PNAS Plus Significance Statements

Vibrational coherence transfer in the ultrafast intersystem crossing of a diplatinum complex in solution

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The observation of vibrational wave packets allows tracking the pathways of energy relaxation in nonadiabatic surface crossing events of (bio)molecular systems, such as conical intersections or charge transfer processes. Here, we identify transfer of vibrational coherence in the course of conversion between electronic excited states of different spin in a diplatinum complex in solution, as an example. Retention of coherence is due to the fact that the conversion rate is dramatically accelerated in an acetonitrile solvent due to the strong solvation of higher-lying electronic states that then provide the channel for the coherent population flow from the singlet to the triplet state. These results highlight the role of the environment in controlling the pathways of energy flow and conversion. (See pp. E6396–E6403.)

Kinetically guided radical-based synthesis of $C(sp^3)$–$C(sp^3)$ linkages on DNA

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Combinatorial synthesis via DNA encoded library (DEL) has evolved as a technology of great importance in drug discovery. However, the idiosyncratic aqueous, dilute, DNA-sensitive parameters and infinitesimal scale of this system present new challenges for traditional organic reactions. A detailed protocol aiding the transition from organic reactions to reactions with DNA-bound molecules was developed using a tactical combination of kinetic analysis and reaction screening. As an example, the venerable Giese addition was applied to forge high-value C–C bonds, including all-carbon quaternary centers, on DNA, representing the first radical-based synthesis in DEL that expands the traditional toolbox beyond pericyclic, carbonyl-based, and two-electron cross-couplings. (See pp. E6404–E6410.)

Learning atoms for materials discovery

Quan Zhou, Peizhe Tang, Shenxiu Liu, Jinbo Pan, Qimin Yan, and Shou-Cheng Zhang

Motivated by the recent achievements of artificial intelligence (AI) in linguistics, we design AI to learn properties of atoms from materials data on its own. Our work realizes knowledge representation of atoms via computers and could serve as a foundational step toward materials discovery and design fully based on machine learning. (See pp. E6411–E6417.)

Developmental prosopagnosics have widespread selectivity reductions across category-selective visual cortex

Guo Jiahui, Hua Yang, and Bradley Duchaine

People with developmental prosopagnosia (DP) have extremely poor face recognition and even have problems recognizing the faces of family and close friends. We carried out a comprehensive investigation of the neural basis of DP by comparing brain responses to multiple visual categories in DPs and people with normal face processing. The DPs showed widespread abnormalities in areas specialized for face processing and areas that respond preferentially to scenes and bodies. The abnormalities in scene and body areas indicate cortical problems in many DPs extend beyond face areas and open the door to investigations of developmental disorders impacting recognition of categories other than faces. (See pp. E6418–E6427.)

Unusual duplication mutation in a surface loop of human transthyretin leads to an aggressive drug-resistant amyloid disease

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We identified a one-of-a-kind duplication mutation in human transthyretin (TTR) that causes unusually aggressive systemic amyloidosis. To understand the poor response to treatment with a drug that stabilizes the TTR tetramer, we explored the structure, stability, and drug binding of recombinant proteins. The results suggested that amyloid formation could stem from global destabilization of the monomeric and tetrameric protein as well as the local disordering near the mutation site. This disordering induced proteolysis with release of aggregation-prone fragments. Alternatively, local disordering could trigger misfolding of the full-length protein by exposing an adhesive segment. Drug binding at a dimer interface distant from the mutation site did not significantly influence these pathological processes, indicating the need for alternative therapeutic targets. (See pp. E6428–E6436.)

Gene expression distribution deconvolution in single-cell RNA sequencing

Jingshu Wang, Mo Huang, Eduardo Torre, Hannah Dueck, Sydney Shaffer, John Murray, Arjun Raj, Mingyao Li, and Nancy R. Zhang

We developed deconvolution of single-cell expression distribution (DESCEND), a method to recover cross-cell distribution of the true gene expression level from observed counts in single-cell RNA sequencing, allowing adjustment of known confounding cell-level factors. With the recovered distribution, DESCEND provides reliable estimates of distribution-based measurements, such as the dispersion of true gene expression and the probability that true gene expression is positive. This is important, as with better estimates of these measurements, DESCEND clarifies and improves many downstream analyses including finding differentially expressed genes, identifying cell types, and selecting differentiation markers. Another contribution is that we verified using nine public datasets a simple "Poisson-alpha" noise model for the technical noise of unique molecular identifier-based single-cell RNA-sequencing data, clarifying the current intense debate on this issue. (See pp. E6437–E6446.)

Reversible inhibition of the ClpP protease via an N-terminal conformational switch

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ClpP is a protease that degrades damaged or misfolded proteins. Consistent with its critical role in maintaining cellular homeostasis, inhibiting and dysregulating ClpP function has shown promise in fighting antibiotic resistance and in targeting cancer cells in acute myeloid leukemia. Here we identify a conformational switch in ClpP that, upon mutagenesis, leads to a catalytically inactive structure that can be reactivated through the binding of small-molecule activators. This functional hotspot therefore represents a drug target for allosteric inhibition of ClpP. The combination of methyl–transverse relaxation-optimized spectroscopy (TROSY) NMR, cryo-EM, and molecular simulation methods employed here provides a detailed characterization of ClpP along with the promise of crucial insights into the structure–function relationship of molecular machines in general. (See pp. E6447–E6456.)

Structural basis for recognition of human 7SK long noncoding RNA by the La-related protein Larp7

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The 7SK ribonucleoprotein (RNP) complex regulates the activity of the kinase positive transcription elongation factor b (P-TEFb), an essential activator of RNA Polymerase II transcription. The human La-related protein group 7 (hLarp7) protein is an essential and constitutively assembled component of the 7SK RNP and is required for 7SK RNA stability and P-TEFb recruitment. We report the structure of the hLarp7 C-terminal RNA recognition motif bound to the 7SK stem-loop 4, revealing a unique binding interface. From this and other available structures, we generate a structural model of hLarp7 bound to the 7SK 3′ end. This work provides seminal insights into the unique recognition of 7SK RNA by hLarp7 and a working model for how hLarp7 assembles with 7SK to form the 7SK RNP. (See pp. E6457–E6466.)

Targeting β1-integrin inhibits vascular leakage in endotoxemia

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Compromised vascular integrity is associated with capillary leakage in sepsis, but effective therapies stabilizing the vasculature are lacking. Here, we show that targeting β1-integrin in vivo with inhibitory antibodies or deletion of a single allele of endothelial β1-integrin inhibits lipopolysaccharide (LPS)-induced vascular leakage in murine endotoxemia. The inflammatory agents IL-1β, thrombin, and LPS induced changes in endothelial cell–extracellular matrix (ECM) adhesion via β1-integrin, angiopoietin-2, and the adapter protein tensin-1, leading to increased endothelial cell contractility and permeability. These results indicate that β1-integrin actively promotes vascular leakage and that targeting β1-integrin signaling could be a novel means of achieving vascular stabilization in pathological vascular leak. (See pp. E6467–E6476.)

PIP30/FAM192A is a novel regulator of the nuclear proteasome activator PA28γ

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The 20S proteasome is a key actor of the control of protein levels and integrity in cells. To perform its multiple functions, it works with a series of regulators, among which is a nuclear complex called PA28γ. In particular, PA28γ participates in the regulation of cell proliferation and nuclear dynamics. We describe here the characterization of a protein, PIP30/FAM192A, which binds tightly to PA28γ and favors its interaction with the 20S proteasome while inhibiting its association with coilin, a central component of nuclear Cajal bodies. Thus, PIP30/FAM192A critically controls the interactome and, consequently, the functions of PA28γ, and appears to be a previously unidentified player in the fine regulation of intracellular proteostasis in the cell nucleus. (See pp. E6477–E6486.)

F-actin homeostasis through transcriptional regulation and proteasome-mediated proteolysis

Masayuki Onishi, Kresti Pecani, Taylor Jones IV, John R. Pringle, and Frederick R. Cross

Cytoskeletal actin microfilaments have roles in cell-shape determination, motility, membrane trafficking, and cell division. Actin filaments respond dynamically to environmental changes and shifting cellular needs, and functionally different actin subtypes may play important roles in such responses. The alga *Chlamydomonas* has two actins: IDA5, an actin of conventional sequence that is expressed in normal growing cells, and NAP1, a divergent actin that is normally not expressed. Disruption of IDA5 filaments results in rapid transcriptional induction of *NAP1* and hundreds of other genes, rapidly replacing all IDA5 filaments with NAP1 filaments, in part by proteasome-mediated degradation of IDA5. This system allows resistance of *Chlamydomonas* to actin-depolymerizing drugs and probably also compensates for other, diverse actin cytoskeletal perturbations, whether intrinsic or induced. (See pp. E6487–E6496.)

Phosphatases control PKA-dependent functional microdomains at the outer mitochondrial membrane

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The selective phosphorylation of spatially distinct PKA targets is key for the pleiotropy of the cAMP cascade. This characteristic of the pathway is currently attributed to the ability of phosphodiesterases or adenylate cyclases to create subcellular sites (microdomains) where the concentration of cAMP is distinct from that of the surrounding areas. The role of phosphatases in this process has not been tested. Here we show that limited access of phosphatases to the PKA targets present at the outer mitochondrial membrane generates distinct microdomains of PKA phosphorylated proteins despite there being no differences in the local cAMP levels. These results describe an alternative mechanism capable of generating functional cAMP/PKA-dependent microdomains and may be extrapolated to the compartmentalization of other kinase-dependent events. (See pp. E6497–E6506.)

Whole-genome data reveal the complex history of a diverse ecological community

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Widespread biological communities are common, but little is known about how they assemble. A key question is how sets of trophically linked species (predators and their prey, hosts and parasites) spread to occupy current distributions. Do they disperse together, preserving ecological interactions, or separately, such that interactions are interrupted? This is central to assessing the potential for coevolution in a system and requires inference of species associations both over space and through time. Here, we use de novo genomic data and likelihood-based approaches to infer the assembly history of a multispecies community of Western Palearctic insect herbivores and parasitoid natural enemies—the two trophic groups that together comprise 50% of all animal species. (See pp. E6507–E6515.)

Real-time dynamics of mutagenesis reveal the chronology of DNA repair and damage tolerance responses in single cells

Stephan Uphoff

A central goal in genetics is to understand how mutation rates are regulated by the genome maintenance system in response to DNA damage or drug treatments. This has been challenging because existing mutation assays only show time and population averages of mutation rates and do not resolve the underlying molecular processes. Toward this goal, I utilized a microscopy-based method which enables relating the creation of DNA mismatches to single-cell gene expression dynamics in real time. I show that DNA alkylation damage causes a distinct pulse of mutagenesis that is shaped by the chronology of constitutive and inducible DNA repair and damage tolerance pathways. Stochastic fluctuations in the expression of these pathways modulated the dynamics of mutagenesis in single *Escherichia coli* cells. (See pp. E6516–E6525.)

Evolutionary genomic dynamics of Peruvians before, during, and after the Inca Empire

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Through the Peruvian Genome Project we generate and analyze the genomes of 280 individuals where the majority have >90% Native American ancestry and explore questions at the interface of evolutionary genetics, history, anthropology, and medicine. This is the most extensive sampling of high-coverage Native American and mestizo whole genomes to date. We estimate an initial peopling of Peru was rapid and began by 12,000 y ago. In addition, the mestizo populations exhibit admixture between Native American groups prior to their Spanish admixture and was likely influenced by the Inca Empire and Spanish conquest. Our results address important Native American population history questions and establish a dataset beneficial to address the underrepresentation of Native American ancestry in sequencing studies. (See pp. E6526–E6535.)

Gentamicin induces *LAMB3* nonsense mutation readthrough and restores functional laminin 332 in junctional epidermolysis bullosa

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Premature termination codons (PTCs) generated by nonsense mutations produce abnormal, short, or diminished proteins. Eighty-three percent of patients with Herlitz junctional epidermolysis bullosa (H-JEB), an inherited, incurable skin disease, harbor nonsense mutation(s) in genes encoding a structural protein (laminin 332) responsible for skin adherence. Gentamicin, a common antibiotic, was shown to induce readthrough of PTCs in various disease models. Using in vitro assays and 3D skin models, we found that H-JEB cells harboring nonsense mutations exposed to gentamicin produce full-length structural protein, deposit it correctly between skin layers, and exhibit reversal of other H-JEB–associated cellular abnormalities. Our findings indicate that gentamicin may present an immediate therapy for this otherwise fatal disease and other skin disorders caused by nonsense mutations. (See pp. E6536–E6545.)

Fatty acid metabolism complements glycolysis in the selective regulatory T cell expansion during tumor growth

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Recent studies have established that metabolic restrains, such as glucose restriction, impair the activities of effector T cells in the tumor microenvironment. In the same context, a huge expansion of activated Treg cells in tumor tissues has been described in mice and humans, contributing to the suppression of protective antitumor immunity. Our data demonstrate that Tregs are

committed to survive and proliferate in such a hostile milieu thanks to a metabolic advantage based on the combination of glycolysis and fatty acid synthesis and oxidation. This allows Tregs to prevail over conventional T cells that rely primarily on the glycolytic pathway for their metabolic demands. Awareness of the metabolic dynamics of Tregs in tumor could provide a means for cancer immunotherapy. (See pp. E6546–E6555.)

Disrupting LXRα phosphorylation promotes FoxM1 expression and modulates atherosclerosis by inducing macrophage proliferation

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To date, the importance of liver X receptors (LXRs) in atherosclerosis development has been gleaned from their pharmacological or genetic manipulation. Here, we show that altering LXRα phosphorylation can shape proatherogenic responses to fat-rich diets, uncovering previously unrecognized mechanisms. Disrupting LXRα phosphorylation in myeloid cells triggers global changes in gene expression in macrophages, including the up-regulation of proliferation-promoting factors, consistent with increased proliferation of lesion-resident cells. This leads to an enhanced atherosclerotic plaque burden and plaques with altered phenotypic features. Notably, novel LXRα-regulated targets revealed by impaired LXRα phosphorylation are markedly distinct from those promoted by LXR ligand activation. Overall, this work reveals LXRα phosphorylation as an important determinant of atherosclerosis development. This could be exploited for the design of novel antiatherosclerotic strategies. (See pp. E6556–E6565.)

Drosophila model of myosin myopathy rescued by overexpression of a TRIM-protein family member

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The majority of mutations residing within the rod domain of myosin have been associated with skeletal myopathy with or without cardiomyopathy. However, the molecular mechanisms underlying the variation in clinical and pathological phenotypes of myopathies associated with the *MYH7* mutation are still poorly understood. We used CRISPR/Cas9-mediated genome engineering to develop a fly model for Laing distal myopathy to investigate the pathological mechanisms of the recurrent L1729del *MYH7* mutation. This study unveils structural and functional phenotypes associated with this mutation in skeletal and heart muscles, and identifies a mechanism that alleviates the pathological phenotype, suggesting that E3-ligase modifier gene activity may reduce or enhance the impact of this myosin mutation in patients. (See pp. E6566–E6575.)

Autoantibodies reactive to adrenocorticotropic hormone can alter cortisol secretion in both aggressive and nonaggressive humans

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The number of inmates imprisoned for violent aggression is increasing, as are the penitentiaries, but still our understanding of mechanisms underlying criminality is limited. Our analysis of violent aggressor inmates reveals unique properties of IgG reactive with adrenocorticotropic hormone (ACTH). We show that these IgGs can regulate ACTH-induced cortisol secretion in the adrenal gland, and they exhibit a clear-cut difference in ACTH epitope binding in violent aggressors vs. controls. Additionally, IgG from a subset of aggressive subjects selectively bind to hypothalamic vasopressin neurons. Thus, using several in vitro and in vivo approaches, the study reveals a molecular mechanism involved in the variability of stress response relevant to the neurobiology of aggression and possibly other stress-related conditions. (See pp. E6576–E6584.)

Coupled laboratory and field investigations resolve microbial interactions that underpin persistence in hydraulically fractured shales

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Microorganisms persisting in hydraulically fractured shales must maintain osmotic balance in hypersaline fluids, gain energy in the absence of electron acceptors, and acquire carbon and nitrogen to synthesize cell building blocks. We provide evidence that that cofermentation of amino acids (Stickland reaction) meets all of these organismal needs, thus functioning as a keystone metabolism in enriched and natural microbial communities from hydraulically fractured shales. This amino acid-based metabolic network can be rationally designed to optimize biogenic methane yields and minimize undesirable chemistries in this engineered ecosystem. Our proposed ecological framework extends to the human gut and other protein-rich ecosystems, where the role of Stickland fermentations and their derived syntrophies play unrecognized roles in carbon and nitrogen turnover. (See pp. E6585–E6594.)

Direct cell–cell contact activates SigM to express the ESX-4 secretion system in *Mycobacterium smegmatis*

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A conjugation model of mycobacterial interaction recently revealed that intercellular communication occurs between donors and recipients. This communication links two ESAT-6 (ESX) (type VII) secretion systems that are both required for conjugation. Functionally distinct ESX secretion systems are found in all mycobacteria, and they serve important virulence functions in pathogenic mycobacteria. We demonstrate that SigM, an extracytoplasmic transcription factor, activates ESX-4. Direct donor-recipient cell contact triggers the recipient cell to release membrane-sequestered SigM, which rapidly induces an ESX-4-focused regulon. The conservation of SigM and ESX-4 throughout mycobacteria suggests that this interaction-response network is intact and active in pathogens. Contact-dependent responses similar to those identified in our model system may therefore also mediate communal processes within infectious mycobacterial populations. (See pp. E6595–E6603.)

Arthropod EVs mediate dengue virus transmission through interaction with a tetraspanin domain containing glycoprotein Tsp29Fb

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So far, no studies have reported whether dengue virus uses arthropod extracellular vesicles (EVs) for its transmission from vector to the mammalian host. Our study reports a very significant

finding on EV-mediated transmission of dengue viruses (serotypes 2 and 3) from mosquito to mammalian, including human cells, through interactions with an arthropod EV-enriched tetraspanin domain-containing glycoprotein, Tsp29Fb. (See pp. E6604–E6613.)

Inhibitor of intramembrane protease RseP blocks the σ^E response causing lethal accumulation of unfolded outer membrane proteins

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The σ^E stress response monitors outer membrane protein (OMP) assembly. Uninduced, σ^E is sequestered to the plasma membrane by its anti-sigma factor, RseA. Mutations perturbing OMP biogenesis induce degradation of RseA by the proteases DegS and RseP, liberating σ^E so it can activate gene expression. σ^E activity is essential in wild-type *Escherichia coli*, although why it is essential has remained unclear. We report that batimastat is an inhibitor of RseP, preventing it from cleaving RseA and thereby causing a lethal decrease in σ^E activity. Surprisingly, lethality is caused by the accumulation of unfolded OMPs, despite a wild-type OMP biogenesis pathway. Hence, σ^E is essential because it must fine-tune OMP synthesis, assembly, and degradation to prevent the appearance of toxic unfolded OMPs. (See pp. E6614–E6621.)

In vivo imaging of the pathophysiological changes and neutrophil dynamics in influenza virus-infected mouse lungs

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We used a state-of-the-art in vivo imaging system and fluorescent influenza viruses (Color-flu) to determine in real time the pathophysiological changes in the lungs of infected mice. We found that influenza virus infections reduced blood flow speed and decreased neutrophil motility. More significantly, infection with a prototypic "bird flu" strain, a highly pathogenic H5N1 influenza virus, caused higher pulmonary permeability than did infection with a mouse-adapted human influenza virus. This in vivo imaging system with quantitative analyses allowed us to reveal the progression of the disease at the cellular level and to perform a multiparameter analysis that is not possible by using conventional histopathology. (See pp. E6622–E6629.)

Piano training enhances the neural processing of pitch and improves speech perception in Mandarin-speaking children

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Musical training is beneficial to speech processing, but this transfer's underlying brain mechanisms are unclear. Using pseudorandomized group assignments with 74 4- to 5-year-old Mandarin-speaking children, we showed that, relative to an active control group which underwent reading training and a no-contact control group, piano training uniquely enhanced cortical responses to pitch changes in music and speech (as lexical tones). These neural enhancements further generalized to early literacy skills: Compared with the controls, the piano-training group also improved behaviorally in auditory word discrimination, which was correlated with their enhanced neural sensitivities to musical pitch changes. Piano training thus improves children's common sound processing, facilitating certain aspects of language development as much as, if not more than, reading instruction. (See pp. E6630–E6639.)

Amyloid clearance defect in ApoE4 astrocytes is reversed by epigenetic correction of endosomal pH

Hari Prasad and Rajini Rao

Alzheimer's disease is the most common cause of dementia in the elderly. Most cases occur sporadically, with 40–65% of patients carrying at least one copy of the E4 allele of Apolipoprotein E. Because no drug exists that can halt disease progress, there is strong interest in understanding the presymptomatic role of endosomes. We show that excessive endosomal acidification in ApoE4 astrocytes is caused by downregulation of the Na^+/H^+ exchanger NHE6 and results in defective clearance of amyloid beta (Aβ) peptide by intracellular sequestration of the LRP1 receptor. Epigenetic modifiers restore NHE6 expression to alkalinize endosomal pH, increase surface expression of LRP1, and correct Aβ clearance in astrocytes. Thus, endosomal pH emerges as a target for the correction of amyloid disorders. (See pp. E6640–E6649.)

Multigenome analysis implicates miniature inverted-repeat transposable elements (MITEs) in metabolic diversification in eudicots

Alexander M. Boutanaev and Anne E. Osbourn

Recently discovered biosynthetic gene clusters in plants are a striking example of the nonrandom complex structure of eukaryotic genomes. The mechanisms underpinning the formation of these clustered pathways are not understood. Here we carry out a systematic analysis of transposable elements associated with clustered terpene biosynthetic genes in plant genomes, and find evidence to suggest a role for miniature inverted-repeat transposable elements in cluster formation in eudicots. Our analyses provide insights into potential mechanisms of cluster assembly. They also shed light on the emergence of a "block" mechanism for the foundation of new terpene clusters in the eudicots in which microsyntenic blocks of terpene synthase and cytochrome P450 gene pairs duplicate, providing templates for the evolution of new pathways. (See pp. E6650–E6658.)

Degradation of unmethylated miRNA/miRNA*s by a DEDDy-type 3′ to 5′ exoribonuclease Atrimmer 2 in *Arabidopsis*

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The steady-state levels of miRNAs are under sophisticated control to ensure their proper functions such as development and responses to environmental stimuli. Nevertheless, enzymes responsible for the degradation of various forms of unmethylated miRNAs remain enigmatic, which largely impedes our understanding of miRNA homeostasis and active turnover. Here we report a 3′ to 5′ exoribonuclease Atrimmer 2 that may degrade unmethylated miRNAs in their miRNA/miRNA* duplex status, at places distinct from their production sites (i.e., Dicing bodies). Our study not only increases the complexity of miRNA surveillance, but also provides clues into how nascent miRNA/miRNA* duplexes undergo methylation and RNA-induced silencing complex loading, which is a big challenge in the plant small RNA field. (See pp. E6659–E6667.)