PNAS Plus Significance Statements

Spatiotemporal dynamics of word retrieval in speech production revealed by cortical high-frequency band activity

Stephanie K. Riès, Rummit K. Dhillon, Alex Clarke, David King-Stephens, Kenneth D. Laxer, Peter B. Weber, Rachel A. Kuperman, Kurtis I. Auguste, Peter Brunner, Gerwin Schalk, Jack J. Lin, Josef Parvizi, Nathan E. Crone, Nina F. Dronkers, and Robert T. Knight

Word retrieval is essential to language production, relying on the activation of conceptual and word representations in memory followed by the selection of the correct word. The detailed spatiotemporal cortical dynamics of this core language process are not well-known. By using direct cortical recordings, we show that the activation of concepts or word representations and their selection co-occur in time and engage widespread brain networks and overlapping brain regions. In contrast with modular brain models of language production, our data do not support a clear division of labor between brain regions during these early stages of language production. Rather, we suggest that overlapping brain mechanisms optimize word retrieval. (See pp. E4530–E4538.)

Mechanistic insight into the nucleus–vacuole junction based on the Vac8p–Nvj1p crystal structure

Hanbin Jeong, Jumi Park, Hye-In Kim, Miriam Lee, Young-Joon Ko, Sanghwa Lee, Youngsoo Jun, and Changwook Lee

Organelle contact sites are specialized intracellular zones called membrane contact sites (MCS), in which two distinct suborganelles are closely apposed in eukaryotic cells. The nucleus–vacuole junction (NVJ) is the first identified interorganellar MCS in the budding yeast *Saccharomyces cerevisiae*, and its formation depends on the nuclear membrane protein Nvj1p and vacuolar membrane protein Vac8p. We present the crystal structure of Vac8p–Nvj1p complex at 2.4-Å resolution. Based on the structure, we propose a molecular mechanism in which Vac8p competitively recognizes Nvj1p or Atg13p and present a model showing how Vac8p facilitates NVJ formation, mediates piecemeal microautophagy of the nucleus, and participates in the cytoplasm-to-vacuole targeting pathway. (See pp. E4539–E4548.)

Multiscale model predicts increasing focal adhesion size with decreasing stiffness in fibrous matrices

Xuan Cao, Ehsan Ban, Brendon M. Baker, Yuan Lin, Jason A. Burdick, Christopher S. Chen, and Vivek B. Shenoy

Focal adhesions play crucial roles in mechanotransduction and regulate processes such as spreading, proliferation, differentiation, and motility. It is well known that cells develop larger adhesions when cultured on stiffer elastic hydrogels, but the native extracellular matrix (ECM) is fibrous, nonlinear, and dissipative. We developed a multiscale model showing that adhesion size decreases with increasing stiffness in fibrous matrices, in excellent agreement with our experiments on engineered fibrous matrices. Our model shows that this is due to the feedback between cell contractility and the physical remodeling of ECM, which does not exist in elastic substrates. The basic stiffness–adhesion size principle uncovered can be applied to understand tumor progression fundamentally or to better design biomaterial scaffolds to control cell behavior. (See pp. E4549–E4555.)

Physicochemical code for quinary protein interactions in *Escherichia coli*

Xin Mu, Seongil Choi, Lisa Lang, David Mowray, Nikolay V. Dokholyan, Jens Danielsson, and Mikael Oliveberg

This study shows that the diffusive motions of proteins in live cells are by no means without control but follow simplistic physical–chemical rules that can be quantified and optimized through surface composition. Most strikingly, human proteins are observed to stick to the "foreign" environment of bacterial cells, whereas the bacterial analogue moves around freely. Even so, the human proteins can predictably be transformed to bacterial behavior with a few structurally benign surface mutations, and, conversely, the bacterial protein can be made to stick. The findings have not only fundamental implications for how protein function is controlled at the physical–chemical level but can also be used to adjust protein motion in *Escherichia coli* at will. (See pp. E4556–E4563.)

Angular measurements of the dynein ring reveal a stepping mechanism dependent on a flexible stalk

Lisa G. Lippert, Tali Dadosh, Jodi A. Hadden, Vishakha Karnawat, Benjamin T. Diroll, Christopher B. Murray, Erika L. F. Holzbaur, Klaus Schulten, Samara L. Reck-Peterson, and Yale E. Goldman

Cytoplasmic dynein is a complex intracellular molecular motor primarily responsible for many crucial retrograde transport functions within cells. Its mechanism is distinct from the mechanism of other transport motors and remains poorly understood. This work examines the structural dynamics of actively translocating dynein

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motors in single-molecule detail to reveal a previously unknown role of stalk flexibility in dynein stepping. By integrating these findings with previously published structural information, we present a unified model of the dynein translocation mechanism. A detailed understanding of this mechanism provides insight into how dynein is able to navigate around obstacles in vivo. The experimental techniques presented here may be broadly applicable to the study of rotational motions in other molecular systems. (See pp. E4564–E4573.)

Selective regulation of Notch ligands during angiogenesis is mediated by vimentin

Daniel Antfolk, Marika Sjöqvist, Fang Cheng, Kimmo Isoniemi, Camille L. Duran, Adolfo Rivero-Muller, Christian Antila, Rasmus Niemi, Sebastian Landor, Carlijn V. C. Bouten, Kayla J. Bayless, John E. Eriksson, and Cecilia M. Sahlgren

Angiogenesis, the formation of new blood vessels from existing ones, is essential for embryonic development and necessary for tumor growth and invasion. The Notch signaling pathway regulates angiogenesis where the two Notch ligands, Dll4 and Jagged 1, exert opposite functions on sprouting. We found that the intermediate filament vimentin balances angiogenesis by binding specifically to the proangiogenic Jagged ligands. This binding provides a force-generating mechanism on the Jagged–Notch complex to ensure efficient transendocytosis and Notch activation. The interaction between vimentin and Jagged constitutes a mechanism behind selective regulation of Notch ligands during angiogenesis. The interaction may be amendable by therapeutic intervention and can facilitate strategies targeting a variety of diseases related to Jagged deregulation. (See pp. E4574–E4581.)

Major contribution of the 3/6/7 class of TRPC channels to myocardial ischemia/reperfusion and cellular hypoxia/reoxygenation injuries

Xiju He, Shoutian Li, Benju Liu, Sebastian Susperreguy, Karina Formoso, Jinghong Yao, Jinsong Kang, Anbing Shi, Lutz Birnbaumer, and Yanhong Liao

Calcium overload has been recognized as a critical cause of the injury tissues suffer after periods of ischemia. The ports that determine calcium entry into tissues subjected to transient hypoxia have not been identified. Here we identify two members of the transient potential receptor channel (TRPC) family of nonselective cation channels that allow passage of calcium, TRPC3 and TRPC6, as major factors causing calcium entry in the heart, which is responsible for ischemia/reperfusion (I/R) injury. Blocking TRPC activity or the genetic ablation of TRPCs markedly protected cardiac tissue and cells from I/R injury. TRPC3 and TRPC6 are promising therapeutic targets. (See pp. E4582–E4591.)

Data-driven modeling reveals cell behaviors controlling self-organization during *Myxococcus xanthus* development

Christopher R. Cotter, Heinz-Bernd Schüttler, Oleg A. Igoshin, and Lawrence J. Shimkets

Coordinated cell movement is critical for a broad range of multicellular phenomena, including microbial self-organization, embryogenesis, wound healing, and cancer metastasis. Elucidating how these complex behaviors emerge within cell populations is frequently obscured by randomness in individual cell behavior and the multitude of internal and external factors coordinating cells. This work describes a technique of combining fluorescent cell tracking with computational simulations driven by the tracking data to identify cell behaviors contributing to an emergent phenomenon. Application of this technique to the model social bacterium *Myxococcus xanthus* suggested key aspects of cell coordination during aggregation without complete knowledge of the underlying signaling mechanisms. (See pp. E4592–E4601.)

Integrative modeling of gene and genome evolution roots the archaeal tree of life

Tom A. Williams, Gergely J. Szöllősi, Anja Spang, Peter G. Foster, Sarah E. Heaps, Bastien Boussau, Thijs J. G. Ettema, and T. Martin Embley

The Archaea represent a primary domain of cellular life, play major roles in modern-day biogeochemical cycles, and are central to debates about the origin of eukaryotic cells. However, understanding their origins and evolutionary history is challenging because of the immense time spans involved. Here we apply a new approach that harnesses the information in patterns of gene family evolution to find the root of the archaeal tree and to resolve the metabolism of the earliest archaeal cells. Our approach robustly distinguishes between published rooting hypotheses, suggests that the first Archaea were anaerobes that may have fixed carbon via the Wood–Ljungdahl pathway, and quantifies the cumulative impact of horizontal transfer on archaeal genome evolution. (See pp. E4602–E4611.)

cGAS is essential for cellular senescence

Hui Yang, Hanze Wang, Junyao Ren, Qi Chen, and Zhijian J. Chen

Cellular senescence is important for the maintenance of tissue homeostasis. Dysregulation of senescence is linked to many human diseases, such as cancer, premature aging, and age-related diseases. Although DNA damage response has been linked to senescence, the underlying mechanism is unknown. Here we show that cGAS is essential for the senescence phenotypes, including expression of inflammatory genes. This finding reveals a molecular mechanism of cellular senescence and suggests that modulation of cGAS activity may be a new strategy to treat senescence-associated human diseases that potentially include cancer, neurodegenerative diseases, cardiovascular diseases, and aging. (See pp. E4612–E4620.)

The FERM protein EPB41L5 regulates actomyosin contractility and focal adhesion formation to maintain the kidney filtration barrier

Christoph Schell, Manuel Rogg, Martina Suhm, Martin Helmstädter, Dominik Sellung, Mako Yasuda-Yamahara, Oliver Kretz, Victoria Küttner, Hani Suleiman, Laxmikanth Kollipara, René P. Zahedi, Albert Sickmann, Stefan Eimer, Andrey S. Shaw, Albrecht Kramer-Zucker, Mariko Hirano-Kobayashi, Takaya Abe, Shinichi Aizawa, Florian Grahammer, Björn Hartleben, Jörn Dengjel, and Tobias B. Huber

Loss of podocyte adhesion is a hallmark of glomerular disease progression. Here we unravel the in vivo composition of the podocyte adhesion machinery by the use of quantitative proteomics and identify the FERM domain protein EPB41L5 as a selectively enriched novel podocyte focal adhesion protein. EPB41L5 is essential to maintaining podocyte adhesion in vivo by recruiting the Rho GEF ARHGEF18, initiating a signaling cascade and ultimately resulting in increased actomyosin activity and focal adhesion stabilization. As EPB41L5 is down-regulated in various glomerular pathologies, these findings offer a perspective for interventions aiming to prevent loss of podocytes in glomerular disease. (See pp. E4621–E4630.)

RNA editing of *SLC22A3* drives early tumor invasion and metastasis in familial esophageal cancer

Li Fu, Yan-Ru Qin, Xiao-Yan Ming, Xian-Bo Zuo, Yu-Wen Diao, Li-Yi Zhang, Jiaoyu Ai, Bei-Lei Liu, Tu-Xiong Huang, Ting-Ting Cao, Bin-Bin Tan, Di Xiang, Chui-Mian Zeng, Jing Gong, Qiangfeng Zhang, Sui-Sui Dong, Juan Chen, Haibo Liu, Jian-Lin Wu, Robert Z. Qi, Dan Xie, Li-Dong Wang, and Xin-Yuan Guan

Familial esophageal squamous cell carcinoma (ESCC) often shows early onset and worse prognosis. Little is known about the genetic basis underlying the pathogenesis of familial ESCC. To identify the genetic alterations associated with familial ESCC susceptibility, we compared the gene-expression profiles of familial and sporadic ESCCs. We found that A-to-I RNA editing–mediated downregulation of *SLC22A3* is almost exclusively present in familial ESCC and may serve as a potential biomarker for familial ESCC patients. Molecular mechanism study further revealed that a single mutation at the RNA level could change the protein structure of SLC22A3, leading to a loss of inhibitory capability for the metastasis-promoting protein ACTN4. Our findings provide insights that may lead to more effective clinical management of individuals at high risk of familial ESCC with *SLC22A3* deregulation. (See pp. E4631–E4640.)

Prognostic and biological significance of the proangiogenic factor EGFL7 in acute myeloid leukemia

Dimitrios Papaioannou, Changxian Shen, Deedra Nicolet, Betina McNeil, Marius Bill, Malith Karunasiri, Matthew H. Burke, Hatice Gulcin Ozer, Selen A. Yilmaz, Nina Zitzer, Gregory K. Behbehani, Christopher C. Oakes, Damian J. Steiner, Guido Marcucci, Bayard L. Powell, Jonathan E. Kolitz, Thomas H. Carter, Eunice S. Wang, Krzysztof Mrózek, Carlo M. Croce, Michael A. Caligiuri, Clara D. Bloomfield, Ramiro Garzon, and Adrienne M. Dorrance

In this work we report on the previously uncharacterized clinical and biological role for EGFL7 in acute myeloid leukemia (AML). Patients with increased *EGFL7* mRNA expression had lower complete remission rates and shorter overall and event-free survival, demonstrating the clinical relevance of *EGFL7* expression in cytogenetically normal AML. Our results show that AML blasts are able to synthesize and secrete EGFL7 protein, promoting autocrine blast cell growth. Inhibition of EGFL7 results in decreased proliferation and induces apoptosis of AML cells. Taken together, our data provide the rationale for targeting EGFL7 using blocking antibodies as a therapy for patients with AML. (See pp. E4641–E4647.)

Selective lowering of synapsins induced by oligomeric α-synuclein exacerbates memory deficits

Megan E. Larson, Susan J. Greimel, Fatou Amar, Michael LaCroix, Gabriel Boyle, Mathew A. Sherman, Hallie Schley, Camille Miel, Julie A. Schneider, Rakez Kayed, Fabio Benfenati, Michael K. Lee, David A. Bennett, and Sylvain E. Lesné

Alzheimer's disease (AD) is the most common form of dementia affecting an estimated 5.3 million Americans based on the 2015 Report of the Alzheimer Association. Our current understanding of the pathogenesis of AD suggests that soluble, nonfibrillar forms of amyloid proteins [e.g. amyloid-β, tau, and α-synuclein (αSyn)] may be responsible for impairing cognition and have therefore been advanced to be the most bioactive species in this brain disorder. We sought to determine the potential contribution of αSyn oligomers to AD-associated cognitive decline. We found that selective αSyn oligomers are elevated in AD brains and that genetically elevating oligomeric αSyn in an AD mouse model led to a selective decrease in presynaptic proteins and cognitive performance. (See pp. E4648–E4657.)

Modulation of sensory information processing by a neuroglobin in *Caenorhabditis elegans*

Shigekazu Oda, Yu Toyoshima, and Mario de Bono

Sensory neurons encode environmental stimuli in their electrical activity and alter behavior and physiology by transmitting this information to downstream circuits. Their response properties can be characterized by tuning curves that relate stimulus parameters to neural responses. Tuning curves identify the response threshold, the stimulus features at the tuning curve peak, and high-slope regions that give maximum stimulus discrimination. Here we show that two antagonistically acting molecular oxygen sensors, a neuroglobin and a soluble guanylate cyclase, sculpt a sharp sigmoidal tuning curve in the URX oxygen sensing neurons of *Caenorhabditis elegans*. By combining experiments with computational modelling, we show that these changes in stimulus-encoding properties broaden *C. elegans*'s O_2 preference. (See pp. E4658–E4665.)

Parallel memory traces are built after an experience containing aversive and appetitive components in the crab *Neohelice*

Martín Klappenbach, Ayelén Nally, and Fernando Federico Locatelli

In nature, animals are exposed to complex situations in which actions and stimuli predict appetitive and aversive consequences at the same time. To study memory formation after a learning episode that represents such real-life experience, we trained crabs in a context in which they found food while they were also threatened by a danger stimulus. We found that crabs build separate appetitive and aversive memories that compete during retrieval. Which memory is expressed depends on the strength of the unconditioned stimuli during training but also on the motivational state of the animal during retrieval. The results support that appetitive and aversive memories acquired during experience are independently stored to be used according to particular requirements during retrieval. (See pp. E4666–E4675.)

KEAP1-modifying small molecule reveals muted NRF2 signaling responses in neural stem cells from Huntington's disease patients

Luisa Quinti, Sharadha Dayalan Naidu, Ulrike Träger, Xiqun Chen, Kimberly Kegel-Gleason, David Llères, Colúm Connolly, Vanita Chopra, Cho Low, Sébastien Moniot, Ellen Sapp, Adelaide R. Tousley, Petr Vodicka, Michael J. Van Kanegan, Linda S. Kaltenbach, Lisa A. Crawford, Matthew Fuszard, Maureen Higgins, James R. C. Miller, Ruth E. Farmer, Vijay Potluri, Susanta Samajdar, Lisa Meisel, Ningzhe Zhang, Andrew Snyder, Ross Stein, Steven M. Hersch, Lisa M. Ellerby, Eranthie Weerapana, Michael A. Schwarzschild, Clemens Steegborn, Blair R. Leavitt, Alexei Degterev, Sarah J. Tabrizi, Donald C. Lo, Marian DiFiglia, Leslie M. Thompson, Albena T. Dinkova-Kostova, and Aleksey G. Kazantsev

Chronic neuroinflammation and oxidative stress are likely complicit in driving disease progression in Huntington's disease (HD). Here, we describe the mechanism of action of a unique chemical scaffold that is highly selective for activation of NRF2, the master transcriptional regulator of cellular antiinflammatory and antioxidant defense genes. The use of this scaffold revealed that NRF2 activation responses were muted in HD patient-derived neural stem cells, suggesting increased susceptibility of this critical renewable cell population to oxidative stress in HD brain. However, pharmacological activation of NRF2 was able to repress inflammatory responses in mouse microglia and astrocytes, the principal cellular mediators of neuroinflammation, and in blood monocytes from HD patients. Our results suggest multiple protective benefits of NRF2 activation for HD patients. (See pp. E4676–E4685.)

HDAC1 links early life stress to schizophrenia-like phenotypes

Sanaz Bahari-Javan, Hristo Varbanov, Rashi Halder, Eva Benito, Lalit Kaurani, Susanne Burkhardt, Heike Anderson-Schmidt, Ion Anghelescu, Monika Budde, Roman M. Stilling, Joan Costa, Juan Medina, Detlef E. Dietrich, Christian Figge, Here Folkerts, Katrin Gade, Urs Heilbronner, Manfred Koller, Carsten Konrad, Sara Y. Nussbeck, Harald Scherk, Carsten Spitzer, Sebastian Stierl, Judith Stöckel, Andreas Thiel, Martin von Hagen, Jörg Zimmermann, Antje Zitzelsberger, Sybille Schulz, Andrea Schmitt, Ivana Delalle, Peter Falkai, Thomas G. Schulze, Alexander Dityatev, Farahnaz Sananbenesi, and André Fischer

Early life stress (ELS) is an important risk factor for schizophrenia. Our study shows that ELS in mice increases the levels of histone-deacetylase (HDAC) 1 in brain and blood. Although altered *Hdac1* expression in response to ELS is widespread, increased *Hdac1* levels in the prefrontal cortex are responsible for the development of schizophrenia-like phenotypes. In turn, administration of an HDAC inhibitor ameliorates ELS-induced schizophrenia-like phenotypes. We also show that *Hdac1* levels are increased in the brains of patients with schizophrenia and in blood from patients who suffered from ELS, suggesting that the analysis of *Hdac1* expression in blood could be used for patient stratification and individualized therapy. (See pp. E4686–E4694.)