PNAS Plus Significance Statements

Properties of real metallic surfaces: Effects of density functional semilocality and van der Waals nonlocality

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It is primarily at their surfaces that solids interact with their environments. What is the physics behind the measurable properties of clean metallic surfaces? To answer this question, we calculate surface energies, work functions, and surface interlayer relaxations for aluminum and seven *d*-electron metals, using a sequence of exchange-correlation density functionals of increasing sophistication. While the simplest one, the local density approximation, works well through error cancellation, the usually more realistic Perdew–Burke–Ernzerhof functional underestimates both surface energies and work functions. The more advanced functionals, including the new strongly constrained and appropriately normed (SCAN) and SCAN+rVV10, demonstrate the unexpected importance of intermediate and long-range van der Waals attraction (seamlessly included in the random phase approximation). (See pp. E9188–E9196.)

In silico evidence for sequence-dependent nucleosome sliding

Joshua Lequieu, David C. Schwartz, and Juan J. de Pablo

The dynamic compaction of DNA into chromatin is essential for gene expression. Errors during compaction are associated with numerous diseases. Several molecular factors are known to affect chromatin dynamics, but their relative importance and the interplay between them are poorly understood. A detailed molecular model is used here to examine chromatin dynamics at the level of its most fundamental building block, namely the nucleosome. Nucleosome dynamics are demonstrated to be encoded in the DNA sequence itself, and key fundamental factors are uncovered that can significantly alter these dynamics at the molecular level. The results serve to complete a hitherto unavailable description of nucleosome dynamics by introducing previously unappreciated molecular processes, with the potential to influence macroscopic chromatin structure and genetics. (See pp. E9197–E9205.)

Methyl-compound use and slow growth characterize microbial life in 2-km-deep subseafloor coal and shale beds

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Microbial cells are widespread in diverse deep subseafloor environments; however, the viability, growth, and ecophysiology of these low-abundance organisms are poorly understood. Using single-cell–targeted stable isotope probing incubations combined with nanometer-scale secondary ion mass spectrometry, we measured the metabolic activity and generation times of thermally adapted microorganisms within Miocene-aged coal and shale bed samples collected from 2 km below the seafloor during Integrated Ocean Drilling Program Expedition 337. Microorganisms from the shale and coal were capable of metabolizing methylated substrates, including methylamine and methanol, when incubated at their in situ temperature of 45 °C, but had exceedingly slow growth, with biomass generation times ranging from less than a year to hundreds of years as measured by the passive tracer deuterated water. (See pp. E9206–E9215.)

The misleading narrative of the canonical faculty productivity trajectory

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Scholarly productivity impacts nearly every aspect of a researcher's career, from their initial placement as faculty to funding and tenure decisions. Historically, expectations for individuals rely on 60 years of research on aggregate trends, which suggest that productivity rises rapidly to an early-career peak and then gradually declines. Here we show, using comprehensive data on the publication and employment histories of an entire field of research, that the canonical narrative of "rapid rise, gradual decline" describes only about one-fifth of individual faculty, and the remaining four-fifths exhibit a rich diversity of productivity patterns. This suggests existing models and expectations for faculty productivity require revision, as they capture only one of many ways to have a successful career in science. (See pp. E9216–E9223.)

PAF1 complex component Leo1 helps recruit *Drosophila* Myc to promoters

Jennifer M. Gerlach, Michael Furrer, Maria Gallant, Dirk Birkel, Apoorva Baluapuri, Elmar Wolf, and Peter Gallant

We identify the PAF1 complex component Leo1 as a factor that helps recruit Myc to its target genes. In particular when Myc is overexpressed, Leo1 becomes limiting for transcriptional regulation by Myc. (See pp. E9224–E9232.)

Trigger loop dynamics can explain stimulation of intrinsic termination by bacterial RNA polymerase without terminator hairpin contact

Ananya Ray-Soni, Rachel A. Mooney, and Robert Landick

RNA polymerase (RNAP), like many cellular processors of information in DNA and RNA, is a complex macromolecular machine whose multiple structural modules and domains undergo poorly understood conformational changes that mediate information processing. We investigated the role of one such mobile module, the polymorphous trigger loop (TL) of RNAP, in intrinsic transcription termination by bacterial RNAP. The TL folds into a helical hairpin to promote RNA synthesis, but also is proposed to aid termination. By separating effects of the TL and of TL variants on termination from effects on RNA synthesis, we established that TL flexibility, not the helical hairpin conformation, facilitates rearrangements of RNAP leading to termination. Our results illustrate how kinetic assays can help dissect complex macromolecular machines. (See pp. E9233–E9242.)

Control of transcriptional activity by design of charge patterning in the intrinsically disordered RAM region of the Notch receptor

Kathryn P. Sherry, Rahul K. Das, Rohit V. Pappu, and Doug Barrick

Charge patterning is a key feature of intrinsically disordered protein regions. Here we test whether charge pattering is important for biochemical and biological function, using the "RAM" disordered region of the Notch receptor. The Notch signaling pathway is important in stem-cell biology and cancer. Using computer design, we built 13 charge permutants that span a broad range of charge segregation. These permutants have profound effects on conformational properties, binding affinity to the downstream transcription factor, CSL, and potency in transcriptional activation. WT Notch has the optimal segregation value for activation, whereas higher levels of segregation disrupt binding and activation. Our study paves the way for control of biological function through redesign of charge patterning. (See pp. E9243–E9252.)

Across the tree of life, radiation resistance is governed by antioxidant Mn^{2+}, gauged by paramagnetic resonance

Ajay Sharma, Elena K. Gaidamakova, Olga Grichenko, Vera Y. Matrosova, Veronika Hoeke, Polina Klimenkova, Isabel H. Conze, Robert P. Volpe, Rok Tkavc, Cene Gostinčar, Nina Gunde-Cimerman, Jocelyne DiRuggiero, Igor Shuryak, Andrew Ozarowski, Brian M. Hoffman, and Michael J. Daly

Decades of functional genomic efforts have failed to predict the ability of cells to survive ionizing radiation (IR). Evidence is mounting that small high-symmetry antioxidant complexes of manganous ions with metabolites (H-Mn^{2+}) are responsible for cellular IR resistance, and that H-Mn^{2+} protects the proteome, not the genome, from IR-induced reactive oxygen species. We show that the amount of H-Mn^{2+} in nonirradiated living cells is readily gauged by electron paramagnetic resonance (EPR) spectroscopy and highly diagnostic of their DNA repair efficiency and survival after gamma-radiation exposure. This spectroscopic measure of cellular H-Mn^{2+} content is the strongest known biological indicator of cellular IR resistance between and within organisms across the three domains of the tree of life, with potential applications including optimization of radiotherapy. (See pp. E9253–E9260.)

Rad52 phosphorylation by Ipl1 and Mps1 contributes to Mps1 kinetochore localization and spindle assembly checkpoint regulation

Gyubum Lim and Won-Ki Huh

Rad52 is a well-known factor in homologous recombination. In this study, we discover functions of Rad52 in spindle assembly checkpoint (SAC) regulation and Mps1 localization for chromosome bi-orientation. Deletion of *RAD52* leads to various phenotypes of inaccurate chromosome segregation that are not observed in another homologous recombination-defective *rad51*Δ mutant. Furthermore, we find that Rad52 is a substrate of mitotic kinases Ipl1/Aurora and Mps1 and that Rad52 phosphorylation by Ipl1 and Mps1 contributes to Mps1 kinetochore localization and SAC regulation. From these findings, we suggest that Rad52 functions as a mediator between Ipl1 and Mps1 in the regulatory pathway of mitosis. Our results provide detailed insights into the molecular basis of chromosome segregation and SAC regulation. (See pp. E9261–E9270.)

Targeting autophagy inhibits melanoma growth by enhancing NK cells infiltration in a CCL5-dependent manner

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The failure in achieving a durable clinical immune response against cancer cells depends on the ability of cancer cells to establish a microenvironment that prevent cytotoxic immune cells to infiltrate tumors and kill cancer cells. Therefore, the key approach to achieving successful antitumor immune response is to harness strategies allowing the reorientation of immune cells to the tumor. Herein we reveal that inhibiting autophagy induces a massive infiltration of natural killer immune cells into the tumor bed, and a subsequent dramatic decrease in the tumor volume of melanomas. These results highlight the role of targeting autophagy in breaking the immunosuppressive tumor microenvironment barrier, thus allowing the infiltration of natural killer cells into the tumor to kill cancer cells. (See pp. E9271–E9279.)

Mib1 contributes to persistent directional cell migration by regulating the Ctnnd1-Rac1 pathway

Takamasa Mizoguchi, Shoko Ikeda, Saori Watanabe, Michiko Sugawara, and Motoyuki Itoh

Cell migration is involved in various biological processes, including animal development and cancer metastasis. Cells show either of two different types of migratory behavior: random or directional. We show that defects in mind bomb 1 (Mib1) ubiquitin ligase, an enzyme that adds ubiquitin to substrates, leads to an increase in random cell migration. Our study revealed that Mib1 ubiquitinates Ctnnd1, a positive regulator of the small GTPase Rac1, in cultured cells. We further found that Mib1-dependent Ctnnd1 ubiquitination decreases Ctnnd1-mediated activation of Rac1, which increases random cell migration, in cultured cells. Therefore, this study shows that Mib1 contributes

to persistent directional cell migration, at least in part, by regulating the Ctnnd1–Rac1 pathway. (See pp. E9280–E9289.)

Control of growth and gut maturation by *HoxD* genes and the associated lncRNA *Haglr*

Jozsef Zakany, Fabrice Darbellay, Bénédicte Mascrez, Anamaria Necsulea, and Denis Duboule

During development, transcription factors are necessary not only to pattern the body plan but also to control growth. However, the link between these two developmental components has been difficult to establish. *Hox* genes are involved in the emergence of a functional digestive system in metazoans, thus providing a potential impact on growth through nutrition. Also, genetic conditions involving these genes lead to important growth retardation. We analyzed several targeted mutant lines at the *HoxD* locus and found that stunted phenotypes can all be explained by the lack of function of *Hoxd3*, whose role seems to be critical in the developing gut of suckling mice, perhaps as an adaptation to the milk-dependent early postnatal period in mammals. (See pp. E9290–E9299.)

Divergence of developmental trajectories is triggered interactively by early social and ecological experience in a cooperative breeder

Stefan Fischer, Lena Bohn, Evelyne Oberhummer, Cecilia Nyman, and Barbara Taborsky

Cooperative breeding represents the pinnacle of vertebrate social evolution. Helpers in cooperatively breeding species are characterized by a life-long potential to reproduce. Therefore it has been predicted that cooperative breeders lack an early specialization into subordinate helpers and dominant breeders. In a 3-year life-history experiment, we manipulated the social and ecological environments jointly during the early postnatal period of a cooperatively breeding vertebrate, the "Princess cichlid" *Neolamprologus pulcher*. We found that individuals did specialize in distinct behavioral competences, which led to either delayed dispersal or early independent breeding. The divergence into different behavioral trajectories became apparent only by manipulating both early social and ecological experiences, highlighting the importance of multivariate influences on the development of social trajectories. (See pp. E9300–E9307.)

WD40-repeat 47, a microtubule-associated protein, is essential for brain development and autophagy

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We present an identification of the relevance of WD40-repeat (WDR) genes in brain connectivity, highlighting the power of unbiased mouse studies in the field of neuroscience. We focus on the poorly studied WDR47 protein sharing structural homology with LIS1, which causes lissencephaly. WDR47 plays a role in progenitor proliferation, neuronal migration, and fiber tract projections in a similar fashion to LIS1 but with the distinctive particularity that WDR47 inhibits autophagic flux. This provides a functional link between autophagy biology and the C-terminal to LisH domain in mammals. Importantly, WDR47 uncovers an aspect of corpus callosum biology pointing toward a link between the regulation of microtubule dynamics and autophagic flux for axonal outgrowth and guidance. (See pp. E9308–E9317.)

Gut dysbiosis breaks immunological tolerance toward the central nervous system during young adulthood

Sudhir K. Yadav, Sridhar Boppana, Naoko Ito, John E. Mindur, Martin T. Mathay, Ankoor Patel, Suhayl Dhib-Jalbut, and Kouichi Ito

Multiple sclerosis (MS) is classified as an autoimmune disease of the central nervous system (CNS). Alterations of gut microbiota (gut dysbiosis) are frequently observed in MS patients. It is still unknown how gut dysbiosis contributes to development of MS. We report here that gut dysbiosis, which we attribute to expansion of enteric pathogenic bacteria, triggers and/or exacerbates the spontaneous development of experimental autoimmune encephalomyelitis, an animal model of MS. This occurs during the period of young adulthood by reducing development of $Foxp3^+$ Treg cells and expression of E3 ubiquitin ligase genes involved in protection from autoimmune diseases. This study suggests that gut dysbiosis may play a pathological role in the initiation and/or progression of MS during a defined age window. (See pp. E9318–E9327.)

PTIP chromatin regulator controls development and activation of B cell subsets to license humoral immunity in mice

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To provide optimal host defense, the full spectrum of antibody-based immunity requires natural antibodies and immunization-induced antigen-specific antibodies. Here we show that the PTIP (Pax transactivation domain-interacting protein) chromatin regulator is induced by B cell activation to potentiate the establishment of steady-state and postimmune serum antibody levels. It does so by promoting activation-associated proliferation and differentiation of all the major B cell subsets, at least in part, through regulating the NF-κB pathway. With the genetic basis still unknown for a majority of patients with common variable immunodeficiency, further work investigating how PTIP controls cell signaling may generate valuable new insight for human health and disease. (See pp. E9328–E9337.)

In vitro reconstitution of T cell receptor-mediated segregation of the CD45 phosphatase

Catherine B. Carbone, Nadja Kern, Ricardo A. Fernandes, Enfu Hui, Xiaolei Su, K. Christopher Garcia, and Ronald D. Vale

The T cell receptor (TCR) and PD-1 signaling cascades have been hypothesized to be triggered by the exclusion of the transmembrane phosphatase CD45 from sites of receptor–ligand engagement at the T cell–antigen-presenting cell interface. We reconstituted TCR–pMHC– and PD1–PD-L1–mediated segregation of CD45 with purified proteins and model membranes, demonstrating that this phenomenon can occur in the absence of any active cellular organization. In this minimal system, two developmentally regulated and different size isoforms of CD45 are differently segregated by TCR–pMHC binding, suggesting a possible mechanism for the fine-tuning of signaling. Collectively, our data show that the binding energy of physiological receptor–ligand pairs is sufficient to create spatial organization in membranes. (See pp. E9338–E9345.)

Identification of a tumor-promoter cholesterol metabolite in human breast cancers acting through the glucocorticoid receptor

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Cholesterol and its transformation into cholesterol-5,6-epoxides (5,6-EC) was long suspected as contributing to breast cancer (BC) pathogenesis, before it was found that 5,6-EC metabolism controls BC development and is deregulated in breast cancers. Herein, we studied in tumor cells and human samples how 5,6-EC metabolism deregulation promotes tumor progression. We have discovered a pathway in BCs producing an oncometabolite derived from 5,6-EC, through the action of the cortisol-inactivating enzyme, and identified the glucocorticoid receptor (GR) as the target mediating its proliferative effects. Inhibition of its production or GR significantly blocked its action on BC progression. Thus, targeting this oncometabolism and GR represents a new opportunity for therapeutic intervention in BCs and potentially other cancers presenting such deregulations. (See pp. E9346–E9355.)

CRISPR/Cas9 knockouts reveal genetic interaction between strain-transcendent erythrocyte determinants of *Plasmodium falciparum* invasion

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During malaria infections, *Plasmodium falciparum* parasites invade RBCs. Identification of host factors for parasite invasion guides the development of vaccines and host-targeted therapeutics. Here we describe the development of an in vitro culture system for the functional analysis of RBC determinants using the immortal erythroleukemia cell line JK-1. JK-1 cells can be induced to differentiate synchronously, support parasite invasion, and are amenable to genetic manipulation. Using this system, we validated two host factors, basigin and CD44, as strain-transcendent host factors for parasite invasion, and we demonstrated a functional interaction between these two proteins. The ability to perform gene editing to produce RBC mutants will augment our ability to study malaria infection. (See pp. E9356–E9365.)

Balanced excitation and inhibition are required for high-capacity, noise-robust neuronal selectivity

Ran Rubin, L. F. Abbott, and Haim Sompolinsky

Neurons and networks in the cerebral cortex must operate reliably despite multiple sources of noise. Using a mathematical analysis and model simulations, we show that noise robustness requires synaptic connections to be in a balanced regime in which excitation and inhibition are strong and largely cancel each other. Our theory predicts an optimal ratio for the number of excitatory and inhibitory synapses that depends on the statistics of afferent activity and is consistent with data. This distinct form of excitation–inhibition balance is essential for robust neuronal selectivity and crucial for stability in associative memory networks, and it emerges automatically from learning in the presence of noise. (See pp. E9366–E9375.)

β-III-spectrin spinocerebellar ataxia type 5 mutation reveals a dominant cytoskeletal mechanism that underlies dendritic arborization

Adam W. Avery, David D. Thomas, and Thomas S. Hays

Neurons are highly polarized cells critical to the processing of signals within the brain, and are distinguished by morphologically and functionally distinct axonal and somatodendritic compartments. Understanding mechanisms dictating neuronal morphological specialization will advance our understanding of neuronal function and neurodegenerative diseases. This report reveals that the spectrin-actin cytoskeleton contributes to the origin of dendritic arbor morphology. We show that this cytoskeleton is required for formation and stabilization of complex dendritic arbors in *Drosophila*. A human spinocerebellar ataxia type 5 mutation causes β-III-spectrin to bind actin with high affinity and disrupts expansion of the spectrin-actin cytoskeleton into distal regions of the arbor. Our data suggest that loss of the spectrin-actin cytoskeleton reduces stabilization of distal dendrites required for arbor outgrowth. (See pp. E9376–E9385.)

Hedgehog signaling regulates ciliary localization of mouse odorant receptors

Devendra Kumar Maurya, Staffan Bohm, and Mattias Alenius

Cells communicate with each other via signaling molecules that bind to surface receptors of responding cells. One example is Hedgehog (Hh) signaling, which is known for its regulatory role of tissue development and maintenance. Here we identify a Hh function, which is to regulate the sense of smell. Hh signaling modulates odorant responsiveness by regulating the accumulation of odorant receptors (ORs) in the olfactory ciliary compartment, which is where odorant detection is initiated. Since the mechanism appears evolutionarily conserved, even though the structures of ORs are not, the results open for the interesting possibility that Hh signaling may regulate protein transport and stimuli responsiveness also in other types of neurons. (See pp. E9386–E9394.)

Site-directed RNA repair of endogenous Mecp2 RNA in neurons

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Rett syndrome (RTT) is a neurological disease caused by mutations in the gene encoding the global transcriptional regulator, Methyl CpG Binding Protein 2 (MECP2). We exploit a strategy to repair mutant *Mecp2* mRNA that if successful should reverse symptoms. The strategy utilizes the catalytic activity of a naturally occurring enzyme, Adenosine Deaminase Acting on RNA (ADAR2), which in brain alters the mRNA sequence and function of proteins. In cultured RTT neurons co-expressing a modified ADAR2 protein and an appropriate RNA guide, a human mutation in *Mecp2* mRNA is repaired efficiently. RNA repair restores MeCP2 function, consistent with reversal of the pathological

consequences of the RTT mutation. Our strategy holds promise for new therapeutic approaches to RTT and other neurological diseases. (See pp. E9395–E9402.)

Root-associated fungal microbiota of nonmycorrhizal *Arabis alpina* and its contribution to plant phosphorus nutrition

Juliana Almario, Ganga Jeena, Jörg Wunder, Gregor Langen, Alga Zuccaro, George Coupland, and Marcel Bucher

Most terrestrial plants live in symbiosis with arbuscular mycorrhizal (AM) fungi and rely on this association to scavenge the macronutrient phosphorus (P) from soil. *Arabis alpina* thrives in P-limited alpine habitats, although, like all Brassicaceae species, it lacks the ability to establish an AM symbiosis. By studying the fungal microbiota associated with *A. alpina* roots we uncovered its association with a beneficial Helotiales fungus capable of promoting plant growth and P uptake, thereby facilitating plant adaptation to low-P environments. (See pp. E9403–E9412.)

Genome of wild olive and the evolution of oil biosynthesis

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We sequenced the genome and transcriptomes of the wild olive (oleaster). More than 50,000 genes were predicted, and evidence was found for two relatively recent whole-genome duplication events, dated at approximately 28 and 59 Mya. Whole-genome sequencing, as well as gene expression studies, provide further insights into the evolution of oil biosynthesis, and will aid future studies aimed at further increasing the production of olive oil, which is a key ingredient of the healthy Mediterranean diet and has been granted a qualified health claim by the US Food and Drug Administration. (See pp. E9413–E9422.)