# PNAS Plus Significance Statements

## Orbital apocenter is not a sufficient condition for HST/STIS detection of Europa's water vapor aurora

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Images of Europa's UV aurora taken by the Hubble Space Telescope in December 2012 have revealed local hydrogen and oxygen emissions in intensity ratios that identify the source as electron impact excitation of water molecules. The existence of water vapor plumes as a source for the detected localized water vapor and the possible accessibility of subsurface liquid water reservoirs at these locations have important implications for the exploration of Europa's potentially habitable environments. The observations reported here (pp. E5123–E5132) tested whether orbital position near the apocenter is an essential requirement for plume activity. Only an upper limit on the amount of water vapor was obtained. Orbital position is therefore not a sufficient condition for detecting plumes and they may be episodic events.

## RecQ helicase and RecJ nuclease provide complementary functions to resect DNA for homologous recombination

Katsumi Morimatsu and Stephen C. Kowalczykowski

Breaks in DNA are repaired by homologous recombination. Because the structure of DNA ends at a break site can be variable, the repair machinery must be designed to act on a variety of heterogeneous DNA break sites. Bacterial RecQ helicase and RecJ nuclease initiate the repair of double-stranded DNA breaks; however, neither protein alone can deal with the broad range of physiological ends. Human Bloom syndrome helicase (BLM) is the homolog of RecQ, and it functions in DNA resection, contributing to genomic stability in humans. We establish (pp. E5133–E5142) that RecQ and RecJ complement one another by acting on DNA ends and intermediates that the other cannot. Thus, by leveraging complementary substrate preferences, recombination initiation from all types of DNA ends, in many organisms, is ensured.

## Relating influenza virus membrane fusion kinetics to stoichiometry of neutralizing antibodies at the single-particle level

Jason J. Otterstrom, Boerries Brandenburg, Martin H. Koldijk, Jarek Juraszek, Chan Tang, Samaneh Mashaghi, Ted Kwaks, Jaap Goudsmit, Ronald Vogels, Robert H. E. Friesen, and Antoine M. van Oijen

We determine the number of broadly neutralizing antibodies required to inhibit influenza virus membrane fusion by simultaneously observing individual viral particles undergoing fusion and counting the number of antibodies bound to them. The viral membrane fusion process is mediated by fusion proteins whose activity is blocked through the binding of these antibodies to evolutionarily conserved epitopes. Surprisingly, the number of antibodies required for inhibition is markedly lower than the number of fusion proteins present, indicating virus neutralization does not require saturation of epitope occupancy. Overall, our results (pp. E5143–E5148) support a model of membrane fusion requiring several fusion proteins working together in a coordinated, stochastic fashion, and the inhibition of this process through disruption of fusion protein coordination.

## Simultaneous sequencing of oxidized methylcytosines produced by TET/JBP dioxygenases in *Coprinopsis cinerea*

Lukas Chavez, Yun Huang, Khai Luong, Suneet Agarwal, Lakshminarayan M. Iyer, William A. Pastor, Virginia K. Hench, Sylvia A. Frazier-Bowers, Evgenia Korol, Shuo Liu, Mamta Tahiliani, Yinsheng Wang, Tyson A. Clark, Jonas Korlach, Patricia J. Pukkila, L. Aravind, and Anjana Rao

A prominent epigenetic mechanism for gene regulation is methylation of cytosine bases in DNA. TET enzymes facilitate DNA demethylation by converting 5-methylcytosine (5mC) to oxidized methylcytosines (oxi-mCs). We show (pp. E5149–E5158) that oxi-mCs are generated by conserved TET/JBP enzymes encoded in the genome of the model organism *Coprinopsis cinerea* and present a method for simultaneous mapping of the three different species of oxi-mCs at near–base-pair resolution. We observe that centromeres and transposable elements exhibit distinctive patterns of 5mC and oxi-mC, and show that gene body 5mC and oxi-mC mark silent paralogous multicopy genes. Our study describes a method to map three species of oxi-mC simultaneously and reveals the colocation of 5mC and oxi-mC at functional elements throughout the *C. cinerea* genome.

## Synaptonemal complex extension from clustered telomeres mediates full-length chromosome pairing in *Schmidtea mediterranea*

Youbin Xiang, Danny E. Miller, Eric J. Ross, Alejandro Sánchez Alvarado, and R. Scott Hawley

In this study we validate a nearly century-old model for chromosome pairing in flatworms and provide a molecular description of meiotic prophase in flatworms. Specifically, we validate József Gelei's proposal that chromosome pairing in flatworms results from the formation of a telomere bouquet followed by the extension of synapsis from the base of the bouquet, thus facilitating homolog pairing in a processive manner. This study (pp. E5159–E5168) further advances the groundwork necessary to establish *Schmidtea mediterranea* as a powerful new meiotic system. The genes identified and the RNAi constructs and antibodies generated during this work help make planarian meiosis a highly tractable model system.

## Basophil-mediated protection against gastrointestinal helminths requires IgE-induced cytokine secretion

Christian Schwartz, Adriana Turqueti-Neves, Susanne Hartmann, Philipp Yu, Falk Nimmerjahn, and David Voehringer

Gastrointestinal worms (helminths) infect more than 2 billion people, and vaccines are not yet available. Helminths elicit a type 2 immune response characterized by high serum IgE levels and increased numbers of IL-4– or IL-13–secreting effector cells including Th2 cells, eosinophils, basophils, and type 2 innate lymphoid cells. We determined the mechanism by which basophils contribute to protection against

secondary infections with gastrointestinal helminths. Here (pp. E5169–E5177) we demonstrate that basophils are recruited into the small intestine of infected mice and orchestrate the local type 2 immune response in this tissue. Basophil-mediated protection required the presence of IgE and the expression of activating Fc receptors and IL-4/IL-13 in basophils. These findings could help the development of new vaccination strategies against helminths.

## Tubulin hyperacetylation is adaptive in cardiac proteotoxicity by promoting autophagy

Patrick M. McLendon, Bradley S. Ferguson, Hanna Osinska, Md. Shenuarin Bhuiyan, Jeanne James, Timothy A. McKinsey, and Jeffrey Robbins

Proteotoxicity, or the accumulation of misfolded protein, can cause heart failure and effective therapeutics are needed to reduce protein accumulation in the myocardium. This study (pp. E5178–E5186) shows that inhibiting tubulin deacetylation by histone deacetylase 6 (HDAC6) is protective in a mouse model of proteinopathy-induced heart failure. Inhibiting tubulin deacetylation using the FDA-approved drug suberoylanilide hydroxamic acid (SAHA) reduced protein aggregates in cardiomyocytes and led to substantial improvement in cardiac function. Mechanistically, we show that inhibiting HDAC6 increases autophagy in cardiomyocytes, and that inducing autophagy with voluntary exercise also induces tubulin acetylation. This study shows that tubulin acetylation is important for autophagy stimulation in the heart and, importantly, sheds new light on the mechanism of autophagy induction with HDAC inhibitors.

## Disruption of Lrp4 function by genetic deletion or pharmacological blockade increases bone mass and serum sclerostin levels

Ming-Kang Chang, Ina Kramer, Thomas Huber, Bernd Kinzel, Sabine Guth-Gundel, Olivier Leupin, and Michaela Kneissel

Targeting WNT (Wingless-type)/β-catenin signaling has emerged as an attractive novel therapeutic approach to the treatment of bone diseases. We previously identified LRP4 (low-density lipoprotein receptor-related protein 4) as a facilitator of action of the WNT signaling antagonist SOST/sclerostin in vitro. Here (pp. E5187–E5195), we generated bone-specific *Lrp4*-deficient mouse lines and anti-LRP4 antibodies selectively disrupting the Lrp4 sclerostin facilitator function. Using these novel genetic and pharmacological tools, we demonstrate that disruption of Lrp4 function induces bone gain in vivo and results in highly elevated circulating sclerostin levels. Together, these findings provide important novel insights into the role of LRP4 as a key regulator of bone homeostasis and into the mode of action of sclerostin and provide a new strategy for promoting bone gain through targeting of the WNT pathway.

## Complex two-component signaling regulates the general stress response in Alphaproteobacteria

Andreas Kaczmarczyk, Ramon Hochstrasser, Julia A. Vorholt, and Anne Francez-Charlot

Bacteria possess many regulatory systems to monitor their environment and adapt their physiology accordingly. Whereas most systems sense one specific signal, the general stress response (GSR) is activated by many signals and protects cells against a wide range of adverse conditions. In Alphaproteobacteria, the GSR is controlled by the response regulator PhyR, but little is known about the upstream pathways. Here (pp. E5196–E5204), we establish the GSR as a complex regulatory network composed of a particular family of partially redundant sensor kinases and of additional response regulators that modulate PhyR activity in *Sphingomonas melonis*. Given the broad conservation of this kinase family, it is possible that it plays a general role in the GSR in Alphaproteobacteria.

## Cytoplasmic HIV-1 RNA is mainly transported by diffusion in the presence or absence of Gag protein

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HIV-1 full-length RNA must go to specific subcellular compartments to carry out its functions as a template for translation of structural and enzymatic proteins and as the genetic material for new virions. RNA mislocalization can affect the functions of the RNA and its encoded proteins, causing defects in viral replication. Currently, little is known about how HIV-1 RNA is transported in the cytoplasm. Here, we demonstrate that HIV-1 full-length RNAs use diffusion as the major mechanism for cytoplasmic transport in the absence of viral group-specific antigen (Gag) proteins and even in the presence of sufficient Gag proteins for virus assembly, indicating that Gag does not alter the RNA transport mechanism. These studies (pp. E5205–E5213) provide insights into mechanisms essential to viral replication.

## The vertical occipital fasciculus: A century of controversy resolved by in vivo measurements

Jason D. Yeatman, Kevin S. Weiner, Franco Pestilli, Ariel Rokem, Aviv Mezer, and Brian A. Wandell

The vertical occipital fasciculus (VOF) is a major white-matter fascicle connecting dorsal and ventral visual cortex. Few vision scientists or cognitive neuroscientists are aware of the VOF's existence. The scarcity of papers on this important pathway stems from the contentious history surrounding its discovery by Wernicke in 1881. We review the conflict surrounding the classic, postmortem, VOF measurements, and we introduce modern, in vivo methods to precisely characterize the VOF's cortical terminations and unique tissue properties (pp. E5214–E5223). The new VOF measurements provide insight into the communication between ventral stream regions involved in form perception and dorsal stream regions involved in eye movements and attention.

## Amazonian landscapes and the bias in field studies of forest structure and biomass

David C. Marvin, Gregory P. Asner, David E. Knapp, Christopher B. Anderson, Roberta E. Martin, Felipe Sinca, and Raul Tupayachi

Although tropical forests absorb more carbon dioxide as biomass than any other terrestrial ecosystem, biomass estimates disagree substantially at landscape-to-regional scales. Current biomass maps rely upon field plots for extrapolations to larger scales, yet whether field plots accurately represent landscape-scale variables has not been assessed. To our knowledge, this is the first study to compare forest structural variables and aboveground biomass derived from field plots to those derived from their host landscapes using airborne 3D remote sensing. We found (pp. E5224–E5232) that typical field plots can produce substantially biased estimates and the number of plots needed to reduce this bias is impractical, positioning airborne remote sensing as a core tool for mapping forest structure and biomass across tropical landscapes.