# PNAS Plus Significance Statements

## Rippling ultrafast dynamics of suspended 2D monolayers, graphene

*Jianbo Hu, Giovanni M. Vanacore, Andrea Cepellotti, Nicola Marzari, and Ahmed H. Zewail*

Rippling is an intrinsic feature of 2D materials, responsible for their structural stability, transport properties, and electron–hole charge redistribution. Modulating these ripples in a controlled manner not only provides a better understanding of their structural properties, but also has potential impact for applications. Here, we examine graphene monolayer as a prototypical 2D material. An ultrafast attenuation of the ripples intrinsically present in the graphene plane is followed by a significant enhancement of the rippling effect on a longer time scale, as driven by the successive excitation of in-plane and out-of-plane phonon modes. The methodology described is of a general nature and is suitable for the investigation of other 2D materials, where we expect to observe similar rippling effects. (See pp. E6555–E6561.)

## Backtracked and paused transcription initiation intermediate of *Escherichia coli* RNA polymerase

*Eitan Lerner, SangYoon Chung, Benjamin L. Allen, Shuang Wang, Jookyung Lee, Shijia W. Lu, Logan W. Grimaud, Antonino Ingargiola, Xavier Michalet, Yazan Alhadid, Sergei Borukhov, Terence R. Strick, Dylan J. Taatjes, and Shimon Weiss*

Transcription initiation by RNA polymerase (RNAP) is a highly regulated rate-limiting step in many genes and involves numerous intermediate states that remain incompletely understood. Here, we report the characterization of a previously hypothesized slow initiation pathway involving RNAP backtracking and pausing. This backtracked and paused state is observed when all nucleoside triphosphates (NTPs) are present at physiologically relevant concentrations, but becomes more prevalent with unbalanced NTP levels, which may occur in vivo under conditions of metabolic stress. Pausing and backtracking in initiation may play an important role in regulating RNAP transcription. Moreover, similar RNA backtracked states may contribute to promoter-proximal pausing among eukaryotic RNA polymerase II enzymes. (See pp. E6562–E6571.)

## Crystal structure of the DNA binding domain of the transcription factor T-bet suggests simultaneous recognition of distant genome sites

*Ce Feng Liu, Gabriel S. Brandt, Quyen Q. Hoang, Natalia Naumova, Vanja Lazarevic, Eun Sook Hwang, Job Dekker, Laurie H. Glimcher, Dagmar Ringe, and Gregory A. Petsko*

The transcription factor T-bet (Tbox protein expressed in T cells), a master regulator of T-cell lineage commitment, is a member of the Tbox family but coordinately regulates many more genes than other Tbox proteins. How T-bet simultaneously recognizes distant elements that may be thousands of base pairs apart is unknown. We have determined the crystal structure of the Tbox DNA binding domain of T-bet complexed with a 24-bp palindromic DNA. The structure shows a dimer where each monomer binds simultaneously to two independent DNA molecules. Fluorescence-based assays show T-bet can synapse two DNA molecules in solution. Chromosome conformation capture assays confirm that T-bet can directly mediate the formation of chromatin loops at the IFN-γ gene locus in the absence of other transcription-related proteins. (See pp. E6572–E6581.)

## Working stroke of the kinesin-14, ncd, comprises two substeps of different direction

*Bert Nitzsche, Elzbieta Dudek, Lukasz Hajdo, Andrzej A. Kasprzak, Andrej Vilfan, and Stefan Diez*

Kinesin, dynein, and myosin motor proteins are best known for their production of linear force along the axes of cytoskeletal filaments. However, many of these motors can also generate torque manifesting itself by filament rotations around their longitudinal axes when gliding on motor-coated surfaces. By combining the measured longitudinal and angular velocities of microtubules gliding on nonprocessive kinesin-14 motors with a theoretical model we here show that the working stroke of this motor comprises at least two distinct conformational changes. Our observations clarify the temporal order of events in the hydrolysis cycle of kinesin-14, which has not been conclusive from previous structural and single-molecule data. Moreover, our results demonstrate how conformational changes in individual enzymes can be deduced from their ensemble properties. (See pp. E6582–E6589.)



www.pnas.org/cgi/doi/10.1073/pnas.ss11343

## Mitotic Golgi disassembly is required for bipolar spindle formation and mitotic progression

*Gianni Guizzunti and Joachim Seemann*

During mammalian cell division, the contiguous Golgi ribbon is first vesiculated and then partitioned into the daughter cells with the aid of the mitotic spindle. Whether Golgi vesiculation is required for cell division is unclear. Here, we show that inhibition of Golgi disassembly by the acute formation of a polymer in the Golgi lumen prior to mitotic entry blocks bipolar spindle formation and arrests cells in early mitosis with an active spindle assembly checkpoint (SAC). Importantly, mitotic progression is fully restored upon depletion of centrosomes. Our work thus reveals that mitotic Golgi disassembly is tightly monitored and acts as an upstream surveillance point for the canonical SAC to ensure the fidelity of Golgi inheritance and cell division. (See pp. E6590–E6599.)

## FOXA1 overexpression mediates endocrine resistance by altering the ER transcriptome and IL-8 expression in ER-positive breast cancer

*Xiaoyong Fu, Rinath Jeselsohn, Resel Pereira, Emporia F. Hollingsworth, Chad J. Creighton, Fugen Li, Martin Shea, Agostina Nardone, Carmine De Angelis, Laura M. Heiser, Pavana Anur, Nicholas Wang, Catherine S. Grasso, Paul T. Spellman, Obi L. Griffith, Anna Tsimelzon, Carolina Gutierrez, Shixia Huang, Dean P. Edwards, Meghana V. Trivedi, Mothaffar F. Rimawi, Dolores Lopez-Terrada, Susan G. Hilsenbeck, Joe W. Gray, Myles Brown, C. Kent Osborne, and Rachel Schiff*

One of the mechanisms of endocrine resistance in estrogen receptor α (ER)-positive ($^+$) breast cancer is the cross-talk between the ER and growth factor receptor pathways leading to altered ER activity and a reprogrammed ER-dependent transcriptome. However, key mediators of this ER-dependent transcriptional reprogramming remain elusive. Here we demonstrate that forkhead box protein A1 (FOXA1) up-regulation via gene amplification or overexpression contributes to endocrine resistance and increased invasiveness phenotypes by altering the ER-dependent transcriptome. We further show that IL-8, one of the top altered FOXA1/ER effectors, plays a key role in mediating these phenotypes and is a potential target to treat ER$^+$/FOXA1-high breast cancer. Our findings provoke a new interplay of FOXA1 in the ER transcriptional program in endocrine-resistant breast cancer. (See pp. E6600–E6609.)

## V-1 regulates capping protein activity in vivo

*Goeh Jung, Christopher J. Alexander, Xufeng S. Wu, Grzegorz Piszczek, Bi-Chang Chen, Eric Betzig, and John A. Hammer*

F-actin is assembled downstream of major actin nucleators like the actin-related proteins 2/3 (Arp2/3) complex and formins. Capping Protein (CP), conversely, halts actin assembly. The ankyrin-repeat protein V-1 has been shown to block CP activity in vitro. Here we show that V-1 overexpression and knockout in *Dictyostelium* amoeba phenocopy CP knockdown and overexpression, respectively. Moreover, V-1 must be capable of binding CP to exhibit overexpression phenotypes and rescue V-1–null cells. Finally, we present evidence that V-1's ability to sequester CP is regulated by phosphorylation. Together, our data demonstrate that V-1 controls major actin-dependent cellular processes in vivo by virtue of its ability to inhibit CP and that its influence may be modulated in cells. (See pp. E6610–E6619.)

## Genetic architecture of natural variation in visual senescence in *Drosophila*

*Mary Anna Carbone, Akihiko Yamamoto, Wen Huang, Rachel A. Lyman, Tess Brune Meadors, Ryoan Yamamoto, Robert R. H. Anholt, and Trudy F. C. Mackay*

Functional decline with age—senescence—is a major determinant of health span in an aging population, but its genetic basis remains largely unknown. In humans, visual decline often heralds the onset of senescence. We used the fruit fly, *Drosophila melanogaster*, to explore the genetic basis of natural variation in phototaxis, the innate tendency to move toward light, and age-dependent decline in phototaxis as a proxy for visual senescence. We found the genetic basis for visual senescence is distinct from that which determines variation in life span. Furthermore, genes shaping early development of the nervous system, in particular the visual system, also contribute to senescence at later age, demonstrating that senescence is part of a genetic continuum throughout the life span. (See pp. E6620–E6629.)

## Architecture of a minimal signaling pathway explains the T-cell response to a 1 million-fold variation in antigen affinity and dose

*Melissa Lever, Hong-Sheng Lim, Philipp Kruger, John Nguyen, Nicola Trendel, Enas Abu-Shah, Philip Kumar Maini, Philip Anton van der Merwe, and Omer Dushek*

T cells initiate and regulate adaptive immune responses when their T-cell antigen receptors recognize antigens. The T-cell response is known to depend on the antigen affinity/dose, but the precise relationship, and the mechanisms underlying it, are debated. To resolve the debate, we stimulated T cells with antigens spanning a 1 million-fold range in affinity/dose. We found that a different antigen (and hence different affinity) produced the largest T-cell response at different doses. Using model identification algorithms, we report a simple mechanistic model that can predict the T-cell response from the physiological low-affinity regime into the high-affinity regime applicable to therapeutic receptors. (See pp. E6630–E6638.)

## Sustained antigen availability during germinal center initiation enhances antibody responses to vaccination

*Hok Hei Tam, Mariane B. Melo, Myungsun Kang, Jeisa M. Pelet, Vera M. Ruda, Maria H. Foley, Joyce K. Hu, Sudha Kumari, Jordan Crampton, Alexis D. Baldeon, Rogier W. Sanders, John P. Moore, Shane Crotty, Robert Langer, Daniel G. Anderson, Arup K. Chakraborty, and Darrell J. Irvine*

We explored the effect of nontraditional vaccine dosing profiles on antibody titers of vaccines and discovered that certain dosing profiles demonstrate >10-fold higher antibody production than the traditional single-dose prime–boost method. We also present a computational model that captures the experimental results and provides a mechanistic understanding of the biology behind the effectiveness of our strategy. This work has clinical significance in vaccine design because it is a simple method to increase the efficacy of subunit vaccines, which may lead to the development of efficacious vaccines for diseases such as HIV. (See pp. E6639–E6648.)

## A conserved αβ transmembrane interface forms the core of a compact T-cell receptor–CD3 structure within the membrane

*Logesvaran Krshnan, Soohyung Park, Wonpil Im, Melissa J. Call, and Matthew E. Call*

The T-cell antigen receptor (TCR) is an eight-subunit modular biosensor that detects the presence of pathogen-derived molecular fragments in infected cells and tissues. No atomic-resolution structure has been determined for the intact receptor complex, and the mechanism by which it transmits signals across the cell membrane remains poorly defined. Using a combination of biochemical, biophysical, and computational approaches to study the assembled complex in cellular membranes, we identified an evolutionarily conserved core transmembrane (TM) structure that organizes the complex into an intimately packed eight-helix bundle within the membrane and is critical for the structural integrity of the intact receptor. Our model provides insights into possible structural pathways involved in TM TCR signaling. (See pp. E6649–E6658.)

## miRNA92a targets KLF2 and the phosphatase PTEN signaling to promote human T follicular helper precursors in T1D islet autoimmunity

*Isabelle Serr, Rainer W. Fürst, Verena B. Ott, Martin G. Scherm, Alexei Nikolaev, Füsun Gökmen, Stefanie Kälin, Stephanie Zillmer, Melanie Bunk, Benno Weigmann, Nicole Kunschke, Brigitta Loretz, Claus-Michael Lehr, Benedikt Kirchner, Bettina Haase, Michael Pfaffl, Ari Waisman, Richard A. Willis, Anette-G. Ziegler, and Carolin Daniel*

The onset of type 1 diabetes autoimmunity is indicated by the development of multiple islet autoantibodies, produced by B cells with the help of T follicular helper (TFH) cells. MicroRNAs (miRNAs) are small noncoding RNAs that regulate cellular states, as immune activation, making them suitable targets for disease intervention. Here, we show an enrichment of insulin-specific C-X-C chemokine receptor type 5 $(CXCR5)^{+}CD4^{+}$ TFH precursors correlating with high miRNA92a abundance during onset of autoimmunity and identify Krueppel-like factor 2 (KLF2) as a target for miRNA92a. We demonstrate that miRNA92a inhibition blocks TFH induction and reduces murine islet autoimmunity in vivo. Therefore, we propose miRNA92a and the phosphatase and tension homolog-phosphoinositol-3-kinase-KLF2 signaling network as possible innovative precision medicine targets to interfere with aberrant immune activation in islet autoimmunity. (See pp. E6659–E6668.)

## ATM/G6PD-driven redox metabolism promotes FLT3 inhibitor resistance in acute myeloid leukemia

*Mark A. Gregory, Angelo D'Alessandro, Francesca Alvarez-Calderon, Jihye Kim, Travis Nemkov, Biniam Adane, Andrii I. Rozhok, Amit Kumar, Vijay Kumar, Daniel A. Pollyea, Michael F. Wempe, Craig T. Jordan, Natalie J. Serkova, Aik Choon Tan, Kirk C. Hansen, and James DeGregori*

FMS-like tyrosine kinase 3 (FLT3) inhibitors have shown impressive activity in clinical trials for acute myeloid leukemia (AML); however, these inhibitors invariably fail to achieve sustained remissions. Here we demonstrate that FLT3 inhibition causes severe deficiencies in redox metabolism and accumulation of reactive oxygen species (ROS) in the mitochondria of AML cells. We discovered that the metabolic regulators ataxia telangiectasia mutated and glucose 6-phosphate dehydrogenase help maintain antioxidant capacity and survival of a subpopulation of AML cells in the face of FLT3 inhibition. Inactivation of these factors escalates mitochondrial ROS and enhances AML cell eradication. Importantly, we show that the use of a drug that increases mitochondrial ROS enhances the efficacy of FLT3 inhibitor therapy, suggesting a combinatorial therapeutic strategy. (See pp. E6669–E6678.)

## Fast fMRI can detect oscillatory neural activity in humans

*Laura D. Lewis, Kawin Setsompop, Bruce R. Rosen, and Jonathan R. Polimeni*

A major challenge in neuroscience is our limited ability to image neural signals noninvasively in humans. Oscillations in brain activity are important for perception, attention, and awareness, and progress in cognitive neuroscience depends on localizing these patterns. fMRI is thought to be too slow to measure brain oscillations because it depends on slow changes in blood flow. Here, we use recently developed imaging techniques to show that fMRI can measure faster neural oscillations than previously thought, and responses are 10 times larger than expected. With computational modeling and simultaneous electroencephalography we show that vascular responses are surprisingly fast when brain activity fluctuates rapidly. These results suggest that fMRI can be used to track oscillating brain activity directly during human cognition. (See pp. E6679–E6685.)

## Orthopedic surgery modulates neuropeptides and BDNF expression at the spinal and hippocampal levels

*Ming-Dong Zhang, Swapnali Barde, Ting Yang, Beilei Lei, Lars I. Eriksson, Joseph P. Mathew, Thomas Andreska, Katerina Akassoglou, Tibor Harkany, Tomas G. M. Hökfelt, and Niccolò Terrando*

Orthopedic surgery sometimes causes persistent pain and, especially in the elderly, delirium and cognitive dysfunction. Using an established mouse bone-fracture model associating with memory deficits, we assessed pain behavior and expression of several molecules in sensory neurons, spinal cord, and brain. An increase in cold sensitivity and up-regulation of several injury markers, including activating transcription factor 3, the neuropeptide galanin, and growth factor brain-derived neurotrophic factor (BDNF), were observed in sensory ganglia. In the hippocampus, BDNF protein levels were increased in mossy fibers. In contrast, the Bdnf transcript was not increased in the parent granule cell bodies, and c-Fos levels were decreased, as was neurogenesis. Thus, impaired hippocampal BDNF signaling may contribute to mental deficits observed after surgery. (See pp. E6686–E6695.)

## Allosteric binding site in a Cys-loop receptor ligand-binding domain unveiled in the crystal structure of ELIC in complex with chlorpromazine

*Mieke Nys, Eveline Wijckmans, Ana Farinha, Özge Yoluk, Magnus Andersson, Marijke Brams, Radovan Spurny, Steve Peigneur, Jan Tytgat, Erik Lindahl, and Chris Ulens*

Cys-loop receptors belong to a family of ion channels that are involved in fast synaptic transmission. Allosteric modulators of Cys-loop receptors hold therapeutic potential as they tweak receptor function while preserving the normal fluctuations in neurotransmitter signaling at the synapse. Here, we take advantage of a model Cys-loop receptor, the *Erwinia* ligand-gated ion channel (ELIC). We determined cocrystal structures of ELIC in complex with chlorpromazine ($IC_{50}$, ~160 μM) and its brominated derivative bromopromazine, which unveil an allosteric binding site localized at the interface between the extracellular ligand-binding domain and the pore-forming transmembrane domain. Our results demonstrate that the different allosteric binding sites present in Cys-loop receptors form an

almost continuous path stretching from top to bottom of the receptor. (See pp. E6696–E6703.)

## Role of DNA methylation in hybrid vigor in *Arabidopsis thaliana*

*Takahiro Kawanabe, Sonoko Ishikura, Naomi Miyaji, Taku Sasaki, Li Min Wu, Etsuko Itabashi, Satoko Takada, Motoki Shimizu, Takeshi Takasaki-Yasuda, Kenji Osabe, W. James Peacock, Elizabeth S. Dennis, and Ryo Fujimoto*

Hybrid vigor is an important phenomenon in basic genetics and in agricultural practice, but the bases of the superior performance of the hybrid relative to its parents in biomass and seed production remain elusive. In recent years, it has been suggested that epigenetic controls on levels of gene action are involved. Using mutants of genes involved in DNA methylation, we show that RNA polymerase IV or methyltransferase I do not contribute to the generation of the heterotic phenotype but that decrease in DNA methylation 1, a nucleosome remodeller with an effect on DNA methylation level, is required to produce a full level of hybrid vigor. (See pp. E6704–E6711.)

## Human attention filters for single colors

*Peng Sun, Charles Chubb, Charles E. Wright, and George Sperling*

The eyes present the brain much more information than it could possibly process. One important way to prioritize information is by selective attention to features, processing only items containing the attended features and blocking others (i.e., forming an attention filter). Here we demonstrate an extremely efficient paradigm and a powerful analysis to quantitatively measure, as accurately as one might measure physical color filters, 32 such human attention filters for single colors. These data are an essential basis for a theory of attention to color. The centroid paradigm itself, because it quickly and quantitatively characterizes basic attention processes, has numerous applications. (See pp. E6712–E6720.)