PNAS Plus Significance Statements

Measuring dynamic cell–material interactions and remodeling during 3D human mesenchymal stem cell migration in hydrogels

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Scaffolds that serve as synthetic mimics of the extracellular matrix have applications in wound healing, tissue engineering, and stem cell expansion. When cells are cultured in these tunable matrices, little is known about local microenvironmental changes during degradation and remodeling. Methods that provide quantitative and predictable information about cell-mediated remodeling could significantly improve the biomaterial design process. We use passive microrheology, a technique that measures rheological properties from Brownian motion of embedded particles, to characterize remodeling of a cell-laden peptide-functionalized poly(ethylene glycol) hydrogel that degrades in response to cell-secreted enzymes. Results show microenvironmental changes at multiple time and size scales, and reveal an interesting degradation gradient, as mesenchymal stem cells attach, spread, and move through these synthetic extracellular matrix mimics. (See pp. E3757–E3764.)

Adolescent impatience decreases with increased frontostriatal connectivity

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Compared with children and adults, teens and young adults often exhibit greater impulsivity and corresponding increases in emergency room visits, accidents from drug or alcohol use, and increased mortality risk. However, it remains poorly understood how increased impulsivity during adolescence may be explained in terms of brain and cognitive development. We focused on impatience, a central component of impulsiveness. We relate impatient behavior on a decision-making task to changes in connectivity within the brain's frontostriatal circuitry. Our results suggest that relative future orientation, not sensitivity to immediate rewards, determines adolescent impatience. These findings may help to design interventions to prevent the detrimental effects of adolescent impulsiveness and serve as a template for understanding neurodevelopmental disorders. (See pp. E3765–E3774.)

Drivers for the renaissance of coal

Jan Christoph Steckel, Ottmar Edenhofer, and Michael Jakob

The current carbonization of the global energy system poses a severe challenge for efforts to reduce carbon emissions. Here we show that the increase in the carbon intensity of energy production is caused mainly by the increased use of coal, not only in China and India but also across a broad range of developing countries, especially poor, fast-growing countries mainly in Asia. The (relatively) low coal prices are an important reason countries choose coal to satisfy their energy needs. This result underlines the importance of cheaply available energy for economic growth and suggests that viable alternatives to cheap coal will be required to ensure the participation of developing countries in global climate change mitigation. (See pp. E3775–E3781.)

Optimized deep-targeted proteotranscriptomic profiling reveals unexplored *Conus* toxin diversity and novel cysteine frameworks

Vincent Lavergne, Ivon Harliwong, Alun Jones, David Miller, Ryan J. Taft, and Paul F. Alewood

Venomous marine cone snails have evolved complex mixtures of fast-acting paralytic cysteine-rich peptides for prey capture and defense able to modulate specific heterologous membrane receptors, ion channels, or transporters. In contrast to earlier studies in which the richness and sequence hypervariability of lowly expressed toxins were overlooked, we now describe a comprehensive deep-targeted proteotranscriptomic approach that provides, to our knowledge, the first high-definition snapshot of the toxin arsenal of a venomous animal, *Conus episcopatus*. The thousands of newly identified conotoxins include peptides with cysteine motifs present in FDA-approved molecules or currently undergoing clinical trials. Further highlights include novel cysteine scaffolds likely to unveil unique protein structure and pharmacology, as well as a new category of conotoxins with odd numbers of cysteine residues. (See pp. E3782–E3791.)

Structure and mechanism of the ATPase that powers viral genome packaging

Brendan J. Hilbert, Janelle A. Hayes, Nicholas P. Stone, Caroline M. Duffy, Banumathi Sankaran, and Brian A. Kelch

Many viruses use a molecular motor to pump DNA into a preformed protein shell called the capsid, a process that is essential for the formation of infectious virus particles. The ATPase machine powering this process is the strongest known biological motor. However, the structure and mechanism of this motor are unknown. Here, we derive a structural model of the ATPase assembly using a combination of X-ray crystallography, small-angle X-ray scattering, molecular modeling, and biochemical data. We identify residues critical for ATP hydrolysis and DNA binding, and derive a mechanistic model for the translocation of DNA into the viral capsid. Our studies introduce a model for ATPase assembly and illustrate how DNA is pumped with high force. (See pp. E3792–E3799.)

Potent organo-osmium compound shifts metabolism in epithelial ovarian cancer cells

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Platinum-based metallodrugs are the most widely used anticancer agents. Their reduced effectiveness after repeat dosing (resistance) constitutes a major clinical problem. We study a potent organo-osmium compound with improved activity over cisplatin and no cross-resistance in platinum-resistant cancers. This compound disrupts metabolism in A2780 human ovarian cancer cells, generating reactive oxygen species and damaging DNA. We identified mutations in complex I of the electron transport chain in A2780 cells and suggest that the osmium compound may exploit these mutations to exert a potent mechanism of action. Such activity increases selectivity toward cancer cells, given that normal-functioning cells can better adapt to drug-induced metabolic perturbations. Therefore, this report highlights a promising strategy to drive the future development of organometallic anticancer compounds. (See pp. E3800–E3805.)

Skip residues modulate the structural properties of the myosin rod and guide thick filament assembly

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Myosins are cellular motors that promote muscle contraction by converting chemical energy into mechanical force. The myosin molecule self-assembles through its coiled-coil rod domain into the highly ordered thick filaments of the sarcomeres, which represent the basic contractile unit of the muscle. Because there is limited information about the mechanisms of filament formation, and mutations in the rod domain cause muscle disease, we investigated the molecular properties and function of four regions of the rod containing an extra amino acid (skip residue) predicted to alter the regular organization of the coiled-coil. To our knowledge, this is the first study reporting that these regions fold into specialized structures engaged in promoting proper myosin assembly into the thick filaments. (See pp. E3806–E3815.)

Sex hormone-dependent tRNA halves enhance cell proliferation in breast and prostate cancers

Shozo Honda, Phillipe Loher, Megumi Shigematsu, Juan P. Palazzo, Ryusuke Suzuki, Issei Imoto, Isidore Rigoutsos, and Yohei Kirino

Although transfer RNAs (tRNAs) are best known as adapter molecules essential for translation, recent biochemical and computational evidence has led to a previously unexpected conceptual consensus that tRNAs are not always end products but can further serve as a source of small functional RNAs. Here we report that a novel type of tRNA-derived small RNA, termed SHOT-RNAs, are specifically and abundantly expressed in sex hormone-dependent breast and prostate cancers. SHOT-RNAs are produced from aminoacylated mature tRNAs by angiogenin-mediated cleavage of the anticodon loop, which is promoted by sex hormones and their receptors. We identified the complete repertoire of SHOT-RNAs, and also found their functional significance in cell proliferation. These results have unveiled a novel tRNA-engaged pathway in tumorigenesis. (See pp. E3816–E3825.)

Biased Brownian motion as a mechanism to facilitate nanometer-scale exploration of the microtubule plus end by a kinesin-8

Yongdae Shin, Yaqing Du, Scott E. Collier, Melanie D. Ohi, Matthew J. Lang, and Ryoma Ohi

The cellular distributions of kinesins are defined in part by their intrinsic biophysical properties. The well-characterized kinesin-8s, for example, translocate exceptionally long distances on a microtubule track, concentrating them at plus ends of long, stable microtubules. Kif18B, a little-studied kinesin-8, targets the plus ends of fast-growing, short-lived microtubules by binding the plus-end tracking protein EB1. Whether ultraprocessivity is conserved among kinesin-8s is thus unclear. Here, we show that Kif18B is not ultraprocessive and that the motor switches frequently between diffusive and directed modes of motility. Our work identifies properties of Kif18B that may have optimized the motor to explore the ∼1-μm domain of microtubule plus ends and show that biophysical motor properties cannot be generalized within any one kinesin subfamily. (See pp. E3826–E3835.)

Jagged mediates differences in normal and tumor angiogenesis by affecting tip-stalk fate decision

Marcelo Boareto, Mohit Kumar Jolly, Eshel Ben-Jacob, and José N. Onuchic

Developing effective antiangiogenesis strategies remains clinically challenging. Unlike physiological angiogenesis, pathological angiogenesis comprises of many microvessels that do not fully mature or develop functionally, because the cell fate decision about which endothelial cells become the tip and lead the following stalk cells is dysregulated. We devised a specific theoretical framework to decipher the cross-talk between two crucial players of the decision-making process of tip and stalk cell fate: VEGF and Notch-Delta-Jagged signaling. We find that high expression of Jagged, but not Delta, can destabilize the terminal differentiation into tip or stalk cells and give rise to a hybrid tip/stalk phenotype, a phenotype that can transform physiological into pathological angiogenesis. Our results offer insights into why tumor-stroma communication often implicates Jagged. (See pp. E3836–E3844.)

Novel protein Callipygian defines the back of migrating cells

Kristen F. Swaney, Jane Borleis, Pablo A. Iglesias, and Peter N. Devreotes

Though the asymmetric distribution of proteins is a crucial first step in establishing polarity and guiding cell migration, the molecular mechanisms regulating many of these localizations are unknown. Our study reports on the novel protein Callipygian (CynA), which localizes to the rear of cells during symmetry breaking, thereby promoting polarity and increasing migration efficiency. Our data indicate that CynA localization is mediated by two distinct mechanisms, which may be important for segregating proteins in other

polarized cell types including epithelial cells, neurons, and immune cells. Thus, our findings have implications for tissue formation during embryonic development, the migration of immune cells during wound healing and infection, and the aberrant migrations associated with arthritis, asthma, atherosclerosis, cancer metastasis, and other diseases. (See pp. E3845–E3854.)

EGFR inhibition evokes innate drug resistance in lung cancer cells by preventing Akt activity and thus inactivating Ets-1 function

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Lung cancer is the leading cause of cancer death worldwide. About 10% harbor mutations in epidermal growth factor receptor (EGFR). Despite remarkable progress in treatment with EGFR inhibitors, only 5% of patients achieve tumor reduction >90%, even though all of those treated have EGFR mutations. Our study addressed this discrepant response by investigating the mechanism of innate drug resistance, i.e. resistance inherent in the tumor cells even before treatment begins and not acquired over its course. Because overcoming innate resistance will increase the primary response, our findings may provide an opportunity to develop new therapies to reduce the probability of emergent resistance to EGFR inhibitors. (See pp. E3855–E3863.)

The RNA-binding protein LIN28B regulates developmental timing in the mammalian cochlea

Erin J. Golden, Ana Benito-Gonzalez, and Angelika Doetzlhofer

The stereotyped cellular organization found within the mammalian auditory epithelium is key to its proper function. Differentiation of this structure occurs under strict spatial and temporal regulation to ensure that proper patterning is achieved. Unlike other neuronal structures (e.g. the retina and cortex), where terminal mitosis and differentiation are linked, these processes remain distinctly separated within the developing auditory epithelium. How coordination is achieved remains largely unknown. Here we show that the RNA-binding protein LIN28B times auditory prosensory cell cycle withdrawal and differentiation through both *let-7*–dependent and *let-7*–independent mechanisms. Additionally, we show that manipulation of the LIN28B/*let-7* axis alters the capacity for postnatal production of sensory hair cells (HC) in the absence of Notch signaling, revealing this axis as a potential candidate for future HC regeneration therapies. (See pp. E3864–E3873.)

Reanalysis of parabiosis of obesity mutants in the age of leptin

Wenwen Zeng, Yi-Hsueh Lu, Jonah Lee, and Jeffrey M. Friedman

As a central hormone in metabolism, leptin functions to suppress food intake and to dissipate energy. Mutations of leptin or its receptor result in profound obesity. Data from classic parabiosis experiments (a procedure to surgically connect the vascular systems of two mice together) by Douglas Coleman over 40 years ago and recent studies of leptin treatment, have suggested that leptin might require a cofactor to exert its full metabolic strength. However, we found that a leptin-binding protein, clusterin, is dispensable for the normal function of leptin. Rather, the parabiosis procedure itself appears to potentiate the metabolic action of leptin, leading to chronic starvation and lethality of the mice. Our results have resolved a long-standing puzzle in leptin biology. (See pp. E3874–E3882.)

Long noncoding RNA derived from CD244 signaling epigenetically controls $CD8^+$ T-cell immune responses in tuberculosis infection

Yang Wang, Huiling Zhong, Xiaodan Xie, Crystal Y. Chen, Dan Huang, Ling Shen, Hui Zhang, Zheng W. Chen, and Gucheng Zeng

Tuberculosis (TB) infection induces up-regulation of T cell-inhibitory molecules on $CD8^+$ T cells, which may induce impairment of $CD8^+$ T-cell immunity. However, how T cell-inhibitory molecules regulate $CD8^+$ T-cell immune responses during TB infection remains unclear. Here, we demonstrate that CD244, a T cell-inhibitory molecule, mediates inhibition of IFN-γ and TNF-α expression through inducing expression of a long noncoding RNA (lncRNA)-CD244. lncRNA-CD244 physically interacts with a chromatin-modification enzyme, enhancer of zeste homolog 2 (EZH2), and mediates modification of a more repressive chromatin state in *infg* and *tnfa* loci. Knock down of lncRNA-CD244 significantly enhances IFN-γ and TNF-α expression and improves protective immunity of $CD8^+$ T cells. This study therefore uncovers a previously unknown mechanism for T-cell immune responses regulated by lncRNA during TB infection. (See pp. E3883–E3892.)

PDGFB-based stem cell gene therapy increases bone strength in the mouse

Wanqiu Chen, David J. Baylink, Justin Brier-Jones, Amanda Neises, Jason B. Kiroyan, Charles H. Rundle, Kin-Hing William Lau, and Xiao-Bing Zhang

Osteoporosis is a morbid disease afflicting millions of people worldwide. To unlock the unique regenerative powers of the skeleton that have not yet been exploited, we used stem cell gene therapy to dramatically increase bone formation at sites where bone is lost during osteoporosis. Our therapy tremendously increased de novo trabecular bone formation and trabecular connections, resulting in a large increase in bone strength. Our therapy has clinical potential, may serve as a prototype for future skeletal stem cell gene therapies, and is a model for mechanistic studies of de novo trabecular bone formation. (See pp. E3893–E3900.)

Dissociation of HSV gL from gH by αvβ6- or αvβ8-integrin promotes gH activation and virus entry

Tatiana Gianni, Raffaele Massaro, and Gabriella Campadelli-Fiume

Entry of enveloped viruses into the cell requires the activation of viral glycoproteins, often mediated by cellular receptors. Herpesviruses infect cells via a multipartite system, which includes species-specific glycoproteins plus conserved apparatus gH/gL and gB. HSV makes use of αvβ6- or αvβ8-integrins as gH/gL receptors. The interaction of HSV gH/gL with integrins resulted in the dissociation of gL. The dissociation took place if all the actors of the entry apparatus were present, i.e., under conditions that lead to glycoprotein

activation and virus entry. We propose that (*i*) gL is a regulator of gH and prevents its activation until integrins promote gL dissociation from gH/gL. (*ii*) Dissociation from an inhibitory regulator represents a previously unidentified mechanism of activation of viral fusion glycoproteins. (See pp. E3901–E3910.)

Distinct roles for GABA across multiple timescales in mammalian circadian timekeeping

Daniel DeWoskin, Jihwan Myung, Mino D. C. Belle, Hugh D. Piggins, Toru Takumi, and Daniel B. Forger

Each day, over 50 billion synaptic signals, mediated by the neurotransmitter GABA, are sent between neurons in the central circadian pacemaker in the mammalian brain to time and coordinate daily events. Although GABA is the only signaling molecule sent and received by most, if not all of these neurons, its role is not well understood. Past studies have shown paradoxically that GABA can synchronize and desynchronize, as well as excite and inhibit, clock neurons. Through experiments and modeling characterizing the role of GABA in timekeeping, we propose the existence of two types of differentially regulated GABA signaling—fast signaling that regulates neuronal output, and slow signaling that modulates synchrony between neurons—a hypothesis that can explain many previous experimental results. (See pp. E3911–E3919.)

GABA-mediated repulsive coupling between circadian clock neurons in the SCN encodes seasonal time

Jihwan Myung, Sungho Hong, Daniel DeWoskin, Erik De Schutter, Daniel B. Forger, and Toru Takumi

How animals track the seasons has long been a mystery. We found a mechanism that explains how day length is encoded within the neuronal network of suprachiasmatic nucleus (SCN). Using an integrated approach combining experiments and modeling, we find evidence for changes in the coupling in the SCN that divides the clock oscillations into two clusters as a function of day length. We show that asymmetric distribution of intracellular chloride across the SCN causes this coupling change. Blocking GABA or chloride import erases the oscillator organization formed by day-length entrainment. These demonstrate that coupling through GABA is a key ingredient of day-length encoding in the SCN. (See pp. E3920–E3929.)

Insular neural system controls decision-making in healthy and methamphetamine-treated rats

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Patients with addiction have a greater tendency to engage in risk-taking behavior. However, the neural substrates responsible for these deficits remain unknown. Here we demonstrated that chronic methamphetamine-treated rats preferred high-risk/high-reward actions and assigned higher value to high returns, indicative of altered decision-making. Pharmacological studies revealed that the insular neural system controls decision-making in both healthy and methamphetamine-treated rats. We further confirmed the role of the insular cortex in decision-making using designer receptor exclusively activated by designer drug technology. Because decision-making is a cognitive process that influences many aspects of daily living and both mental and physical health, the findings of this study have broader implications. (See pp. E3930–E3939.)

Behavioral consequences of selective damage to frontal pole and posterior cingulate cortices

Farshad A. Mansouri, Mark J. Buckley, Majid Mahboubi, and Keiji Tanaka

Frontal pole cortex (FPC) refers to the most anterior part of prefrontal cortex, a region that is highly developed in anthropoid primates. However, because of technical difficulties in studying this area, its role in primate cognition had remained largely unknown. We studied effects of selective bilateral lesions within FPC on monkeys' cognitive flexibility. FPC lesion did not impair the performance in well-learned cognitively demanding tasks. However, FPC-lesioned monkeys remained more focused than control monkeys in exploiting the current task when they faced newly introduced interruptions by a simple secondary task or free rewards. This unique pattern of behavioral changes in FPC-lesioned monkeys suggests that FPC is involved in redistribution of cognitive resources from the current task to novel opportunities. (See pp. E3940–E3949.)

Spectrum of power laws for curved hand movements

Dongsung Huh and Terrence J. Sejnowski

In curved hand movements around ellipses, the speed tends to scale inversely with the curvature with a power law having an exponent of −1/3. We examined whether this well-known regularity in motor planning holds for more general shapes. Using an optimality principle, we identified a set of basis shapes, each with a single characteristic angular frequency, which subjects drew with power laws whose exponents ranged from 0 to −2/3. More general movements exhibited linear mixtures of power laws. The speed of arbitrary doodling movements with a broad spectrum of frequencies could also be predicted from the curvature with high accuracy. (See pp. E3950–E3958.)

Conserved regulatory mechanism controls the development of cells with rooting functions in land plants

Thomas Ho Yuen Tam, Bruno Catarino, and Liam Dolan

This work describes the discovery of an ancient genetic mechanism that was used to build rooting systems when plants colonized the relatively dry continental surfaces >470 million years ago. We demonstrate that a group of basic helix–loop–helix transcription factors—the LOTUS JAPONICUS ROOTHAIRLESS1-LIKE proteins—is part of a conserved auxin-regulated gene network that controls the development of tip-growing cells with rooting functions among extant land plants. This result suggests that this mechanism was active in the common ancestor of most land plants and facilitated the development of early land plant filamentous rooting systems, crucial for the successful colonization of the land by plants. (See pp. E3959–E3968.)