PNAS Plus Significance Statements

A network perspective reveals decreasing material diversity in studies on nanoparticle interactions with dissolved organic matter

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Understanding the environmental fate of engineered nanoparticles (ENPs) is essential to the risk assessment of nanotechnology. Dissolved organic matter (DOM) strongly influences the behavior of ENPs in aqueous systems. This influence depends on many factors and, in particular, the types of ENPs and DOM. Accordingly, recent reviews have called repeatedly to expand the range of DOM and ENPs studied. Here, we use a network perspective to assess, in terms of the material types studied, the current state of research into DOM–ENP interactions. We identify global tendencies in the choice of materials and experimental approaches that have contributed to an undesirable discrepancy between the ever-increasing number of published experiments and the persisting call for investigation of more material types. (See pp. E1756–E1765.)

Monkeys choose as if maximizing utility compatible with basic principles of revealed preference theory

Alexandre Pastor-Bernier, Charles R. Plott, and Wolfram Schultz

Revealed preference theory scrutinizes utility maximization based on tradeoffs between goods. This notion concerns the transition from biological rewards (necessary for survival) to tradable economic goods (beneficial for welfare and evolutionary fitness). However, these assumptions have never been tested empirically in species closely related to humans, as would be necessary to infer a general biological mechanism. In this experiment, rhesus monkeys repeatedly chose between bundles of two goods. Their choice frequencies conformed to curves of equal choice frequency (indifference curves) and satisfied crucial consistency and axiomatic tests involving out-of-sample prediction from modeled indifference curves, transitivity, and axiomatic change of option set size. In satisfying stringent theoretical criteria, the data suggest the existence of well-structured preferences consistent with utility maximization. (See pp. E1766–E1775.)

Social–ecological network analysis of scale mismatches in estuary watershed restoration

Jesse S. Sayles and Jacopo A. Baggio

Spatial misalignments between governance and environmental systems, often called spatial scale mismatch, are a key sustainability challenge. Collaboration and coordination networks can help overcome scale mismatch problems and should align with the environmental system. Using an approach based on network science, this paper advances scale mismatch analysis by explicitly considering collaborations among local and regional organizations doing estuary watershed restoration (i.e., multilevel governance) and how these collaborations align with environmental patterns. Collaboration quality is considered to inform network-based theories for natural resource governance. Integrating network analysis results with ecological habitat data further provides a social– environmental restoration planning perspective. This research can help policymakers allocate resources and is a fundamental step toward addressing scale mismatch while considering multilevel governance. (See pp. E1776–E1785.)

Conformational dynamics of a neurotransmitter:sodium symporter in a lipid bilayer

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Most studies of neurotransmitter:sodium symporter (NSS) function and dynamics have been carried out in detergent even though the activity of these integral membrane proteins is heavily modulated by surrounding lipids. Here, we reconstituted the prokaryotic homolog LeuT into nanodiscs and subjected the preparation to hydrogen–deuterium exchange mass spectrometry to reveal a global view of the hallmarks of the transporter in two disparate conformations. The data were interpreted with the aid of molecular dynamics simulations, allowing unprecedented atomic-level insights into the dynamics of an unmodified, unlabeled NSS in a native-like lipid bilayer environment. (See pp. E1786–E1795.)

Heart failure drug changes the mechanoenzymology of the cardiac myosin powerstroke

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Heart failure is the leading cause of mortality in the United States, despite sustained efforts to develop effective small-molecule treatments. The biophysical

characterization of existing therapies will drive development of next-generation approaches for treating heart failure. Furthermore, small molecules can be powerful probes for dissecting protein structure function relationships. We used an innovative FRET-based spectroscopic approach to determine that the small-molecule heart disease therapeutic omecamtiv mecarbil (OM) changes how myosin's working powerstroke is coordinated with actin-activated phosphate release, the biochemical step associated with force generation. This result explains how OM alters cardiac contractility at the molecular level, forcing the accumulation of actin-bound prepowerstroke cross-bridges. (See pp. E1796–E1804.)

Crystal structure of *Aquifex aeolicus* σ^N bound to promoter DNA and the structure of σ^N-holoenzyme

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The bacterial σ factors confer promoter specificity to the RNA polymerase (RNAP). One σ factor, σ^N, is unique in its structure and functional mechanism, forming transcriptionally inactive promoter complexes with RNAP that require activation by specialized ATPases. The structural basis for σ^N function is of great interest but poorly understood. Here, we determined an X-ray crystal structure of a σ^N fragment bound to promoter DNA, revealing the molecular details of promoter recognition by σ^N. Moreover, the new structure allowed us to build and refine a corrected σ^N-holoenzyme (σ^N/RNAP complex) model using previously published X-ray data. This work overall provides a solid structural framework with which to address further the poorly understood mechanism of activator function in ATP hydrolysis-dependent promoter opening. (See pp. E1805–E1814.)

Structural basis of jasmonate-amido synthetase FIN219 in complex with glutathione S-transferase FIP1 during the JA signal regulation

Chun-Yen Chen, Sih-Syun Ho, Tzu-Yen Kuo, Hsu-Liang Hsieh, and Yi-Sheng Cheng

Far-red (FR) insensitive 219 (FIN219) is the main jasmonate (JA)-amido synthetase that activates the systemic synthesis of bioactive JAs in *Arabidopsis*. FIN219 is involved in FR light signaling and interacts with another signaling component, FIN219-interacting protein 1 (FIP1). To extend our understanding of the regulatory mechanism between FR light signaling and the JA response, we determine the crystal structures of the FIN219–FIP1 complex with substrates and show that interaction with FIP1 triggers enhanced activity of FIN219. FIN219 conformational changes driven by FIP1 are observed in the C-terminal domain and show a relatively occluded form of the active site. By measuring the FIN219–FIP1 interaction and adenylation function, this study reveals that FIP1 may regulate FIN219 activity and further alters the level of JA signaling. (See pp. E1815–E1824.)

Inhibition of α9α10 nicotinic acetylcholine receptors prevents chemotherapy-induced neuropathic pain

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This study addresses the need to phase out opioids as the major analgesic drugs for moderate to severe chronic pain. We establish that a highly selective and potent inhibitor of the α9α10 nicotinic acetylcholine receptor (nAChR) subtype prevents the expression of chemotherapy-induced neuropathic pain. Thus, selective antagonists of the α9α10 nAChR are potential leads for nonopioid analgesic drug development. The effects of inhibitors of the α9α10 receptor, together with genetic studies, suggest a key role for the α9α10 nAChR subtype in an intercellular signaling network that can be activated by diverse insults (e.g., chemotherapy, nerve injury, and diabetes). (See pp. E1825–E1832.)

Integrated view of internal friction in unfolded proteins from single-molecule FRET, contact quenching, theory, and simulations

Andrea Soranno, Andrea Holla, Fabian Dingfelder, Daniel Nettels, Dmitrii E. Makarov, and Benjamin Schuler

The dynamics of proteins, which are essential for both folding and function, are known to be strongly dependent on solvent viscosity and friction. However, an increasing number of experiments have demonstrated the importance of a contribution to protein dynamics independent of solvent friction. Such "internal friction" has recently been detected even in unfolded proteins, although they are more expanded and solvent-accessible than folded proteins. Based on two complementary experimental methods, simulations, and theory, our results provide a coherent view of internal friction in unfolded proteins and constitute an important basis for understanding the molecular origin of this phenomenon and its role for the folding of proteins and for the functional dynamics of intrinsically disordered proteins. (See pp. E1833–E1839.)

Identification of productive and futile encounters in an electron transfer protein complex

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Paramagnetic NMR spectroscopy is exquisitely sensitive for sparsely populated states in protein–protein interactions, and thus, it can provide important information on how protein–protein complexes form and evolve toward their productive state. However, the description of ensembles of protein–protein orientations is nontrivial, and great care must be taken when deriving biologically relevant results. We have applied an algorithm that restricts the conformational space sampled by the two partners to the maximum allowed for by the data. These ensembles can then be reduced assuming the principle of scarcity. We found that some states are linked to the main state through electrostatic pathways. Such paths help to identify those minor states that are able to evolve into the productive complex. (See pp. E1840–E1847.)

Cryo-EM structure of the replisome reveals multiple interactions coordinating DNA synthesis

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The antiparallel nature of the two strands in duplex DNA poses a topological problem for their simultaneous synthesis. The "trombone" model of the replication fork postulates that the lagging-strand forms a loop such that the leading- and lagging-strand replication proteins contact one another. The replisome then can move in one direction along the DNA while synthesizing both strands. Physical interactions between the replication proteins and DNA coordinate processive synthesis

of the leading and lagging strands. Here, we present the structure of a functional replisome from bacteriophage T7. Our structural and biochemical analyses provide an explanation of the mechanisms governing coordination of leading- and lagging-strand synthesis. (See pp. E1848–E1856.)

Mapping of voltage sensor positions in resting and inactivated mammalian sodium channels by LRET

Tomoya Kubota, Thomas Durek, Bobo Dang, Rocio K. Finol-Urdaneta, David J. Craik, Stephen B. H. Kent, Robert J. French, Francisco Bezanilla, and Ana M. Correa

Physical activities of our body and extremities are achieved by the propagation of electrical signals called action potentials from our brain, through nerves, to skeletal muscles. Voltage-gated sodium channel (Navs) play essential roles in the generation and propagation of action potentials in such excitable cells. Although mammalian Nav function has been studied comprehensively, the precise structural basis for the gating mechanisms has not been fully clarified. In this study, we have used lanthanide-based resonance energy transfer to obtain dynamic structural information in mammalian Nav gating. Our data define a geometrical map of Navs with the bound toxins and reveal voltage-induced structural changes related to channel gating, which lead us further toward an understanding of the gating mechanism in mammalian Navs. (See pp. E1857–E1865.)

Nondestructive nanostraw intracellular sampling for longitudinal cell monitoring

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Cell content analysis has rapidly become one of the most important new tools for measuring cell phenotype and behavior. However, the central limitation of current sampling technologies is they are destructive and must lyse the cells to measure the contents. This destruction prevents knowledge of prior or future states of the cell, which is particularly important for dynamic cell processes, such as development and differentiation. Here, we show a nondestructive longitudinal sampling and analysis platform that can sample repeatedly and accurately from the same single cell or group of cells over a long time period. We demonstrate sampling of both proteins and mRNA for cell lines as well as human-derived cardiomyocytes and astrocytes. (See pp. E1866–E1874.)

Imaging mRNA and protein interactions within neurons

Carolina Eliscovich, Shailesh M. Shenoy, and Robert H. Singer

Gene expression is regulated by interactions between mRNAs and RNA-binding proteins. This functionality depends on their ability to specifically recognize and bind RNAs within the cell. Thus, elucidation of RNA–protein interactions is an area of active research. Technological developments allowed the study of these associations in living cells by ensemble biochemistry approaches. However, these traditional methods to investigate RNA–protein association may report adventitious associations and importantly, lack information about cellular spatial information. In this work, we provide an approach that can integrate information from biochemical interactions with visualization of these physical contacts within neurons. (See pp. E1875–E1884.)

Intragenic CpG islands play important roles in bivalent chromatin assembly of developmental genes

Sun-Min Lee, Jungwoo Lee, Kyung-Min Noh, Won-Young Choi, Sejin Jeon, Goo Taeg Oh, Jeongsil Kim-Ha, Yoonhee Jin, Seung-Woo Cho, and Young-Joon Kim

The decision-making process of cellular phenotype specification is controlled by the interplay between genetic and epigenetic elements. Intragenic CGIs (iCGIs) associated with developmental regulators have sequence features that favor DNA methylation and bivalent histone modification, i.e., both activating histone H3 lysine 4 trimethylation and repressing H3K27me3 marks. The epigenetic transition from bivalent modification to DNA methylation on iCGIs during differentiation results in cell type-specific activation of their associated genes. This process is accompanied by loss of physical interactions with promoter regions, and the motifs of developmental regulators are enriched at iCGIs, indicating involvement of these regulators in the epigenetic transition. Our work uncovers the role of iCGIs in cell type-specific differentiation. (See pp. E1885–E1894.)

Transcriptome-wide microRNA and target dynamics in the fat body during the gonadotrophic cycle of *Aedes aegypti*

Xiufeng Zhang, Emre Aksoy, Thomas Girke, Alexander S. Raikhel, and Fedor V. Karginov

A potential avenue to control the spread of mosquito disease vectors lies in reproductive events that follow a blood meal. A key component is the massive production of yolk proteins in the fat body tissue, governed by regulatory networks triggered by the available nutrients. MicroRNAs play a critical role in mosquito egg maturation, and deciphering their dynamics and targets is necessary to fully realize these regulatory processes. We carried out a tissue-specific and time-resolved characterization of microRNA expression in the *Aedes aegypti* fat body and integrated these results with transcriptome-wide determination of their mRNA targets, followed by validation. This extensive analysis lays the groundwork for a systemic understanding of the gene regulation that underpins reproductive events in the female mosquito. (See pp. E1895–E1903.)

Specificity of genome evolution in experimental populations of *Escherichia coli* evolved at different temperatures

Daniel E. Deatherage, Jamie L. Kepner, Albert F. Bennett, Richard E. Lenski, and Jeffrey E. Barrick

Organisms evolve and adapt via changes in their genomes that improve survival and reproduction in the context of their environment. Few experiments have examined how these genomic signatures of adaptation, which may favor mutations in certain genes or molecular pathways, vary across a set of similar environments that have both shared and distinctive characteristics. We sequenced complete genomes from 30 *Escherichia coli* lineages that evolved for 2,000 generations in one of five environments that differed only in the temperatures they experienced. Particular "signature" genes acquired mutations in these bacteria in response to selection imposed by specific temperature treatments. Thus, it is sometimes possible to predict aspects of the environment recently experienced by microbial populations from changes in their genome sequences. (See pp. E1904–E1912.)

Clustered brachiopod Hox genes are not expressed collinearly and are associated with lophotrochozoan novelties

Sabrina M. Schiemann, José M. Martín-Durán, Aina Børve, Bruno C. Vellutini, Yale J. Passamaneck, and Andreas Hejnol

Hox genes pattern the anteroposterior axis of all animals that have left and right body sides. In many animals, Hox genes are clustered along the chromosomes and expressed in spatial and temporal order. This coordinated regulation is thought to have preserved the cluster through a developmental constraint. Our study of the genomic organization and the embryonic spatial and temporal expression of Hox genes in sessile marine animals called lampshells (brachiopods) shows that along with having a broken Hox cluster, they lack both temporal and spatial collinearity. Furthermore, we present molecular evidence that the hard tissues (chaetae and shells) of segmented worms, mollusks, and brachiopods share a common origin that dates back to the Early Cambrian. (See pp. E1913–E1922.)

PEMapper and PECaller provide a simplified approach to whole-genome sequencing

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PEMapper and PECaller are paired software programs that simplify mapping and variant calling for whole-genome datasets. Whole-genome sequencing data are fast becoming the most natural dataset for all genetic studies. Analysis tools for data at this scale are essential. This manuscript describes tools, which solve the challenges of data analysis at whole-genome scale, using an approach involving 16-mer mapping and SNP calling based on a Pólya–Eggenberger distribution for SNP genotypes. We show that our software package is faster (cheaper to run), uses much less disk space (cheaper to store results), requires no previous knowledge of existing genetic variation (easier to deploy to nonhuman species), and achieves calling results that are as good as Genome Analysis Toolkit best practices. (See pp. E1923–E1932.)

Clinical, genetic, and structural basis of congenital adrenal hyperplasia due to 11β-hydroxylase deficiency

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Congenital adrenal hyperplasia resulting from mutations in the *CYP11B1* gene, which encodes a steroidogenic enzyme 11β-hydroxylase, is a rare inherited disorder associated with hyperandrogenemia, short stature, hypertension, and virilization of female newborns. We present a comprehensive clinical, genetic, and hormonal characterization for 68 of 108 patients with a genotype from an International Consortium on Rare Steroid Disorders. We also use computational modeling to define the effect of each of the missense mutations on the structure of 11β-hydroxylase, information that can be used to predict clinical severity prenatally in high-risk mothers. (See pp. E1933–E1940.)

Systemic delivery of factor IX messenger RNA for protein replacement therapy

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Abnormal gene expression is the underlying cause for many pathological states, and restoring normalcy through overexpression or knockdown is a conceptually simple solution. Despite the advantages over DNA and viral vectors, RNA-based therapeutics have been plagued by problems of poor translatability, stability, and adverse immune reactions. Efficient in vivo delivery has also been challenging because currently available lipid nanoparticles (LNPs) can induce liver damage and elicit a strong immune response. In this study, we demonstrate the successful use of LUNAR—a safe, reproducible, and effective LNP mRNA delivery platform that can be used to treat diseases requiring protein replacement. We achieve therapeutic delivery of mRNAs in a preclinical model of hemophilia and demonstrate alleviation of disease symptoms. (See pp. E1941–E1950.)

Activation of contact-dependent antibacterial tRNase toxins by translation elongation factors

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Contact-dependent growth inhibition (CDI) systems enable cells to bind competing bacteria and deliver toxins that cleave nucleic acids or form membrane pores. Here, we characterize a CDI toxin that specifically cleaves transfer RNA (tRNA), thereby blocking protein synthesis and inhibiting bacterial growth. Remarkably, two highly conserved and essential translation factors, EF-Ts and EF-Tu, are critical for this toxic nuclease activity. The toxin binds EF-Tu with high affinity and only cleaves tRNA in complex with the translation factor. EF-Ts appears to increase the rate of tRNA turnover. The activities of two other distinct CDI toxins are also regulated by EF-Ts. We propose that the regulation of toxin activity by the protein synthesis apparatus may play a role in intercellular communication. (See pp. E1951–E1957.)

A signal sequence suppressor mutant that stabilizes an assembled state of the twin arginine translocase

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The twin-arginine translocation (Tat) system transports folded proteins across the prokaryotic inner membrane and the thylakoid membrane of plant chloroplasts. Proteins are targeted to the Tat system by signal peptides containing a highly conserved twin arginine motif. We isolated suppressors in the TatB component that allowed a Tat substrate with a defective twin arginine motif to be transported. The strongest of these suppressors, TatB F13Y, resulted in the constitutive assembly of the Tat translocase in the absence of signal peptide binding. These results show that Tat signal peptides have two separable roles: they target their passenger proteins to the Tat machinery but they also trigger the assembly of the active Tat transporter. (See pp. E1958–E1967.)

Serum-borne bioactivity caused by pulmonary multiwalled carbon nanotubes induces neuroinflammation via blood–brain barrier impairment

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Inhaled particulates, such as multiwalled carbon nanotubes, can induce neuroinflammatory outcomes. The present study shows that acute neuroinflammation is dependent on the impairment of blood-brain barrier function. Pharmacologic restoration of blood-brain barrier integrity prevented the neuroinflammatory responses to pulmonary multiwalled carbon nanotube exposure. Circulating factors, including possibly thrombospondin-1, recapitulate inflammatory responses in cultured cerebrovascular endothelial cells, suggesting a mechanism for indirect systemic effects of inhaled nanoparticles. (See pp. E1968–E1976.)

Synchronous spikes are necessary but not sufficient for a synchrony code in populations of spiking neurons

Jan Grewe, Alexandra Kruscha, Benjamin Lindner, and Jan Benda

Populations of sensory neurons convey information about the outside world to the brain. Postsynaptic neurons may read out their total activity or, alternatively, by focusing only on synchronous activity, they might extract specific features from the same sensory information. But does synchronous activity always encode special features of the stimulus? This question was experimentally addressed in in vivo recordings from two closely related populations of electrosensory neurons of a weakly electric fish. Despite having similar amounts of synchronous activity, only in one population of neurons did synchronous spikes carry specific information about the stimulus. A detailed spectral analysis reveals that too low levels of intrinsic noise paired with too little frequency locking of the neural oscillator destroys a synchrony code. (See pp. E1977–E1985.)

Distance-dependent gradient in NMDAR-driven spine calcium signals along tapering dendrites

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Neurons in the brain extend long dendrites that are packed with small protrusions, or spines, responsible for receiving information from presynaptic axons. Although we know much about the workings of spines, we know little about the large-scale distribution of the properties of these compartments along dendritic trees. Here, we provide a map of the structure and function of spines along dendrites. We find that synapses are not randomly distributed, but show a gradual decrease in size with distance along a dendrite, which is matched by an increase in the amplitude of neurotransmitter-evoked calcium signals. This distance-dependent gradient in calcium signals will have important implications for neuronal integration of synaptic information and for the rules behind the calcium-driven plasticity of spines. (See pp. E1986–E1995.)

Targeting human Mas-related G protein-coupled receptor X1 to inhibit persistent pain

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Chronic pain is a major health and economic problem worldwide with an estimated prevalence reaching epidemic levels of >25% of the population. Most drugs on the market for chronic pain have undesired side effects because their targets exist both inside and outside the pain pathways. Human Mas-related G protein-coupled receptor X1 (MRGPRX1) is a promising target of novel pain inhibitors, mainly because of its restricted expression in primary nociceptive neurons. Our humanized mouse model expressing MRGPRX1 in native nociceptive neurons allowed us to examine physiological roles of MRGPRX1 and to develop therapeutic agents for pain treatment in patients. Our studies suggest that both agonists and positive allosteric modulators of MRGPRX1 may be promising novel drug candidates for managing persistent pain conditions. (See pp. E1996–E2005.)

Secretagogin-dependent matrix metalloprotease-2 release from neurons regulates neuroblast migration

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Persisting proliferative capacity and regeneration in the adult brain are confined to a minority of regions. In the murine brain, the olfactory system is supplied by thousands of newly born neuroblasts daily to support sensory plasticity. Here, we reveal a neuronal scaffold that resides within and alongside this migratory route [termed the "rostral migratory stream" (RMS)] to guide forward migration of newly born neuroblasts. These neurons can externalize the enzyme matrix metalloprotease-2 to loosen the extracellular matrix, thus producing permissive corridors for migrating neuroblasts. This mechanism is likely phylogenetically conserved because it exists in the RMS equivalent in human fetal brains. This inducible mechanism might be pharmacologically targeted for therapeutic benefit. (See pp. E2006–E2015.)

A higher-order theory of emotional consciousness

Joseph E. LeDoux and Richard Brown

Although emotions, or feelings, are the most significant events in our lives, there has been relatively little contact between theories of emotion and emerging theories of consciousness in cognitive science. In this paper we challenge the conventional view, which argues that emotions are innately programmed in subcortical circuits, and propose instead that emotions are higher-order states instantiated in cortical circuits. What differs in emotional and nonemotional experiences, we argue, is not that one originates subcortically and the other cortically, but instead the kinds of inputs processed by the cortical network.

We offer modifications of higher-order theory, a leading theory of consciousness, to allow higher-order theory to account for self-awareness, and then extend this model to account for conscious emotional experiences. (See pp. E2016–E2025.)

Selective autophagy limits cauliflower mosaic virus infection by NBR1-mediated targeting of viral capsid protein and particles

Anders Hafrén, Jean-Luc Macia, Andrew J. Love, Joel J. Milner, Martin Drucker, and Daniel Hofius

Autophagy contributes to innate immune responses in metazoans by targeted elimination of intracellular pathogens, including viruses, in a process termed "xenophagy." Whether autophagy has a similar role in plant immunity is unknown. Here we demonstrate that the selective autophagy receptor NEIGHBOR OF BRCA1 (NBR1) binds the viral capsid protein and particles of cauliflower mosaic virus (CaMV) and mediates their autophagic degradation. We further demonstrate that this antiviral xenophagy is counteracted by protective functions of autophagy-resistant CaMV inclusion bodies. Finally, we show that a second, nonselective NBR1-independent autophagy pathway promotes plant viability during infection and serves as a proviral mechanism to extend the timespan for virus production and potential CaMV transmission. Thus autophagy exhibits important pro- and antiviral roles in compatible plant–virus interactions. (See pp. E2026–E2035.)

Two tonoplast MATE proteins function as turgor-regulating chloride channels in *Arabidopsis*

Haiwen Zhang, Fu-Geng Zhao, Ren-Jie Tang, Yuexuan Yu, Jiali Song, Yuan Wang, Legong Li, and Sheng Luan

Turgor pressure is the driving force for cell growth in plants, and the large central vacuole provides the major space for turgor regulation. However, the molecular identity and function of many transporters that control water and solute fluxes in and out of vacuoles remain unknown. We report here that two Multidrug and Toxic Compound Extrusion (MATE)-type transporters show previously unrecognized function as chloride channels essential for turgor regulation in *Arabidopsis*. The MATE transporters are highly conserved from bacteria, fungi, plants, to animals, and widely accepted as transporters of organic compounds. This study, showing some MATE transporters as chloride channels, thus breaks the old dogma on the functional definition of this large family of transporters. (See pp. E2036–E2045.)

Multiple functional self-association interfaces in plant TIR domains

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Toll/interleukin-1 receptor/resistance protein (TIR) domains are present in plant and animal innate immunity receptors and appear to play a scaffold function in defense signaling. In both systems, self-association of TIR domains is crucial for their function. In plants, the TIR domain is associated with intracellular immunity receptors, known as nucleotide-binding oligomerization domain-like receptors (NLRs). Previous studies from several plant NLRs have identified two distinct interfaces that are required for TIR:TIR dimerization in different NLRs. We show that the two interfaces previously identified are both important for self-association and defense signaling of multiple TIR–NLR proteins. Collectively, this work suggests that there is a common mechanism of TIR domain self-association in signaling across the TIR–NLR class of receptor proteins. (See pp. E2046–E2052.)

TIR-only protein RBA1 recognizes a pathogen effector to regulate cell death in *Arabidopsis*

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Multicellular organisms must have complex immune systems to detect and defeat pathogens. Plants rely on nucleotide binding site leucine rich repeat (NLR) intracellular receptors to detect pathogens. For hundreds of years, plant breeders have selected for disease-resistance traits derived from NLR genes. Despite the molecular cloning of the first NLRs more than 20 y ago, we still do not understand how these sensors function at a mechanistic level. Here, we identified a truncated NLR protein that activates cell death in response to a specific pathogen effector. Understanding how truncated NLRs function will provide a better mechanistic understanding of the plant immune system and an expanded toolkit with which to engineer disease resistance rationally in crops. (See pp. E2053–E2062.)