage for small AAAs. A deeper understanding of the mechanisms that underlie disease pathogenesis could suggest strategies for medical intervention. Herein we identify (pp. E4335–E4344) a natural mouse IgG antibody that recognizes fibrinogen deposited in human AAA tissues and induces aneurysm in a mouse model through activation of the complement lectin pathway (LP). Conversely, absence of antibody or complement proteins abrogates AAA development. These results support the concept that antibody directed against aortic wall epitopes initiates LP activation that culminates in AAA formation. The antibody response and complement LP may provide therapeutic targets for halting disease progression.

Intracellular *Shigella* remodels its LPS to dampen the innate immune recognition and evade inflammasome activation

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During bacterial infection, the eukaryotic innate immune system detects a restricted number of bacterial structures, such as LPSs, and activates signaling pathways conveying an inflammatory reaction aimed at eradication of the pathogen. *Shigella* spp. are human enteropathogens that invade colonic and rectal mucosa, where they cause deleterious inflammation. Here (pp. E4345–E4354) we show that *Shigella* drastically modifies the degree of acylation of the lipid A moiety of LPS during host cell invasion. The purified hypoacylated LPS displays a reduced inflammatory potential that allows the bacteria to lower the sensing activity of the immune system and to escape from downstream effector mechanisms.

A neuropeptide speeds circadian entrainment by reducing intercellular synchrony

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Daily rhythms in behavior depend on the coordinated cycling of circadian neurons in the brain. Here, we found that a neuropeptide that is required for synchrony among circadian neurons also, surprisingly, dose-dependently reduces synchrony among circadian cells. We find this allows the system to adjust how quickly it entrains to environmental cycles. We propose (pp. E4355–E4361) that treatments that enhance this signaling pathway could reduce jet lag associated with shift work and travel across time zones.

Intracellular pH reduction prevents excitotoxic and ischemic neuronal death by inhibiting NADPH oxidase

Tina I. Lam, Angela M. Brennan-Minnella, Seok Joon Won, Yiguo Shen, Colleen Hefner, Yejie Shi, Dandan Sun, and Raymond A. Swanson

Activation of NMDA-type glutamate receptors produces neuronal excitotoxicity, a primary cause of cell death in stroke and other neurological disorders. This cell death process requires superoxide release by neuronal NADPH oxidase. Results presented here (pp. E4362–E4368) show that small reductions in intracellular pH uncouple neuronal NADPH oxidase from NMDA receptor activation, and thereby prevent neuronal death. The findings establish a link between metabolic activity and excitotoxicity, and identify a mechanism by which mild acidosis improves outcome after excitotoxic and ischemic brain insults. The findings also suggest that variations in intracellular pH associated with physiological brain activity may likewise influence the cell-to-cell signaling normally mediated by neuronal superoxide release.

Human genome–guided identification of memory-modulating drugs

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In the last decade there has been an exponential increase in knowledge about the genetic basis of complex human traits. It is not clear, however, to what extent this knowledge can be used as a starting point for drug identification, one of the central hopes of the human genome project. Here, we report that by using genomic information related to aversive memory—a trait central to post-traumatic stress disorder—we identified several potential drug targets and compounds. In a subsequent pharmacological study with one of the identified compounds, we found a drug-induced reduction of aversive memory. These findings (pp. E4369–E4374) indicate that genomic information can be used as a starting point for the identification of memory-modulating compounds.

Region-specific restoration of striatal synaptic plasticity by dopamine grafts in experimental parkinsonism

Daniella Rylander, Vincenza Bagetta, Valentina Pendolino, Elisa Zianni, Shane Grealish, Fabrizio Gardoni, Monica Di Luca, Paolo Calabresi, M. Angela Cenci, and Barbara Picconi

This paper (pp. E4375–E4384) identifies long-term synaptic plasticity restoration as an underlying mechanism of progressive motor improvement after neuronal transplantation in a rat Parkinson model. A Parkinson-associated loss of plasticity in the host striatum could be restored by transplanted dopamine neurons with sufficient fiber innervation, suggesting that functional innervation with possible synapse formation is required for the long-term effect of neural transplants. These data support a multisite-grafting procedure to more extensively restore the plasticity in the host parkinsonian brain. Dopamine neuron transplantation could be a future therapy for Parkinson disease and is currently being evaluated in a European Union-sponsored project.

C1q induction and global complement pathway activation do not contribute to ALS toxicity in mutant SOD1 mice

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Activation of the immune system within the nervous system is widely found in neurodegenerative diseases, including ALS. In mice that develop fatal paralytic disease from ALS-causing superoxide dismutase (SOD1) mutants, motor neurons activate expression of C1q, the initiating component of the classic complement system. As C1q and complement play a central role in developmental synapse elimination, disease-linked activation has suggested that it drives motor neuron denervation. Instead, suppressing C1q induction by gene deletion is shown to enhance loss, not retention, of synapses, whereas elimination of global complement activation by C1q or C3 gene deletions leave onset and progression of paralytic disease unaffected (pp. E4385–E4392). Thus, C1q induction and complement activation are not significant contributors to SOD1 mutant-mediated ALS disease mechanism in mice.

Yeast metabolic and signaling genes are required for heat-shock survival and have little overlap with the heat-induced genes

Patrick A. Gibney, Charles Lu, Amy A. Caudy, David C. Hess, and David Botstein

We used the model eukaryote *Saccharomyces cerevisiae* to investigate which genes are important for survival of heat stress. Previously, this question was addressed by examining which genes are turned on by mild heat stress; in this study, we examined gene-deletion mutants for increased sensitivity to lethal heat stress. This approach (pp. E4393–E4402) reveals that these two sets of genes are largely nonoverlapping, demonstrating that mutant analysis is a powerful complementary approach to gene-expression analysis. In addition, many of the genes identified as important for heat survival are involved in metabolism or signaling, or their function is completely uncharacterized, suggesting that our understanding of the systems-level response to heat stress is incomplete.