PNAS Plus Significance Statements

Controlling orientational order in block copolymers using low-intensity magnetic fields

Manesh Gopinadhan, Youngwoo Choo, Kohsuke Kawabata, Gilad Kaufman, Xunda Feng, Xiaojun Di, Yekaterina Rokhlenko, Lalit H. Mahajan, Dennis Ndaya, Rajeswari M. Kasi, and Chinedum O. Osuji

Magnetic field interactions with condensed matter can produce orientationally ordered states that are important for fundamental research and technological applications. Block copolymer (BCP) mesophases typically exhibit weak field coupling, requiring high-intensity fields generated by superconducting magnets to produce such states. This work advances a strategy for circumventing such field intensity limitations and creates highly aligned mesophases using fields an order of magnitude smaller than typically required and that can be produced by simple permanent magnets. We elucidate the roles of molecular mobility, grain size, and ordering kinetics on the mesophase field response. Low-intensity field-directed BCP ordering has potentially profound implications for processing functional materials and developing complex textures by field shaping. (See pp. E9437–E9444.)

Clinical validation of a nanodiamond-embedded thermoplastic biomaterial

Dong-Keun Lee, Theodore Kee, Zhangrui Liang, Desiree Hsiou, Darron Miya, Brian Wu, Eiji Osawa, Edward Kai-Hua Chow, Eric C. Sung, Mo K. Kang, and Dean Ho

There is a continued need to advance novel nanomedicine platforms into the clinic to address treatment challenges in oncology, infection, and regenerative medicine, among other areas. As such, this work demonstrates the in-human validation of nanodiamonds through their incorporation into gutta percha [nanodiamond-embedded gutta percha (NDGP)], a polymer that repairs root canal treatment sites following tissue disinfection. A randomized, dual-arm clinical trial was implemented, and study endpoints included confirmation of lesion healing, postoperative pain reduction, and the absence of reinfection. To date, the NDGP-treated patients successfully met the study endpoints. Therefore, these findings support the potential expansion of nanodiamonds, and the broader nanomedicine field, into other disease indications. (See pp. E9445–E9454.)

Three-dimensional mesostructures as high-temperature growth templates, electronic cellular scaffolds, and self-propelled microrobots

Zheng Yan, Mengdi Han, Yan Shi, Adina Badea, Yiyuan Yang, Ashish Kulkarni, Erik Hanson, Mikhail E. Kandel, Xiewen Wen, Fan Zhang, Yiyue Luo, Qing Lin, Hang Zhang, Xiaogang Guo, Yuming Huang, Kewang Nan, Shuai Jia, Aaron W. Oraham, Molly B. Mevis, Jaeman Lim, Xuelin Guo, Mingye Gao, Woomi Ryu, Ki Jun Yu, Bruno G. Nicolau, Aaron Petronico, Stanislav S. Rubakhin, Jun Lou, Pulickel M. Ajayan, Katsuyo Thornton, Gabriel Popescu, Daining Fang, Jonathan V. Sweedler, Paul V. Braun, Haixia Zhang, Ralph G. Nuzzo, Yonggang Huang, Yihui Zhang, and John A. Rogers

Exploiting advanced 3D designs in micro/nanomanufacturing inspires potential applications in various fields including biomedical engineering, metamaterials, electronics, electromechanical components, and many others. The results presented here provide enabling concepts in an area of broad, current interest to the materials community—strategies for forming sophisticated 3D micro/nanostructures and means for using them in guiding the growth of synthetic materials and biological systems. These ideas offer qualitatively differentiated capabilities compared with those available from more traditional methodologies in 3D printing, multiphoton lithography, and stress-induced bending—the result enables access to both active and passive 3D mesostructures in state-of-the-art materials, as free-standing systems or integrated with nearly any type of supporting substrate. (See pp. E9455–E9464.)

Neurobiology of culturally common maternal responses to infant cry

Marc H. Bornstein, Diane L. Putnick, Paola Rigo, Gianluca Esposito, James E. Swain, Joan T. D. Suwalsky, Xueyun Su, Xiaoxia Du, Kaihua Zhang, Linda R. Cote, Nicola De Pisapia, and Paola Venuti

We undertook an interdisciplinary exploration that unites evolutionary biology, neuroscience, and developmental cultural psychology. Based on extensive and detailed behavioral analyses of 684 new mothers in 11 countries and complementary functional magnetic resonance imaging (fMRI) analyses of brain responses in 43 first-time new US mothers to their own infants' cries, 44 experienced Chinese mothers to infant cries and control emotional sounds, and 12 Italian mothers and nonmothers to generic infant cries, we identified specific behavior repertoires and specific corresponding activated brain regions in human caregivers that constitute primary responses to infant distress. This study set will appeal to scientific and general audiences because it elucidates the foundations of core

www.pnas.org/cgi/doi/10.1073/pnas.ss11445

Downloaded by guest on October 25, 2020

parenting practices in response to infant vocal distress. (See pp. E9465–E9473.)

Reconstitution of *UCP1* using CRISPR/Cas9 in the white adipose tissue of pigs decreases fat deposition and improves thermogenic capacity

Qiantao Zheng, Jun Lin, Jiaojiao Huang, Hongyong Zhang, Rui Zhang, Xueying Zhang, Chunwei Cao, Catherine Hambly, Guosong Qin, Jing Yao, Ruigao Song, Qitao Jia, Xiao Wang, Yongshun Li, Nan Zhang, Zhengyu Piao, Rongcai Ye, John R. Speakman, Hongmei Wang, Qi Zhou, Yanfang Wang, Wanzhu Jin, and Jianguo Zhao

Uncoupling protein 1 (UCP1) is responsible for brown adipose tissue-mediated thermogenesis and plays a critical role in protecting against cold and regulating energy homeostasis. Modern pigs lack functional UCP1, which makes them susceptible to cold and prone to fat deposition and results in neonatal mortality and decreased production efficiency. In the current study, a CRISPR/Cas9-mediated homologous recombination-independent approach was established, and mouse adiponectin-UCP1 was efficiently inserted into the porcine endogenous *UCP1* locus. The resultant UCP1 KI pigs showed an improved ability to maintain body temperature, decreased fat deposition, and increased carcass lean percentage. UCP1 KI pigs are a potentially valuable resource for the pig industry that can improve pig welfare and reduce economic losses. (See pp. E9474–E9482.)

Identification and characterization of *Sr13*, a tetraploid wheat gene that confers resistance to the Ug99 stem rust race group

Wenjun Zhang, Shisheng Chen, Zewdie Abate, Jayaveeramuthu Nirmala, Matthew N. Rouse, and Jorge Dubcovsky

Wheat provides a substantial proportion of the calories and proteins consumed by humans, but further production increases are necessary to feed a growing human population. Reducing yield losses caused by pathogens can contribute to these increases. In this study, we report the identification of *Sr13*, a gene from pasta wheat that confers resistance to the new virulent races of the stem rust pathogen that appeared in Africa at the beginning of this century. We identified three different resistance forms of *Sr13* and developed a diagnostic marker to accelerate their deployment in wheat breeding programs. In addition, *Sr13* can be a useful component of transgenic cassettes including multiple resistance genes. (See pp. E9483–E9492.)

A unique surface on Pat1 C-terminal domain directly interacts with Dcp2 decapping enzyme and Xrn1 5′–3′ mRNA exonuclease in yeast

Clément Charenton, Claudine Gaudon-Plesse, Zaineb Fourati, Valerio Taverniti, Régis Back, Olga Kolesnikova, Bertrand Séraphin, and Marc Graille

Control of mRNA synthesis and decay is crucial for cells to adapt to their environment and for proper development. The 5′ end of eukaryotic mRNAs is modified by a structure called cap that protects them from rapid and uncontrolled decay. During mRNA decay, this cap is removed by a specialized and finely regulated multiprotein factory called decapping complex. Our results support a model in which the two major enzymes responsible for mRNA decapping (Dcp2) and decay (Xrn1) are sequentially recruited to mRNAs by the same surface from Pat1, a scaffolding protein central for decapping. As this Pat1 region is important for growth and specific to fungi, this is a potential target for the development of drugs against pathogenic yeasts. (See pp. E9493–E9501.)

Crystal structures of Mmm1 and Mdm12–Mmm1 reveal mechanistic insight into phospholipid trafficking at ER-mitochondria contact sites

Hanbin Jeong, Jumi Park, Youngsoo Jun, and Changwook Lee

The endoplasmic reticulum (ER) forms membrane contact sites (MCSs) with other organelles such as mitochondria, endosomes, and peroxisomes in eukaryotic cells. The MCS plays a pivotal role in exchanging cellular materials such as ions and lipids. More importantly, nonvesicular lipid trafficking occurring at the ER-mitochondria MCS is essential for the biogenesis of the mitochondrial membrane. In yeast, the ER-mitochondria encounter structure (ERMES) complex comprising the ER proteins Mmm1 and cytosolic Mdm12 and the mitochondria proteins Mdm34 and Mdm10 provides a tethering force between the ER and the mitochondria and mediates lipid trafficking. Here, we present two crystal structures of Mmm1 and the Mdm12–Mmm1 complex. Based on these structures, we propose the model by which the Mdm12–Mmm1 complex contributes to phospholipid trafficking at the ER-mitochondria MCS. (See pp. E9502–E9511.)

Bacteriorhodopsin-like channelrhodopsins: Alternative mechanism for control of cation conductance

Oleg A. Sineshchekov, Elena G. Govorunova, Hai Li, and John L. Spudich

Two well-characterized carboxylate residues catalyze vectorial proton transport in rhodopsin proton pumps, such as bacteriorhodopsin. We report the mechanism of a type of rhodopsin, a channelrhodopsin unique in that it exploits the same residues to accomplish a completely different process: opening and closing of a cation channel. This finding promises leaps in our understanding of how evolution modifies shared protein scaffolds to create new protein chemistry in homologous membrane proteins. Moreover, algal channelrhodopsins have become widespread molecular tools to manipulate membrane potential in cells with light (optogenetics). The structurally distinct, independently evolved channelrhodopsins studied here open the possibility for discovery of optogenetic tools with new properties. Our results provide insights into the conductance mechanisms of these channelrhodopsins. (See pp. E9512–E9519.)

Structural insights into binding of STAC proteins to voltage-gated calcium channels

Siobhan M. Wong King Yuen, Marta Campiglio, Ching-Chieh Tung, Bernhard E. Flucher, and Filip Van Petegem

Skeletal muscle contraction is a tightly orchestrated event that starts with the depolarization of the T-tubular membrane. At the center is a functional and mechanical coupling between two membrane proteins: L-type voltage-gated calcium channels, located in the plasma membrane, and ryanodine receptors, located in the membrane of the sarcoplasmic reticulum. How exactly these proteins associate has remained a mystery, but recent reports have highlighted a key role for the STAC3 adaptor protein in this process. Here, we provide structural snapshots of the three STAC isoforms and identify a cytosolic loop of two Ca_V isoforms as a functional interaction site. A mutation linked to Native American myopathy is at the interface and abolishes the interaction. (See pp. E9520–E9528.)

Cryo-EM structure of Mcm2-7 double hexamer on DNA suggests a lagging-strand DNA extrusion model

Yasunori Noguchi, Zuanning Yuan, Lin Bai, Sarah Schneider, Gongpu Zhao, Bruce Stillman, Christian Speck, and Huilin Li

During initiation of DNA replication in eukaryotes, the origin recognition complex, with Cdc6 and Cdt1, assembles an inactive

Downloaded by guest on October 25, 2020

Mcm2-7 double hexamer on the dsDNA. Later, the double hexamer recruits Cdc45 and GINS to form two active and separate DNA helicases. The active Cdc45–Mcm2-7–GINS helicase encircles the leading strand while excluding the lagging strand. One of the fundamental unanswered questions is how each Mcm2-7 hexamer converts from binding dsDNA to binding one of the single strands. The structure of the double hexamer on dsDNA reveals how DNA interacts with key elements inside the central channel, leading us to propose a lagging-strand extrusion mechanism. This work advances our understanding of eukaryotic replication initiation. (See pp. E9529–E9538.)

Structural basis of human kinesin-8 function and inhibition

Julia Locke, Agnel Praveen Joseph, Alejandro Peña, Martin M. Möckel, Thomas U. Mayer, Maya Topf, and Carolyn A. Moores

Kinesins are a superfamily of ATP-dependent motors important for many microtubule-based functions, including multiple roles in mitosis. Small-molecule inhibitors of mitotic kinesins disrupt cell division and are being developed as antimitotic therapies. We investigated the molecular mechanism of the multitasking human mitotic kinesin Kif18A and its inhibition by the small molecule BTB-1. We used cryo-electron microscopy to visualize nucleotide-dependent conformational changes in microtubule-bound Kif18A, and the conformation of microtubule-bound, BTB-1-bound Kif18A. We calculated a putative BTB-1–binding site and validated this site experimentally to reveal the BTB-1 inhibition mechanism. Our work points to a general mechanism of kinesin inhibition, with wide implications for a targeted blockade of these motors in both dividing and interphase cells. (See pp. E9539–E9548.)

Evolutionary and molecular foundations of multiple contemporary functions of the nitroreductase superfamily

Eyal Akiva, Janine N. Copp, Nobuhiko Tokuriki, and Patricia C. Babbitt

Functionally diverse enzyme superfamilies are sets of homologs that conserve a structural fold and mechanistic details but perform various distinct chemical reactions. What are the evolutionary routes by which ancestral proteins diverge to produce extant enzymes? We present an approach that combines experimental data with computational tools to trace these sequence–structure–function transitions in a model system, the functionally diverse flavin mononucleotide-dependent nitroreductases (NTRs). Our results suggest an evolutionary model in which contemporary NTR classes have diverged in a radial manner from a minimal flavin-binding scaffold via insertions at key positions and fixation of functional residues, yielding the reaction versatility of contemporary enzymes. These principles will facilitate rational design of NTRs and advance general approaches for delineating the emergence of functional diversity in enzyme superfamilies. (See pp. E9549–E9558.)

Large G protein α-subunit XLαs limits clathrin-mediated endocytosis and regulates tissue iron levels in vivo

Qing He, Richard Bouley, Zun Liu, Marc N. Wein, Yan Zhu, Jordan M. Spatz, Chia-Yu Wang, Paola Divieti Pajevic, Antonius Plagge, Jodie L. Babitt, and Murat Bastepe

Mutations in the gene encoding XLαs and Gsα (*GNAS*) cause several genetic diseases and various tumors. Although alterations in XLαs activity/levels are implicated in some of these disorders, cellular actions of XLαs have remained poorly defined. We identified dynamins and sorting nexin-9, key components of clathrin-mediated endocytosis, as binding partners of XLαs and showed that XLαs, but not Gsα, restricts clathrin-mediated endocytosis and plays a role in iron/transferrin uptake in vivo. Thus, impaired or enhanced endocytosis may be involved in the pathogenesis of some of the *GNAS*-related diseases. Our findings also provide insights into the roles of heterotrimeric G proteins and the mechanisms underlying endocytosis, a fundamental cellular process required for nutrient uptake and regulation of cell signaling. (See pp. E9559–E9568.)

Numerous interactions act redundantly to assemble a tunable size of P bodies in *Saccharomyces cerevisiae*

Bhalchandra S. Rao and Roy Parker

RNA–protein (RNP) granules contribute to spatiotemporal regulation of gene expression in eukaryotes. RNP granules have also been implicated in the pathology of neurodegenerative diseases. Insights into mechanisms of assembly and disassembly are fundamental to understanding the biology of RNP granules. In this manuscript, we provide evidence to support a model for P-body assembly which suggests that a summation of scaffolding interactions drives P-body assembly and scales the size of P body-related assemblies. Since the multivalent nature of factors and scaffolding interactions is a conserved feature of most RNP granules, our model provides an overarching theme for how other RNP granules in biology could assemble and function in vivo. (See pp. E9569–E9578.)

GATA2/3-TFAP2A/C transcription factor network couples human pluripotent stem cell differentiation to trophectoderm with repression of pluripotency

Christian Krendl, Dmitry Shaposhnikov, Valentyna Rishko, Chaido Ori, Christoph Ziegenhain, Steffen Sass, Lukas Simon, Nikola S. Müller, Tobias Straub, Kelsey E. Brooks, Shawn L. Chavez, Wolfgang Enard, Fabian J. Theis, and Micha Drukker

This study provides a mechanistic explanation for the differentiation of trophoblasts from human pluripotent stem cells, a process relying on BMP morphogens. We found that a network of the transcription factors GATA2, GATA3, TFAP2A, and TFAP2C regulates early trophoblast progenitor specification by activating placental genes and inhibiting the pluripotency gene *OCT4*, thus acting to couple trophoblast specification with exit from pluripotency. To demonstrate the relevance of our findings in vivo, we show that down-regulating *GATA3* in primate embryos prevents trophectoderm specification. In addition, we present a genome-wide analysis of active and inactive chromatin during trophoblast progenitor specification. These results provide a basis to guide investigations of human trophectoderm development. (See pp. E9579–E9588.)

Survival and divergence in a small group: The extraordinary genomic history of the endangered Apennine brown bear stragglers

Andrea Benazzo, Emiliano Trucchi, James A. Cahill, Pierpaolo Maisano Delser, Stefano Mona, Matteo Fumagalli, Lynsey Bunnefeld, Luca Cornetti, Silvia Ghirotto, Matteo Girardi, Lino Ometto, Alex Panziera, Omar Rota-Stabelli, Enrico Zanetti, Alexandros Karamanlidis, Claudio Groff, Ladislav Paule, Leonardo Gentile, Carles Vilà, Saverio Vicario, Luigi Boitani, Ludovic Orlando, Silvia Fuselli, Cristiano Vernesi, Beth Shapiro, Paolo Ciucci, and Giorgio Bertorelle

A small and relict population of brown bears lives in complete isolation in the Italian Apennine Mountains, providing a unique opportunity to study the impact of drift and selection on the genomes of a large endangered mammal and reconstruct the phenotypic consequences and the conservation implications of such evolutionary processes. The Apennine bear is highly inbred

Downloaded by guest on October 25, 2020

and harbors very low genomic variation. Several deleterious mutations have been accumulated by drift. We found evidence that this is a consequence of habitat fragmentation in the Neolithic, when human expansion and land clearance shrank its habitat, and that retention of variation at immune system and olfactory receptor genes as well as changes in diet and behavior prevented the extinction of the Apennine bear. (See pp. E9589–E9597.)

Induction of H3K9me3 and DNA methylation by tethered heterochromatin factors in *Neurospora crassa*

Jordan D. Gessaman and Eric U. Selker

Chemical modifications to histones and DNA are critical for the establishment of distinct chromatin states and the regulation of the underlying DNA sequence. Aberrant heterochromatin, often with hyper- or hypomethylated DNA, is associated with many human disease states, including cancers, but the mechanisms controlling heterochromatin establishment are not fully understood. We developed in vivo protein tethering in *Neurospora crassa*, a filamentous fungus harboring many aspects of heterochromatin found in higher eukaryotes, and used it to direct heterochromatin to normally active, euchromatic loci. Testing tethered heterochromatin factors in various mutant backgrounds revealed interrelationships among the classic hallmarks of heterochromatin: DNA methylation, histone deacetylation, and H3K9me3. We also found evidence of complex regulation of the DIM-2 DNA methyltransferase. (See pp. E9598–E9607.)

Quantitative proteomics identifies STEAP4 as a critical regulator of mitochondrial dysfunction linking inflammation and colon cancer

Xiang Xue, Bryce X. Bredell, Erik R. Anderson, Angelical Martin, Christopher Mays, Hiroko Nagao-Kitamoto, Sha Huang, Balázs Győrffy, Joel K. Greenson, Karin Hardiman, Jason R. Spence, Nobuhiko Kamada, and Yatrik M. Shah

Inflammation is a major risk factor for many cancers and the role of metabolic reprogramming in the inflammatory progression of cancer is not clear. We used a quantitative proteomic approach to identify mitochondrial proteins that are altered early in intestinal inflammation. We show that mitochondrial iron dysregulation is an early event that initiates mitochondrial dysfunction. Through the proteomic analysis, we identified a mitochondrial iron reductase, six-transmembrane epithelial antigen of prostate 4 (STEAP4), as being highly elevated during inflammation. Using intestinal epithelial-specific STEAP4 mice, we show that an increase in STEAP4 is sufficient to alter mitochondrial iron homeostasis. Chronic increase in mitochondrial iron leads to tissue injury and potentiates colon cancer, whereas mitochondrial iron chelation is protective in colitis and colitis-associated colon cancer models. (See pp. E9608–E9617.)

Necroptosis controls NET generation and mediates complement activation, endothelial damage, and autoimmune vasculitis

Adrian Schreiber, Anthony Rousselle, Jan Ulrich Becker, Anne von Mässenhausen, Andreas Linkermann, and Ralph Kettritz

In this report, we provide evidence of a mechanistic link between antineutrophil cytoplasmic antibody (ANCA)-induced neutrophil activation, regulated necrosis (necroptosis), generation of neutrophil extracellular traps, complement activation, and endothelial cell damage with consecutive vasculitis and glomerulonephritis in autoimmune ANCA-induced vasculitis (AAV). We now show that inhibition of necroptosis-inducing kinases completely prevents ANCA vasculitis and establish a link to activation of the complement system. We suggest that these findings significantly extend our understanding of the pathogenesis of AAV and especially the tight regulation of neutrophil cell death therein. In addition, specific necroptosis inhibitors are currently being evaluated in clinical studies and can possibly complement existing therapeutic strategies in AAV. (See pp. E9618–E9625.)

Humanized mouse model supports development, function, and tissue residency of human natural killer cells

Dietmar Herndler-Brandstetter, Liang Shan, Yi Yao, Carmen Stecher, Valerie Plajer, Melanie Lietzenmayer, Till Strowig, Marcel R. de Zoete, Noah W. Palm, Jie Chen, Catherine A. Blish, Davor Frleta, Cagan Gurer, Lynn E. Macdonald, Andrew J. Murphy, George D. Yancopoulos, Ruth R. Montgomery, and Richard A. Flavell

Humanized mice represent a promising approach to study the human immune system in health and disease. However, insufficient development and function of human lymphocytes limit the applicability of humanized mice for cancer biology and therapy. We demonstrate that human *SIRPA* and *IL15* knock-in (SRG-15) mice support efficient development of circulating and tissue-resident natural killer (NK) cells, intraepithelial lymphocytes, and innate lymphoid cell subsets. In contrast to previous humanized mouse models, human NK cells in SRG-15 mice mediate efficient antibody-dependent cellular cytotoxicity and thereby enable NK cell-targeted cancer immunotherapy of tumor xenografts. As such, SRG-15 humanized mice may facilitate translational research by enabling the development of novel NK and $CD8^+$ T cell-based therapeutic approaches that target human infections and malignancies. (See pp. E9626–E9634.)

Off-tumor targets compromise antiangiogenic drug sensitivity by inducing kidney erythropoietin production

Masaki Nakamura, Yin Zhang, Yunlong Yang, Ceylan Sonmez, Wenyi Zheng, Guichun Huang, Takahiro Seki, Hideki Iwamoto, Bo Ding, Linlin Yin, Theodoros Foukakis, Thomas Hatschek, Xuri Li, Kayoko Hosaka, Jiaping Li, Guohua Yu, Xinsheng Wang, Yizhi Liu, and Yihai Cao

Understanding the molecular mechanisms underlying drug resistance of antiangiogenic therapy is crucial to improvement of therapeutic efficacy in cancer patients. Our data uncover a mechanism by which the off-tumor targets compromise anti-VEGF drug sensitivity. The therapeutic implication of our findings poses a concept that blocking the off-tumor targets of antiangiogenic drugs are crucial for improvement of therapeutic efficacy. Based on our findings, modest inhibition of excessive EPO production is recommended for improvement of antiangiogenic therapy. Our work will result in a significant paradigm shift and conceptual advances as to improvement of both quality-of-life and overall survivals of antiangiogenic drug-treated cancer patients. (See pp. E9635–E9644.)

Neuron-specific methylome analysis reveals epigenetic regulation and tau-related dysfunction of BRCA1 in Alzheimer's disease

Tatsuo Mano, Kenichi Nagata, Takashi Nonaka, Airi Tarutani, Tomohiro Imamura, Tadafumi Hashimoto, Taro Bannai, Kagari Koshi-Mano, Takeyuki Tsuchida, Ryo Ohtomo, Junko Takahashi-Fujigasaki, Satoshi Yamashita, Yasumasa Ohyagi, Ryo Yamasaki, Shoji Tsuji, Akira Tamaoka, Takeshi Ikeuchi, Takaomi C. Saido, Takeshi Iwatsubo, Toshikazu Ushijima, Shigeo Murayama, Masato Hasegawa, and Atsushi Iwata

To extract critical information from Alzheimer's disease (AD) postmortem brains that may otherwise be lost, we chose to screen epigenetic signatures. Epigenome analysis is a robust methodology in

Downloaded by guest on October 25, 2020

terms of its cell type and gene specificity, suitability for high-throughput analysis, and resistance to postmortem degradation. Analysis of the neuron-specific methylome revealed a variety of differentially methylated genes, including *BRCA1*. We demonstrate the pathogenic relevance of compromised genomic integrity by analyzing the neuroprotective function of BRCA1 against amyloid β (Aβ)-induced DNA double-strand breaks. Furthermore, insolubility of BRCA1 under the presence of aggregated tau suggested the reason for its dysfunction despite enhanced expression. We provide insight into the pathomechanism of AD and demonstrate the potential of screening neuron-specific methylome to reveal new pathogenic contributors. (See pp. E9645–E9654.)

Taurine ameliorates particulate matter-induced emphysema by switching on mitochondrial NADH dehydrogenase genes

Xiaobo Li, Hongbao Yang, Hao Sun, Runze Lu, Chengcheng Zhang, Na Gao, Qingtao Meng, Shenshen Wu, Susanna Wang, Michael Aschner, Jiong Wu, Boping Tang, Aihua Gu, Steve A. Kay, and Rui Chen

Exposure to high levels of particulate matter (PM) poses a major threat to human health. Cigarette smoke is the most common irritant that causes chronic obstructive pulmonary disease (COPD); however, at least one-fourth of patients with COPD are nonsmokers, and their disease is largely attributed to air pollution. The occurrence of pollution episodes in China has raised an emergent question of how PM leads to the pathogenesis of COPD. In this paper, we show that deregulation of mitochondrial NADH dehydrogenase gene expression levels plays a key role in the aggravation of COPD during air pollutant exposure, which can be rescued by taurine and 3-MA treatments in both mammalian cells and animals. (See pp. E9655–E9664.)

Lack of BACE1 S-palmitoylation reduces amyloid burden and mitigates memory deficits in transgenic mouse models of Alzheimer's disease

Robert J. Andrew, Celia G. Fernandez, Molly Stanley, Hong Jiang, Phuong Nguyen, Richard C. Rice, Virginie Buggia-Prévot, Pierre De Rossi, Kulandaivelu S. Vetrivel, Raza Lamb, Arnau Argemi, Emilie S. Allaert, Elle M. Rathbun, Sofia V. Krause, Steven L. Wagner, Angèle T. Parent, David M. Holtzman, and Gopal Thinakaran

Alzheimer's disease (AD) is a devastating neurodegenerative disorder for which no preventative drug or cure is currently available. BACE1 is a key enzyme that initiates the production of β-amyloid peptides (Aβ), which accumulate in AD brain and contribute to cognitive decline. BACE1 undergoes S-palmitoylation, a lipid modification that is known to regulate neuronal protein trafficking. We investigated the in vivo significance of BACE1 S-palmitoylation by generating knock-in mice selectively lacking this modification. The lack of BACE1 S-palmitoylation impaired synaptic activity-induced Aβ production, significantly reduced cerebral amyloid burden in AD mouse models, and mitigated cognitive deficits. Using transgenic mouse models, our results demonstrate that intrinsic posttranslational S-palmitoylation of BACE1 has a significant impact on amyloid pathogenesis and the consequent cognitive decline. (See pp. E9665–E9674.)

Dephosphorylation by protein phosphatase 2A regulates visual pigment regeneration and the dark adaptation of mammalian photoreceptors

Alexander V. Kolesnikov, Tivadar Orban, Hui Jin, Celine Brooks, Lukas Hofmann, Zhiqian Dong, Maxim Sokolov, Krzysztof Palczewski, and Vladimir J. Kefalov

Resetting G-protein–coupled receptors (GPCRs) from their active state to their biologically inert ground state driven by reversible phosphorylation and arrestin binding is an integral part of GPCR signaling. Visual pigments in retinal rod and cone photoreceptors represent a classic example of GPCR signaling. Although pigment inactivation by phosphorylation is well understood, the enzyme(s) responsible for pigment dephosphorylation and the functional significance of this reaction remain largely unknown. Here, we show that protein phosphatase 2A (PP2A) is expressed in mouse photoreceptors and that its targeted ablation compromises, but does not fully block, their pigment dephosphorylation, visual chromophore recycling, and dark adaptation after >90% bleach. We conclude that visual pigments are dephosphorylated by PP2A and that this reaction regulates dark adaptation of photoreceptors. (See pp. E9675–E9684.)

Thin myelin sheaths as the hallmark of remyelination persist over time and preserve axon function

Ian D. Duncan, Rachel L. Marik, Aimee T. Broman, and Moones Heidari

The hallmark of remyelination in the CNS has been proposed to be the presence of thin myelin sheaths. This has been demonstrated in multiple experimental models and in multiple sclerosis. It is the only surrogate marker of remyelination, and therefore a crucial fingerprint of myelin repair. However, this has been challenged recently in separate studies, in which, by implication, the degree or presence of remyelinated axons has been underestimated. In this article, we provide evidence from two different models that thin myelin sheaths and short internodes persist almost indefinitely. Future attempts to promote myelin repair in models of multiple sclerosis will be crucially dependent on a definitive marker of repair. We propose that the thin myelin sheath remains the gold standard. (See pp. E9685–E9691.)

Organizing principles for the cerebral cortex network of commissural and association connections

Larry W. Swanson, Joel D. Hahn, and Olaf Sporns

The cerebral cortex supports cognition and is a structure common to all mammals. The major cortical subdivisions (its gray matter regions) are connected by a complex network of axonal connections that includes connections between regions in the same hemisphere (association connections on the right or left side) and those between hemispheres (commissural connections between opposite sides). A database of over 5,000 connections in the cortical network was extracted from the literature, and network analysis revealed three identical cortical modules (neural subsystems) on each side. One appears to deal especially with the external world, one with the viscera, and one with planning, prioritization, and self-awareness. A set of general organizing principles for association and commissural connections also emerged from the analysis. (See pp. E9692–E9701.)

PIP2 mediates functional coupling and pharmacology of neuronal KCNQ channels

Robin Y. Kim, Stephan A. Pless, and Harley T. Kurata

Despite the availability of many drugs to treat epilepsy, nearly one-third of patients are not responsive to pharmacotherapy. Retigabine (RTG) is the first approved antiepileptic drug that acts by promoting activation of potassium channels, specifically targeting neuronal KCNQ channels that are regulated by both voltage and the membrane phospholipid PIP2. A deeper understanding of the mechanism of action of RTG will enable future development of this unique drug class. In this study, we combine electrophysiology recordings with fluorometric measurements of

Downloaded by guest on October 25, 2020

KCNQ channel conformation to reveal channel features that contribute to the dramatic effects of RTG. Our findings demonstrate that a PIP2-dependent interaction between the pore-forming and voltage-sensing components of the channel is required for optimal RTG action. (See pp. E9702–E9711.)

Albendazole and antibiotics synergize to deliver short-course anti-*Wolbachia* curative treatments in preclinical models of filariasis

Joseph D. Turner, Raman Sharma, Ghaith Al Jayoussi, Hayley E. Tyrer, Joanne Gamble, Laura Hayward, Richard S. Priestley, Emma A. Murphy, Jill Davies, David Waterhouse, Darren A. N. Cook, Rachel H. Clare, Andrew Cassidy, Andrew Steven, Kelly L. Johnston, John McCall, Louise Ford, Janet Hemingway, Stephen A. Ward, and Mark J. Taylor

Filarial nematode infections, caused by *Wuchereria bancrofti*, *Brugia malayi* (elephantiasis), and *Onchocerca volvulus* (river blindness) infect 150 million of the world's poorest populations and cause profound disability. Standard treatments require repetitive, long-term, mass drug administrations and have failed to interrupted transmission in certain sub-Saharan African regions. A drug cure using doxycycline, which targets the essential filarial endosymbiont *Wolbachia*, is clinically effective but programmatically challenging to implement due to long treatment durations and contraindications. Here we provide proof-of-concept of a radical improvement of targeting *Wolbachia* via identification of drug synergy between the anthelmintic albendazole and antibiotics. This synergy enables the shortening of treatment duration of macrofilaricidal anti-*Wolbachia* based treatments from 4 wk to 7 d with registered drugs ready for clinical testing. (See pp. E9712–E9721.)

Reciprocal cross-regulation of VND and SND multigene TF families for wood formation in *Populus trichocarpa*

Ying-Chung Jimmy Lin, Hao Chen, Quanzi Li, Wei Li, Jack P. Wang, Rui Shi, Sermsawat Tunlaya-Anukit, Peng Shuai, Zhifeng Wang, Hongyan Ma, Huiyu Li, Ying-Hsuan Sun, Ronald R. Sederoff, and Vincent L. Chiang

Wood is a widely used renewable feedstock for industrial production and energy generation. The secondary cell wall (SCW) is the major component of wood. Two key transcription factor families, Vascular-Related NAC-Domain (VND) and Secondary Wall-Associated NAC Domain (SND), are master gene regulators for SCW biosynthesis. However, plants exhibit stunted growth or abnormal SCW development under excess VND or SND gene expression. In this study, we show that two splice variants, PtrVND6-C1^{IR} and PtrSND1-A2^{IR}, each from VND and SND families, act as negative regulators. We propose that PtrVND6-C1^{IR} and PtrSND1-A2^{IR} function together for reciprocal cross-regulation of VND and SND families to maintain homeostasis for xylem differentiation and plant development. (See pp. E9722–E9729.)

Similarity between soybean and *Arabidopsis* seed methylomes and loss of non-CG methylation does not affect seed development

Jer-Young Lin, Brandon H. Le, Min Chen, Kelli F. Henry, Jungim Hur, Tzung-Fu Hsieh, Pao-Yang Chen, Julie M. Pelletier, Matteo Pellegrini, Robert L. Fischer, John J. Harada, and Robert B. Goldberg

We describe the spatial and temporal profiles of soybean and *Arabidopsis* seed methylomes during development. CHH methylation increases globally from fertilization through dormancy in all seed parts, decreases following germination, and targets primarily transposons. By contrast, CG- and CHG-context methylation remains constant throughout seed development. Mutant seeds lacking non-CG methylation develop normally, but have a set of up-regulated transposon RNAs suggesting that the CHH methylation increase may be a failsafe mechanism to reinforce transposon silencing. Major classes of seed genes have similar methylation profiles, whether they are active or not. Our results suggest that soybean and *Arabidopsis* seed methylomes are similar, and that DNA methylation does not play a significant role in regulating many genes important for seed development. (See pp. E9730–E9739.)

Framework and resource for more than 11,000 gene-transcript-protein-reaction associations in human metabolism

Jae Yong Ryu, Hyun Uk Kim, and Sang Yup Lee

Alternative splicing is a regulatory mechanism by which multiple protein isoforms can be generated from one gene. Despite its biological importance, there has been no systematic approach that facilitates characterizing functional roles of protein isoforms in human metabolism. To this end, we present a systematic framework for the generation of gene-transcript-protein-reaction associations (GeTPRA) in human metabolism. The framework involves a generic human genome-scale metabolic model (GEM) that is an excellent framework to investigate genotype–phenotype associations. We show that a biochemically consistent and transcript-level data-compatible human GEM can be used to generate GeTPRA, which can be deployed to further upgrade the human GEM. Personal GEMs generated with GeTPRA information enabled more accurate simulation of cancer metabolism and prediction of anticancer targets. (See pp. E9740–E9749.)

Downloaded by guest on October 25, 2020