# PNAS Plus Significance Statements

## Quantum indistinguishability in chemical reactions

*Matthew P. A. Fisher and Leo Radzihovsky*

Counter to conventional approaches that treat nuclear coordinates classically, we explore quantum indistinguishability of nuclei in enzymatic chemical reactions of small symmetric molecules. Supported by several physical arguments, we conjecture a far-reaching "quantum dynamical selection" (QDS) rule that precludes enzymatic chemical bond-breaking reactions from orbitally nonsymmetric molecular states. We propose and discuss experimental implications of QDS, such as (*i*) differential chemical reactivity in ortho- and parahydrogen, (*ii*) a mass-independent mechanism for isotope fractionation, (*iii*) an explanation of the enhanced chemical activity of "reactive oxygen species", (*iv*) a route to parahydrogen-induced hyperpolarization important for zero-field NMR spectroscopy, and (*v*) critical quantum-to-biochemical linkage in the nuclear spin model of the (putative) quantum brain, among others. (See pp. E4551–E4558.)

## Green function of correlated genes in a minimal mechanical model of protein evolution

*Sandipan Dutta, Jean-Pierre Eckmann, Albert Libchaber, and Tsvi Tlusty*

Many protein functions involve large-scale motion of their amino acids, while alignment of their sequences shows longrange correlations. This has motivated search for physical links between genetic and phenotypic collective behaviors. The major challenge is the complex nature of protein: nonrandom heteropolymers made of 20 species of amino acids that fold into a strongly coupled network. In light of this complexity, simplified models are useful. Our model describes protein in terms of the Green function, which directly links the gene to force propagation and collective dynamics in the protein. This allows for derivation of basic determinants of evolution, such as fitness landscape and epistasis, which are often hard to calculate. (See pp. E4559–E4568.)

## Local initiation conditions for water autoionization

*Mahmoud Moqadam, Anders Lervik, Enrico Riccardi, Vishwesh Venkatraman, Bjørn are Alsberg, and Titus S. van Erp*

The dissociation of water is arguably the most fundamental chemical reaction occurring in the aqueous phase. Despite that the splitting of a water molecule very seldom occurs, the reaction is of major importance in many areas of chemistry and biology. Direct experimental probing of the event is still impossible and also simulating the event via accurate computer simulations is challenging. Here, we achieved the latter via specialized rare-event algorithms estimating rates of dissociation in agreement with indirect experimental measurements. Even more interestingly, by a rigorous analysis of our results we identified anomalies in the water structure that act as initiators of the reaction, a finding that suggests paradigms for steering and catalyzing chemical reactions. (See pp. E4569–E4576.)

## Tunneling explains efficient electron transport via protein junctions

*Jerry A. Fereiro, Xi Yu, Israel Pecht, Mordechai Sheves, Juan Carlos Cuevas, and David Cahen*

Investigation of the charge transport mechanism across a monolayer of a redox active protein is important for the fundamental understanding of the naturally occurring electron transfer processes, such as those in photosynthesis or respiration. Inelastic electron tunneling spectroscopy measurements of a redox active protein may provide direct experimental evidence that the tunneling charges are, in fact, passing through the protein molecules. Results of our study of conductance via well-controlled azurin monolayer solid-state junctions show the direct involvement of the Cu(II) site in assisting electron transport, underscoring this site's vibronic characteristics associated with the charge transport mechanism. Our study widens the scope of currently available methodologies and also adds to the potential of using proteins in bioelectronics. (See pp. E4577–E4583.)

## Histone demethylase JMJD1A promotes alternative splicing of AR variant 7 (AR-V7) in prostate cancer cells

*Lingling Fan, Fengbo Zhang, Songhui Xu, Xiaolu Cui, Arif Hussain, Ladan Fazli, Martin Gleave, Xuesen Dong, and Jianfei Qi*

Formation of androgen receptor splicing variant 7 (AR-V7), a constitutively active form of AR, plays a key role in the resistance of prostate cancer to hormone therapy. However, the mechanisms that regulate AR-V7 generation are poorly understood. Here, we identified a new role for histone demethylase JMJD1A (Jumonji domain containing 1A) in the formation of AR-V7 in prostate cancer cells. We found that JMJD1A

facilitated recruitment of a splicing factor, heterogeneous nuclear ribonucleoprotein F, for alternative splicing and generation of AR-V7. The findings suggest that targeting JMJD1A may provide new therapeutic opportunity for prostate cancer. (See pp. E4584–E4593.)

## An endogenous dAMP ligand in *Bacillus subtilis* class Ib RNR promotes assembly of a noncanonical dimer for regulation by dATP

*Mackenzie J. Parker, Ailiena O. Maggiolo, William C. Thomas, Albert Kim, Steve P. Meisburger, Nozomi Ando, Amie K. Boal, and JoAnne Stubbe*

Negative feedback regulation of ribonucleotide reductase (RNR) activity by dATP is important for maintaining balanced intracellular 2′-deoxynucleoside triphosphate (dNTP) pools essential for the high fidelity of DNA replication and repair. To date, this type of allostery has been nearly universally associated with dATP binding to the N-terminal ATP-cone domain of the class Ia RNR large subunit (canonical $\alpha_2$), resulting in an altered quaternary structure that is unable to productively bind the second subunit ($\beta_2$). Here, we report our studies on activity inhibition by dATP of the *Bacillus subtilis* class Ib RNR, which lacks a traditional ATP-cone domain. This unprecedented allostery involves deoxyadenosine 5′-monophosphate (dAMP) binding to a newly identified site in a partial N-terminal cone domain, forming an unprecedented noncanonical $\alpha_2$. (See pp. E4594–E4603.)

## Mechanistic studies of a small-molecule modulator of SMN2 splicing

*Jingxin Wang, Peter G. Schultz, and Kristen A. Johnson*

The development of small-molecule therapeutics that act by targeting defined DNA or RNA sequences associated with human disease remains a challenge. RG-7916, a small-molecule drug candidate for the treatment of spinal muscular atrophy (SMA), selectively regulates the alternative splicing (AS) of the *SMN2* gene. Herein, we show that SMN-C2 and -C3, close analogs of RG-7916, act by binding SMN2 pre-mRNA and thereby increasing the affinity of the RNA binding proteins far upstream element binding protein 1 (FUBP1) and KH-type splicing regulatory protein (KHSRP) to the SMN2 pre-mRNA complex. These results suggest that nucleic acid targeted small molecules may have untapped potential for modulating disease processes at the level of pre-mRNA splicing. (See pp. E4604–E4612.)

## KIF15 nanomechanics and kinesin inhibitors, with implications for cancer chemotherapeutics

*Bojan Milic, Anirban Chakraborty, Kyuho Han, Michael C. Bassik, and Steven M. Block*

Eg5, a mitotic kinesin, has long been an anticancer drug target. However, a different kinesin motor, KIF15, can rescue cell division when Eg5 is incapacitated, leading to chemotherapeutic resistance. We characterized KIF15 motor mechanics at the single-molecule level and studied the effects of combinations of small-molecule inhibitors of KIF15 and Eg5 on admixtures of motors in a motility assay, as well as on cancer cell proliferation. Taken together, our results point the way toward a strategy of combination drug therapy targeting both Eg5 and KIF15 as a means of overcoming KIF15-mediated cancer resistance. This work highlights the importance of understanding the molecular physiology of different kinesins and of exploring inhibitors that target additional mitotic kinesins. (See pp. E4613–E4622.)

## Distinct gating mechanism of SOC channel involving STIM–Orai coupling and an intramolecular interaction of Orai in *Caenorhabditis elegans*

*Kyu Min Kim, Tharaka Wijerathne, Jin-Hoe Hur, Uk Jung Kang, Ihn Hyeong Kim, Yeong Cheon Kweon, Ah Reum Lee, Su Ji Jeong, Sang Kwon Lee, Yoon Young Lee, Bo-Woong Sim, Jong-Hee Lee, Chunggi Baig, Sun-Uk Kim, Kyu-Tae Chang, Kyu Pil Lee, and Chan Young Park*

Store-operated calcium entry (SOCE) is a widespread, essential signaling mechanism for cellular functions in both invertebrates and vertebrates and is controlled by two membrane proteins, STIM in the endoplasmic reticulum (ER) and Orai, in the plasma membrane (PM). How these proteins residing in two different compartments have evolved to interact with each other has not been elucidated. We show that *Caenorhabditis elegans* has a distinct mechanism of SOCE in which the 2–3 loop is regulated by STIM1 and the N and C termini of Orai1 by intramolecular interaction, differing from the previously reported mechanism of human SOCE. Therefore our studies suggest that, while the STIM–Orai interaction has been conserved from invertebrates to mammals, the gating mechanism for Orai has evolved considerably. (See pp. E4623–E4632.)

## *Insulin* promoter in human pancreatic β cells contacts diabetes susceptibility loci and regulates genes affecting insulin metabolism

*Xing Jian and Gary Felsenfeld*

We show that in a human pancreatic β cell line the human insulin gene promoter on chromosome 11 physically contacts sites on other chromosomes. Many of these contacted sites contain type 1 or type 2 diabetes susceptibility loci. We find that insulin gene expression can affect expression of contacted genes on other chromosomes. Some of these genes, in turn, regulate insulin secretion. These results reveal physical regulatory mechanisms in which the level of insulin expression controls expression of genes involved in insulin transport and metabolism. We study the properties of one such gene, somatostatin receptor 5 antisense (*SSTR5-AS1*), and show that it regulates *SSTR5* expression, which affects insulin secretion. Analysis of insulin contacts thus may reveal new insulin metabolic pathways. (See pp. E4633–E4641.)

## *Escherichia coli* cultures maintain stable subpopulation structure during long-term evolution

*Megan G. Behringer, Brian I. Choi, Samuel F. Miller, Thomas G. Doak, Jonathan A. Karty, Wanfeng Guo, and Michael Lynch*

Understanding how microbes adapt to novel environments is essential to understanding acute bacterial infection and long-term disease, as genetic architecture underlying the production and maintenance of genetic variation influences a population's potential for adaptation. In this in-depth analysis of a highly replicated *Escherichia coli* long-term evolution experiment, we observe rapid diversification into stable subpopulations in response to several environmental variables. This niche separation creates novel genetic backgrounds upon which new traits, such as differential nutrient utilization or antimicrobial resistance, can arise. The observed genetic changes, in a simple and tractable

experimental system, mimic events known to occur during bacterial infections. (See pp. E4642–E4650.)

### Metabolic control of T cell immune response through glycans in inflammatory bowel disease

*Ana M. Dias, Alexandra Correia, Márcia S. Pereira, Catarina R. Almeida, Inês Alves, Vanda Pinto, Telmo A. Catarino, Nuno Mendes, Magdalena Leander, M. Teresa Oliva-Teles, Luís Maia, Cristina Delerue-Matos, Naoyuki Taniguchi, Margarida Lima, Isabel Pedroto, Ricardo Marcos-Pinto, Paula Lago, Celso A. Reis, Manuel Vilanova, and Salomé S. Pinho*

Our findings demonstrate that metabolic supplementation of mucosal T cells, isolated from patients with active ulcerative colitis (UC), with *N*-acetylglucosamine (GlcNAc) leads to the enhancement of branched N-glycosylation on the T cell receptor, which was associated with the control of T cell activation and function. These results were validated in "glycoengineered" mouse models with severe colitis. Overall, our results open new avenues for a targeted-specific therapy in inflammatory bowel disease (IBD). The therapeutic use of GlcNAc (either alone or in combination with other antiinflammatory therapies) represents a simple immunomodulatory strategy in IBD, with absence of side effects, low costs, and the possibility of being used as a simple rescue therapy to avoid unnecessary toxic effects and step-up therapies in IBD. (See pp. E4651–E4660.)

### Distinct roles of resident and nonresident macrophages in nonischemic cardiomyopathy

*Xudong Liao, Yuyan Shen, Rongli Zhang, Keiki Sugi, Neelakantan T. Vasudevan, M. Amer Alaiti, David R. Sweet, Lin Zhou, Yulan Qing, Stanton L. Gerson, Chen Fu, Anthony Wynshaw-Boris, Rui Hu, Martin A. Schwartz, Hisashi Fujioka, Brian Richardson, Mark J. Cameron, Hiroki Hayashi, Jonathan S. Stamler, and Mukesh K. Jain*

Pressure overload triggers responses in cardiomyocytes and noncardiomyocytes, leading to pressure overload hypertrophy (POH). Here, we show that cardiac resident macrophages regulate compensatory myocardial adaptation to POH, while nonresident infiltrating macrophages are detrimental. At early-phase POH, pressure overload induces cardiac resident macrophage proliferation, which is regulated by Kruppel-like factor 4. At late-phase POH, pressure overload also induces Ly6C$^{hi}$ monocyte infiltration, and its blockade improves myocardial angiogenesis and preserves cardiac function. Mechanistically, the differential impact of these two macrophage subsets on myocardial angiogenesis may underlie the cardiac phenotype. These findings provide insights regarding the role of cardiac resident and nonresident macrophages, conceptually update the view of myocardial angiogenesis, and identify monocyte infiltration as a therapeutic target for nonischemic cardiomyopathy. (See pp. E4661–E4669.)

### Structural homo- and heterosynaptic plasticity in mature and adult newborn rat hippocampal granule cells

*Tassilo Jungenitz, Marcel Beining, Tijana Radic, Thomas Deller, Hermann Cuntz, Peter Jedlicka, and Stephan W. Schwarzacher*

The lifelong genesis of hippocampal granule cells (abGCs) enables specific forms of spatial learning. We analyzed which forms of synaptic plasticity are present in abGCs in comparison with mature granule cells (mGCs). We found structural equivalents of homosynaptic long-term potentiation and heterosynaptic long-term depression, two fundamental forms of cellular learning. abGCs and mGCs showed spine enlargement on stimulated segments and spine shrinkage on nonstimulated segments concurrently present on dendrites of individual cells, indicating a sharpening and a homeostatic regulation of synaptic efficacy. abGCs expressed homo- and heterosynaptic spine plasticity with a clear onset between 4–5 wk of cell age, demonstrating increasing synaptic plasticity during the phase of abGC integration on the structural level. (See pp. E4670–E4679.)

### Cell-specific discrimination of desmosterol and desmosterol mimetics confers selective regulation of LXR and SREBP in macrophages

*Evan D. Muse, Shan Yu, Chantle R. Edillor, Jenhan Tao, Nathanael J. Spann, Ty D. Troutman, Jason S. Seidman, Adam Henke, Jason T. Roland, Katherine A. Ozeki, Bonne M. Thompson, Jeffrey G. McDonald, John Bahadorani, Sotirios Tsimikas, Tamar R. Grossman, Matthew S. Tremblay, and Christopher K. Glass*

The beneficial effects of LXR-pathway activation have long been appreciated, but clinical application of synthetic LXR ligands has been limited by coactivation of SREBP1c and consequent hypertriglyceridemia. Natural LXR ligands such as desmosterol do not promote hypertriglyceridemia because of coordinate downregulation of the SREBP pathway. Here we demonstrate that synthetic desmosterol mimetics activate LXR in macrophages both in vitro and in vivo while suppressing SREBP target genes. Unexpectedly, desmosterol and synthetic desmosterol mimetics have almost no effect on LXR activity in hepatocytes in comparison with conventional synthetic LXR ligands. These findings reveal cell-specific differences in LXR responses to natural and synthetic ligands in macrophages and liver cells that provide a conceptually new basis for future drug development. (See pp. E4680–E4689.)

### Loss of a highly conserved sterile alpha motif domain gene (*WEEP*) results in pendulous branch growth in peach trees

*Courtney A. Hollender, Thierry Pascal, Amy Tabb, Toto Hadiarto, Chinnathambi Srinivasan, Wanpeng Wang, Zhongchi Liu, Ralph Scorza, and Chris Dardick*

Trees' branches grow against the pull of gravity and toward light. Although gravity and light perception have been studied in model species, much is unknown about how trees detect and respond to these signals. Here, we report the identification of a gene (*WEEP*) that controls lateral branch orientations and is directly or indirectly required for gravity responses in trees. Loss or reduction of WEEP expression produced branches that grow outward and downward and did not exhibit normal gravitropism responses when displaced. WEEP is conserved throughout the plant kingdom and may be involved in gravity perception. WEEP may also be a valuable target for breeding or engineering trees with improved shapes for agricultural and landscaping applications. (See pp. E4690–E4699.)

### Comparative genomics of the nonlegume *Parasponia* reveals insights into evolution of nitrogen-fixing rhizobium symbioses

*Robin van Velzen, Rens Holmer, Fengjiao Bu, Luuk Rutten, Arjan van Zeijl, Wei Liu, Luca Santuari, Qingqin Cao, Trupti Sharma, Defeng Shen, Yuda Roswanjaya, Titis A. K. Wardhani, Maryam Seifi Kalhor, Joelle Jansen, Johan van den Hoogen, Berivan Güngör, Marijke Hartog, Jan Hontelez, Jan Verver, Wei-Cai Yang, Elio Schijlen, Rimi Repin, Menno Schilthuizen, M. Eric Schranz, Renze Heidstra, Kana Miyata, Elena Fedorova, Wouter Kohlen, Ton Bisseling, Sandra Smit, and Rene Geurts*

Fixed nitrogen is essential for plant growth. Some plants, such as legumes, can host nitrogen-fixing bacteria within cells in root organs called nodules. Nodules are considered to have evolved in parallel in different lineages, but the genetic changes underlying this evolution

remain unknown. Based on gene expression in the nitrogen-fixing nonlegume *Parasponia andersonii* and the legume *Medicago truncatula*, we find that nodules in these different lineages may share a single origin. Comparison of the genomes of *Parasponia* with those of related nonnodulating plants reveals evidence of parallel loss of genes that, in legumes, are essential for nodulation. Taken together, this raises the possibility that nodulation originated only once and was subsequently lost in many descendant lineages. (See pp. E4700–E4709.)

## MYB30 links ROS signaling, root cell elongation, and plant immune responses

*Kaho Mabuchi, Hiromasa Maki, Tomotaka Itaya, Takamasa Suzuki, Mika Nomoto, Satomi Sakaoka, Atsushi Morikami, Tetsuya Higashiyama, Yasuomi Tada, Wolfgang Busch, and Hironaka Tsukagoshi*

Plant roots tune their growth to the environment. An important class of molecules involved in environmental responses as well as in root growth regulation is composed of reactive oxygen species (ROS). By making use of a comprehensive transcriptome atlas capturing ROS responses in different developmental zones of the root, we uncovered a regulatory network that is involved in root-growth regulation and responses to biotic stress. This network is composed of the ROS-responsive transcription factor MYB30, which regulates multiple genes involved in the transport of very-long-chain fatty acids (VLCFAs). Overall, our findings show that *Arabidopsis* uses the same *MYB30*-dependent regulatory network for root-growth and immunity responses, processes that were considered largely independent of each other. (See pp. E4710–E4719.)

## FACT complex is required for DNA demethylation at heterochromatin during reproduction in *Arabidopsis*

*Jennifer M. Frost, M. Yvonne Kim, Guen Tae Park, Ping-Hung Hsieh, Miyuki Nakamura, Samuel J. H. Lin, Hyunjin Yoo, Jaemyung Choi, Yoko Ikeda, Tetsu Kinoshita, Yeonhee Choi, Daniel Zilberman, and Robert L. Fischer*

The chromatin remodeling activities of the FACT (facilitates chromatin transactions) complex are required for many cellular functions, including transcription, DNA replication, and repair. Here, we demonstrate that the two FACT subunits, SSRP1 and SPT16, are also required for genome-wide DNA demethylation and regulation of gene imprinting during *Arabidopsis* reproduction. Without FACT, *Arabidopsis* seeds undergo abnormal development and exhibit aberrant DNA hypermethylation, including at imprinting control region loci. We show that FACT associates with the DEMETER (DME) DNA demethylase, facilitating DNA demethylation at over half of DME's targets, specifically those which reside in heterochromatin. These results provide insight into upstream events in the DNA demethylation pathway and reveal the importance of chromatin remodeling for DNA demethylation during *Arabidopsis* reproduction. (See pp. E4720–E4729.)