PNAS Plus Significance Statements

Flow-induced gelation of microfiber suspensions

Antonio Perazzo, Janine K. Nunes, Stefano Guido, and Howard A. Stone

Suspensions of flexible fibers usually behave as shear thinning fluids; that is, their effective viscosity, or resistance to flow, decreases as they are exposed to higher shear stresses. Here we demonstrate that for suspensions created with very-high-aspect-ratio fibers, which are highly flexible, shear thickening behavior of a fiber suspension is obtained. Such a property can be exploited to produce a biocompatible hydrogel by injecting the suspension from a standard needle and syringe without any chemical reactions (unlike a chemically cross-linked hydrogel) or chemical interactions (unlike a traditional physical hydrogel). Once extruded, the hydrogel is a yield-stress material with potentially useful mechanical properties for bioengineering and biomedical applications. (See pp. E8557–E8564.)

Lipidomics reveals diurnal lipid oscillations in human skeletal muscle persisting in cellular myotubes cultured in vitro

Ursula Loizides-Mangold, Laurent Perrin, Bart Vandereycken, James A. Betts, Jean-Philippe Walhin, Iain Templeman, Stéphanie Chanon, Benjamin D. Weger, Christine Durand, Maud Robert, Jonathan Paz Montoya, Marc Moniatte, Leonidas G. Karagounis, Jonathan D. Johnston, Frédéric Gachon, Etienne Lefai, Howard Riezman, and Charna Dibner

Our experiments provide the analysis of lipid metabolite circadian oscillations in a cellular system synchronized in vitro, suggesting cell-autonomous diurnal changes in lipid profiles independent of feeding. Moreover, our work represents a comprehensive comparison between the lipid composition of human skeletal muscle derived from sedentary healthy adults, receiving hourly isocaloric solutions, and human primary skeletal myotubes cultured in vitro. A substantial number of lipid metabolites, in particular membrane lipids, exhibited oscillatory patterns in muscle tissue and in myotube cells, where they were blunted upon cell-autonomous clock disruption. As lipid oscillations in skeletal muscle membrane lipids may impact on insulin signaling and on the development of insulin resistance, studying the temporal lipid composition of human muscle is therefore of utmost importance. (See pp. E8565–E8574.)

A peptide extension dictates IgM assembly

Dzana Pasalic, Benedikt Weber, Chiara Giannone, Tiziana Anelli, Roger Müller, Claudio Fagioli, Manuel Felkl, Christine John, Maria Francesca Mossuto, Christian F. W. Becker, Roberto Sitia, and Johannes Buchner

How protein assemblies with complex topologies are formed is an important question in structural biology. An intriguing example is IgM, a complex of over 1,200 kDa consisting of six antibody subunits (or five in the presence of the J-chain protein). These are arranged in a ring-like structure connected by disulfide bonds. Here, we show that in vitro and in cell culture, a short peptide extension of the IgM heavy chain is sufficient to steer the formation of the hexameric complex. The formation of a disulfide bond triggers conformational changes in the peptide extensions, which involve specific hydrophobic residues. Our study reveals the redox-controlled assembly of a large protein complex via structural rearrangements in a peptide as a design principle. (See pp. E8575–E8584.)

A protean clamp guides membrane targeting of tail-anchored proteins

Un Seng Chio, SangYoon Chung, Shimon Weiss, and Shu-ou Shan

To maintain cellular organization, many chaperones and targeting factors escort nascent proteins to membrane destinations. It was generally thought that substrate proteins preferably bind conformationally closed chaperones. Here, we used single-molecule spectroscopy to study the conformation and dynamics of the ATPase guided entry of tail-anchored protein 3 (Get3), which delivers an essential class of tail-anchored proteins (TAs) to the endoplasmic reticulum. Contrary to previous models, TA-bound Get3 rapidly fluctuates between open and closed conformations, forming a "protean clamp" that stably traps substrates. Biochemical data showed that this dynamic opening primes Get3 for targeting of TAs to membrane receptor sites. Analogous protean traps may operate in other chaperones, targeting factors, and transporters, providing a dual mechanism to both stably retain substrates and vectorially drive cellular processes. (See pp. E8585–E8594.)

TPC2 polymorphisms associated with a hair pigmentation phenotype in humans result in gain of channel function by independent mechanisms

Yu-Kai Chao, Verena Schludi, Cheng-Chang Chen, Elisabeth Butz, O. N. Phuong Nguyen, Martin Müller, Jens Krüger, Claudia Kammerbauer, Manu Ben-Johny, Angelika M. Vollmar, Carola Berking, Martin Biel, Christian A. Wahl-Schott, and Christian Grimm

Polymorphisms in the endolysosomal cation channel TPC2 have been suggested to lead to a shift in human hair color from brown to blond. In two further studies a role for TPC2 in melanosomal pH regulation was

postulated. Electrophysiological data on how these polymorphisms affect channel gating and activity are, however, missing. We show here that both polymorphisms lead to a gain of channel function by different mechanisms. In M484L sensitivity to its endogenous ligand $PI(3,5)P_2$ is strongly increased while in G734E channel inactivation by ATP is reduced. These findings are corroborated by molecular dynamics and ion substitution experiments. Furthermore, >100 blond- and brown/black-haired human individuals were genotyped and fibroblasts isolated from selected donors to confirm key in vitro findings. (See pp. E8595–E8602.)

Miscoding-induced stalling of substrate translocation on the bacterial ribosome

Jose L. Alejo and Scott C. Blanchard

Using single-molecule FRET imaging, we show that programmed base-pair mismatches between the peptidyl-tRNA anticodon and the mRNA codon dramatically prolong elongation factor G (EF-G)–catalyzed translocation. Mismatched peptidyl-tRNA–mRNA pairing within the pretranslocation complex specifically inhibits peptidyl-tRNA engagement of the small subunit P site, a rate-limiting process in translocation characterized by large-scale, intramolecular conformational changes within the EF-G(GDP)–bound ribosome complex. Consistent with the E site being vacant during this period, we find that elongation factor P (EF-P) can rescue this translocation defect. These findings reveal an unexpected relationship between tRNA decoding at the A site and translocation, and suggest an alternative mode of action for miscoding-inducing drugs as well as a novel function of EF-P in the cell to rescue ribosomes stalled by miscoding errors. (See pp. E8603–E8610.)

Molecular origin of the weak susceptibility of kinesin velocity to loads and its relation to the collective behavior of kinesins

Qian Wang, Michael R. Diehl, Biman Jana, Margaret S. Cheung, Anatoly B. Kolomeisky, and José N. Onuchic

Successful functioning of biological systems depends on efficient cellular transport supported by several classes of active biological molecules known as motor proteins. Although they have been intensively studied using various experimental methods, their molecular properties remain not fully understood. We developed a theoretical approach by using structure-based molecular dynamics simulations. It allowed us to understand at the molecular level the effect of external forces on kinesin motor proteins. It is shown that a force-regulated coupling between the neck linker and the ATP binding site of a kinesin accounts for experimentally observed weak susceptibility to loads. Our framework helps us to rationalize the low cooperativity among kinesins. The presented method is a powerful tool in clarifying microscopic features of motor proteins. (See pp. E8611–E8617.)

Cell volume change through water efflux impacts cell stiffness and stem cell fate

Ming Guo, Adrian F. Pegoraro, Angelo Mao, Enhua H. Zhou, Praveen R. Arany, Yulong Han, Dylan T. Burnette, Mikkel H. Jensen, Karen E. Kasza, Jeffrey R. Moore, Frederick C. Mackintosh, Jeffrey J. Fredberg, David J. Mooney, Jennifer Lippincott-Schwartz, and David A. Weitz

Cell volume is thought to be a well-controlled cellular characteristic, increasing as a cell grows, while macromolecular density is maintained. We report that cell volume can also change in response to external physical cues, leading to water influx/efflux, which causes significant changes in subcellular macromolecular density. This is observed when cells spread out on a substrate: Cells reduce their volume and increase their molecular crowding due to an accompanying water efflux. Exploring this phenomenon further, we removed water from mesenchymal stem cells through osmotic pressure and found this was sufficient to alter their differentiation pathway. Based on these results, we suggest cells chart different differentiation and behavioral pathways by sensing/altering their cytoplasmic volume and density through changes in water influx/efflux. (See pp. E8618–E8627.)

Critical role for PI3-kinase in regulating the use of proteins as an amino acid source

Wilhelm Palm, Jingwen Araki, Bryan King, Raymond G. DeMatteo, and Craig B. Thompson

Mammalian cells can take up monomeric amino acids through cell-surface transporters or recover amino acids through macropinocytosis and lysosomal catabolism of extracellular proteins. In mammalian cells, nutrient uptake is regulated by growth factors. Yet how growth-factor signaling orchestrates different nutrient uptake routes is unclear. Here, we establish a central role for growth-factor–activated phosphatidylinositol 3-kinase (PI3-kinase) signaling in promoting cellular amino acid uptake and show that distinct effector branches regulate expression of amino acid transporters, and macropinocytosis and lysosomal catabolism of ingested proteins. Therefore, PI3-kinase signaling supports cell proliferation in the presence of various amino acid sources. These findings suggest that oncogenic PI3-kinase pathway activation is a selective advantage for tumor cells proliferating in fluctuating nutrient environments. (See pp. E8628–E8636.)

Rewiring a Rab regulatory network reveals a possible inhibitory role for the vesicle tether, Uso1

Hua Yuan, Saralin Davis, Susan Ferro-Novick, and Peter Novick

Members of the Rab family act as molecular switches to control distinct stages of vesicular traffic into and out of a eukaryotic cell. Many Rabs are linked to the adjacent Rab by their regulators, generating circuits that direct transport along the pathway. We have rewired one such regulatory circuit by exploiting a chimeric Rab that acts early on the pathway but can be activated by a regulator that is situated late on the pathway. Expression of this chimeric Rab leads to a block late on the pathway. Our findings confirm the importance of Rabs in establishing membrane identity. (See pp. E8637–E8645.)

Speed regulation of genetic cascades allows for evolvability in the body plan specification of insects

Xin Zhu, Heike Rudolf, Lucas Healey, Paul François, Susan J. Brown, Martin Klingler, and Ezzat El-Sherif

How a homogeneous group of cells is partitioned into domains of different identities is a common problem in embryogenesis. Partitioning, in some cases, takes places within a static tissue field and, in other cases, in a progressively growing tissue. A curious case is the partitioning of insect bodies into a head, thorax, and abdomen, which may take place in an elongating or in a non-elongating embryo (short- vs. long-germ insects). Through evolution, the first type of segmentation can easily evolve into the second. In our studies of *Tribolium* segmentation, we elucidated a patterning mechanism based on speed regulation of genetic cascades. The mechanism functions in both elongating and

nonelongating tissues, and could potentially have parallels in other tissues and organisms. (See pp. E8646–E8655.)

Ideal crop plant architecture is mediated by *tassels replace upper ears1*, a BTB/POZ ankyrin repeat gene directly targeted by TEOSINTE BRANCHED1

Zhaobin Dong, Wei Li, Erica Unger-Wallace, Jinliang Yang, Erik Vollbrecht, and George Chuck

Teosinte, the wild ancestor of maize, is a highly branched and low-yielding plant. Branch suppression in maize was achieved through selection for overexpression of the *teosinte branched1* (*tb1*) transcription factor that acts as a repressor of axillary branching. Here, we show a molecular mechanism for how TB1 transformed teosinte into a viable crop plant. The *tassels replace upper ears1* (*tru1*) mutant causes maize to revert back to its highly branched ancestral state, much like *tb1*. We demonstrate that *tru1* is a direct target of TB1 and is overexpressed in modern maize. The genetic mechanism underlying the *tb1* and *tru1* pathway reveals a blueprint for domesticating new grass species. (See pp. E8656–E8664.)

Network of nutrient-sensing pathways and a conserved kinase cascade integrate osmolarity and carbon sensing in *Neurospora crassa*

Lori B. Huberman, Samuel T. Coradetti, and N. Louise Glass

Microbes have evolved complex signaling networks to identify and prioritize utilization of available energy sources. For many fungi, such as *Neurospora crassa*, this entails distinguishing between an array of carbon sources, including insoluble carbohydrates in plant cell walls. Here, we identified a repressor of the cellulose-response pathway in *N. crassa*. Using this derepressed mutant, we implicated the conserved hyperosmotic-response MAP kinase pathway in regulating the response of *N. crassa* to insoluble carbohydrates. We hypothesize that fungal species that degrade plant biomass use osmolarity as a proxy for soluble sugar in the environment to regulate their nutritional responses, enabling tailored production of lignocellulases. This finding could help in battling fungal plant diseases and in the production of second-generation biofuels. (See pp. E8665–E8674.)

Autophagy is required for endothelial cell alignment and atheroprotection under physiological blood flow

Anne-Clemence Vion, Marouane Kheloufi, Adel Hammoutene, Johanne Poisson, Juliette Lasselin, Cecile Devue, Isabelle Pic, Nicolas Dupont, Johanna Busse, Konstantin Stark, Julie Lafaurie-Janvore, Abdul I. Barakat, Xavier Loyer, Michele Souyri, Benoit Viollet, Pierre Julia, Alain Tedgui, Patrice Codogno, Chantal M. Boulanger, and Pierre-Emmanuel Rautou

Atherosclerotic plaques tend to develop preferentially in areas of the vasculature exposed to low and disturbed shear stress (SS), but the mechanisms are not fully understood. In this study, we demonstrate that inefficient autophagy contributes to the development of atherosclerotic plaques in low-SS areas. Defective endothelial autophagy not only curbs endothelial alignment with the direction of blood flow, but also promotes an inflammatory, apoptotic, and senescent phenotype. Furthermore, genetic inactivation of endothelial autophagy in a murine model of atherosclerosis increases plaque burden exclusively in high-SS areas that are normally resistant to atherosclerotic plaque development. Altogether, these findings underline the role of endothelial autophagic flux activation by SS as an atheroprotective mechanism. (See pp. E8675–E8684.)

Cross-activating c-Met/β1 integrin complex drives metastasis and invasive resistance in cancer

Arman Jahangiri, Alan Nguyen, Ankush Chandra, Maxim K. Sidorov, Garima Yagnik, Jonathan Rick, Sung Won Han, William Chen, Patrick M. Flanigan, Dina Schneidman-Duhovny, Smita Mascharak, Michael De Lay, Brandon Imber, Catherine C. Park, Kunio Matsumoto, Kan Lu, Gabriele Bergers, Andrej Sali, William A. Weiss, and Manish K. Aghi

Invasion is a major cause of cancer mortality, as exemplified by metastatic spread of peripheral malignancies or local intracranial invasion of glioblastoma. While individual mediators of invasion are identified, functional or structural interactions between these mediators remain undefined. We identified a structural cross-activating c-Met/β1 integrin complex that promotes breast cancer metastases and invasive resistance of glioblastoma to the antiangiogenic therapy bevacizumab. We show that tumor cells adapt to their microenvironmental stressors by usurping c-Met and β1 integrin, with c-Met displacing α5 integrin from β1 integrin to form a c-Met/β1 complex with far greater fibronectin affinity than α5β1 integrin. These findings challenge conventional thinking about integrin–ligand interactions and define a molecular target for disrupting metastases or invasive oncologic resistance. (See pp. E8685–E8694.)

Mapping allele with resolved carrier status of Robertsonian and reciprocal translocation in human preimplantation embryos

Jiawei Xu, Zhen Zhang, Wenbin Niu, Qingling Yang, Guidong Yao, Senlin Shi, Haixia Jin, Wenyan Song, Lei Chen, Xiangyang Zhang, Yihong Guo, Yingchun Su, Linli Hu, Jun Zhai, Yile Zhang, Fangli Dong, Yumei Gao, Wenhui Li, Shiping Bo, Mintao Hu, Jun Ren, Lei Huang, Sijia Lu, X. Sunney Xie, and Yingpu Sun

In in vitro fertilization, it is difficult, if not impossible, with current methods to determine whether an embryo carries a chromosomal translocation. We have established a method for diagnosing chromosome abnormality named "Mapping Allele with Resolved Carrier Status" (MaReCs), which enables simultaneous screening of chromosomal ploidy and translocation in an embryo by next-generation sequencing. We demonstrate and validate that MaReCs allows accurate selection of translocation-free embryos, preventing the transmission of chromosomal translocations to future generations. (See pp. E8695–E8702.)

Inhibition of EBV-mediated membrane fusion by anti-gHgL antibodies

Karthik Sathiyamoorthy, Jiansen Jiang, Britta S. Möhl, Jia Chen, Z. Hong Zhou, Richard Longnecker, and Theodore S. Jardetzky

Herpesviruses infect a large percentage of the human population and are responsible for a significant human health disease burden. EBV, like other herpesviruses, expresses a set of glycoproteins—gH, gL, and gB—responsible for virus entry into cells, which are targets of protective antibody responses and potential candidates for vaccine development. Here we study the interactions and mechanisms of two anti-EBV antibodies that recognize the gHgL complex, providing a foundation for understanding herpesvirus neutralization. (See pp. E8703–E8710.)

Mycobacterium tuberculosis is protected from NADPH oxidase and LC3-associated phagocytosis by the LCP protein CpsA

Stefan Köster, Sandeep Upadhyay, Pallavi Chandra, Kadamba Papavinasasundaram, Guozhe Yang, Amir Hassan, Steven J. Grigsby, Ekansh Mittal, Heidi S. Park, Victoria Jones, Fong-Fu Hsu, Mary Jackson, Christopher M. Sassetti, and Jennifer A. Philips

Mycobacterium tuberculosis (Mtb), the causative agent of the disease tuberculosis, grows in macrophages, cells that normally

kill bacteria. Recent work has defined a macrophage pathway called "LC3-associated phagocytosis" (LAP) that can eliminate other microbes. LAP is characterized by the recruitment of NADPH oxidase to phagosomes, followed by phagosomal association with LC3 and delivery of the bacteria to a degradative lysosome. Here, we show that LAP does not effectively clear Mtb. The ability of Mtb to inhibit LAP and therefore cause disease depends upon CpsA, a member of the LytR-CpsA-Psr (LCP) protein family, which has previously been implicated in cell-wall metabolism. We demonstrate that Mtb CpsA plays an unexpected role in antagonizing host innate immunity by inhibiting NADPH oxidase and LAP. (See pp. E8711–E8720.)

Catheterization alters bladder ecology to potentiate *Staphylococcus aureus* infection of the urinary tract

Jennifer N. Walker, Ana L. Flores-Mireles, Chloe L. Pinkner, Henry L. Schreiber IV, Matthew S. Joens, Alyssa M. Park, Aaron M. Potretzke, Tyler M. Bauman, Jerome S. Pinkner, James A. J. Fitzpatrick, Alana Desai, Michael G. Caparon, and Scott J. Hultgren

Staphylococcus aureus is a cause of catheter-associated urinary tract infections (CAUTIs). *S. aureus* CAUTIs are problematic because they are usually caused by antibiotic-resistant strains, and patients who develop these infections have a high risk of developing serious complications. Catheterization in humans and mice causes damage in the bladder that results in the release of host protein fibrinogen (Fg). This study suggests that *S. aureus* exploits the presence of Fg via interactions mediated by the Fg-binding protein ClfB to facilitate colonization of the bladder and the catheter to cause a persistent infection in both mice and humans. Insights into *S. aureus* CAUTI pathogenesis is facilitating the development of more-targeted therapies to better treat these infections. (See pp. E8721–E8730.)

Limits of variation, specific infectivity, and genome packaging of massively recoded poliovirus genomesy

Yutong Song, Oleksandr Gorbatsevych, Ying Liu, JoAnn Mugavero, Sam H. Shen, Charles B. Ward, Emmanuel Asare, Ping Jiang, Aniko V. Paul, Steffen Mueller, and Eckard Wimmer

We constructed viable poliovirus (PV) variants carrying up to 2,104 synonymous mutations in the ORF. We studied proliferation phenotypes, particularly the specific infectivity (*si*) that defines the probability of a single virion to initiate a single cell infection. A recent hypothesis proposes that dozens of loosely conserved hairpins, formed within the viral genome, determine assembly specificity of +ssRNA viruses. Our analysis of recoded PVs does not support this hypothesis. We propose that the progression of +ssRNA virus assembly follows two steps. (*i*) Specificity: recognition between either (*a*) replication protein and capsid precursor or (*b*) single RNA packaging signal and cognate capsid precursor. (*ii*) Cocondensation, occurring between single genome/single capsid precursor complex and multiple cognate capsids aided by multiple genome-specific hairpins. (See pp. E8731–E8740.)

Substance P induces plasticity and synaptic tagging/capture in rat hippocampal area CA2

Ananya Dasgupta, Nimmi Baby, Kumar Krishna, Muhammad Hakim, Yuk Peng Wong, Thomas Behnisch, Tuck Wah Soong, and Sreedharan Sajikumar

The hippocampal area *Cornu Ammonis* (CA) CA2 is a small region interposed between CA1 and CA3. For a long time, there has been a lack of information on the CA2 area's role in memory formation. This area is innervated by supramammillary axonal fibers that are rich with Substance P (SP), which acts as a neurotransmitter and neuromodulator. We show that SP induces an NMDA receptor- and protein synthesis-dependent potentiation of CA2 synapses that requires kinases such as CaMKIV and PKMζ. The SP-induced effects on Schaffer collateral-CA2 synapses transform entorhinal cortical-CA2 short-term potentiation into long-term potentiation, thereby expressing synaptic tagging and capture, an associative property of neuronal populations that engage in consolidation. (See pp. E8741–E8749.)

Nucleus accumbens feedforward inhibition circuit promotes cocaine self-administration

Jun Yu, Yijin Yan, King-Lun Li, Yao Wang, Yanhua H. Huang, Nathaniel N. Urban, Eric J. Nestler, Oliver M. Schlüter, and Yan Dong

The nucleus accumbens (NAc) regulates cue-induced motivated behaviors, a process that requires fine-tuned functional output of medium spiny principal neurons (MSNs). Our present study reveals that, although contributing <1% of the NAc shell (NAcSh) neuronal population, fast-spiking interneurons (FSIs) form an inhibitory feedforward circuit to dictate the functional output of NAc MSNs. Cocaine self-administration, a rodent model mimicking human cocaine seeking and taking, persistently potentiates the driving force to this FSI-embedded feedforward circuit. Functionally mimicking this effect of cocaine in vivo expedites the acquisition of cocaine self-administration. These results demonstrate a previously unidentified role of an FSI-mediated NAcSh feedforward circuit in cue-induced cocaine seeking and taking. (See pp. E8750–E8759.)

Proteasome-independent polyubiquitin linkage regulates synapse scaffolding, efficacy, and plasticity

Qi Ma, Hongyu Ruan, Lisheng Peng, Mingjie Zhang, Michaela U. Gack, and Wei-Dong Yao

A well-investigated mechanism regulating synapse protein turnover and remodeling is the classical ubiquitin–proteasome system by which polyubiquitin (polyUb) chains linked through ubiquitin lysine 48 (K48) tag substrates for proteasomal degradation. Little is known about the role of nondegradable polyUb linkages, e.g., K63-linked chains, in the synapse, although these chains represent the second most abundant polyUb linkage in mammalian brain. We identified a bona fide K63-polyUb substrate in PSD-95, a major postsynaptic scaffold. K63-polyUb conjugation markedly augments PSD-95's scaffolding capability and promotes its synaptic targeting and anchorage. We identify a K63-linkage–specific E3 ligase–deubiquitinase complex that controls activity-dependent assembly and disassembly of K63-polyUb chains on PSD-95. We show that this unconventional polyUb linkage promotes synapse maturation, efficacy, and plasticity. (See pp. E8760–E8769.)

Simultaneous two-photon imaging of intracellular chloride concentration and pH in mouse pyramidal neurons in vivo

Sebastian Sulis Sato, Pietro Artoni, Silvia Landi, Olga Cozzolino, Riccardo Parra, Enrico Pracucci, Francesco Trovato, Joanna Szczurkowska, Stefano Luin, Daniele Arosio, Fabio Beltram, Laura Cancedda, Kai Kaila, and Gian Michele Ratto

The control of intracellular Cl^- and pH plays a crucial role in several neuronal functions, and the study of these processes would be helped by tools for their noninvasive optical measurement in vivo. In this study, we have performed combined measurements of Cl^- and pH of individual pyramidal neurons by means of in vivo

two-photon imaging, and we provide direct experimental demonstration for the presence of the postnatal developmental shift to lower intraneuronal Cl^-. Moreover, we introduce an approach for dynamic and simultaneous monitoring of intraneuronal Cl^- and pH in vivo. These methods will open a window for the study of the roles of intraneuronal pH and Cl^- in neuronal signaling, plasticity, and disease. (See pp. E8770–E8779.)

Temporal calcium profiling of specific circadian neurons in freely moving flies

Fang Guo (郭方), Xiao Chen (陈霄), and Michael Rosbash

Monitoring neuronal activity in freely moving, behaving animals is a holy grail of neuroscience. Here we present a noninvasive tool that links the calcium profiles of specific fly neurons to real-time behavior. Optogenetic manipulation of two groups of circadian neurons indicates that they drive sleep or locomotor activity. The two calcium patterns are also distinct and couple well to the sleep/activity phases of fly behavior. The sleep-promoting neurons appear more active when the flies initiate daytime sleep, whereas the activity-promoting neurons appear to fire more strongly coincident with the evening locomotor activity peak. This new approach is complementary to electrophysiological recording and GCaMP imaging, especially for small organisms and behavorial paradigms for which these more traditional methods are not practical. (See pp. E8780–E8787.)

RIPK1 mediates a disease-associated microglial response in Alzheimer's disease

Dimitry Ofengeim, Sonia Mazzitelli, Yasushi Ito, Judy Park DeWitt, Lauren Mifflin, Chengyu Zou, Sudeshna Das, Xian Adiconis, Hongbo Chen, Hong Zhu, Michelle A. Kelliher, Joshua Z. Levin, and Junying Yuan

Dysfunction of microglia plays a fundamental role in the pathogenesis of Alzheimer's disease (AD), the most common form of dementia. However, there is a lack of knowledge about targets that can be safely manipulated for modulating microglia for the treatment of AD. The presence of a unique subtype of disease-associated microglia (DAM) has recently been implicated in mediating pathogenesis of AD. However, the mechanism that promotes the development of DAM is unclear, nor is it known how DAM may modulate the progression of AD. This study demonstrates that RIPK1-dependent transcription promotes DAM and lysosomal defects to mediate the accumulation of amyloid plaques in AD. Thus, targeting RIPK1 may provide an important therapeutic strategy for the treatment of AD. (See pp. E8788–E8797.)