PNAS Plus Significance Statements

Mechanistic insights into electrochemical reduction of CO_2 over Ag using density functional theory and transport models

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Chemical storage of solar energy can be achieved by electrochemical reduction of CO_2 to CO and H_2, and subsequent conversion of this mixture to fuels. Identifying optimal conditions for electrochemical cell operation requires knowledge of the CO_2 reduction mechanism and the influence of all factors controlling cell performance. We report a multiscale model for predicting the current densities for H_2 and CO formation from first principles. Our approach brings together a quantum-chemical analysis of the reaction pathway, a microkinetic model of the reaction dynamics, and a continuum model for mass transport of all species through the electrolyte. This model is essential for identifying a physically correct representation of product current densities dependence on the cell voltage and CO_2 partial pressure. (See pp. E8812–E8821.)

Evolutionary dynamics of language systems

Simon J. Greenhill, Chieh-Hsi Wu, Xia Hua, Michael Dunn, Stephen C. Levinson, and Russell D. Gray

Do different aspects of language evolve in different ways? Here, we infer the rates of change in lexical and grammatical data from 81 languages of the Pacific. We show that, in general, grammatical features tend to change faster and have higher amounts of conflicting signal than basic vocabulary. We suggest that subsystems of language show differing patterns of dynamics and propose that modeling this rate variation may allow us to extract more signal, and thus trace language history deeper than has been previously possible. (See pp. E8822–E8829.)

Cavity hydration dynamics in cytochrome c oxidase and functional implications

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Cytochrome c oxidase (CcO) reduces molecular oxygen to generate the proton motive force across the membrane that drives ATP synthesis. Internal water molecules in and near a central cavity play important roles in mediating the proton transfers. Molecular simulations of CcO reveal reversible transitions between wet and dry configurations of this internal cavity in response to the charge state of key cofactors and residues. Quantitative analysis of the free energy change and timescale of the transition suggests that hydration-level change of the central cavity is an essential feature that contributes to the vectorial efficiency of proton pumping in CcO. Thus, wetting transition of protein internal cavities can be functionally significant, especially for the transport of charged species. (See pp. E8830–E8836.)

Visualizing nuclear RNAi activity in single living human cells

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RNA interference (RNAi) is a natural process occurring in cells, and is used to silence genes. Typically, RNAi occurs via small RNA molecules generated in the cell nucleus, which are exported to the cytoplasm where they silence messenger RNA (mRNA) molecules. However, RNAi is thought to occur in the nucleus as well. To demonstrate that this process can occur in the nucleus and to determine its dynamics, we generated human cell systems that enabled us to image living cells and to track gene silencing as it transpired in real time. We found that the RNAi machinery can target the mRNA as it is being transcribed, and that silencing is mediated through modifications occurring on histone proteins bound to the DNA. (See pp. E8837–E8846.)

Cytoplasmic MTOCs control spindle orientation for asymmetric cell division in plants

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Cell division axis orientation is critical for differentiation and morphogenesis. In animal cells, centrosome-driven mitotic spindle orientation is key to orient cell divisions. However, in naturally acentrosomal plants, the mechanism underlying spindle orientation is poorly understood. Using two model systems, asymmetrically dividing cells in the moss *Physcomitrella patens* and tobacco tissue culture cells, we identified de novo assembled microtubule organizing centers during mitotic prophase as a common critical mechanism for spindle orientation. Disruption of these microtubule organizing centers caused misoriented spindles and cell plates. The "phragmoplast guidance" mechanism, which directs cell plate orientation, could not fully restore initial spindle orientation defects. Thus, this study identifies spindle orientation as a conserved factor in land plants to assist division plane orientation. (See pp. E8847–E8854.)

Period2 3′-UTR and microRNA-24 regulate circadian rhythms by repressing PERIOD2 protein accumulation

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The circadian oscillator is a cell-autonomous biological timer driving daily physiological rhythms to ensure fitness and health. Regulatory mechanisms of the oscillator are complex and not fully understood. We previously generated two circadian reporter mouse lines that differ only in the 3′-UTR region of the core clock gene *Per2*. Interestingly, substitution of the endogenous *Per2* 3′-UTR with an SV40 late poly(A) signal led to a lengthened period, enhanced PER2 protein level, and more robust oscillatory amplitude. PER2 also displayed a positive role in circadian transcription. Molecular and genetic studies showed the microRNA miR-24 binds to the *Per2* 3′-UTR to attenuate rhythmic PER2 accumulation. These results identified an important posttranscriptional regulatory mechanism of PER2 expression required for normal circadian timekeeping. (See pp. E8855–E8864.)

FOXO1 opposition of CD8$^+$ T cell effector programming confers early memory properties and phenotypic diversity

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Acute infection with intracellular pathogen results in the expansion and effector differentiation of pathogen-specific CD8$^+$ T cells, most of which die after pathogen clearance. The factors and steps controlling CD8$^+$ T cell differentiation in response to acute infection are not fully understood. We find FOXO1 and TCF7 drive postinfection immune memory characteristics in cells expressing low TIM3, where TCF7 actively down-regulates the cytotoxic molecule GZMB and reduces terminally differentiated KLRG1^{high} effector abundance. We further report the creation of a continuum of CD8$^+$ T cell phenotypes, ranging from terminal effector cells to long-lived immune-memory cells, dependent on FOXO1 and TCF7. We conclude FOXO1 and TCF7 are essential to oppose cytotoxic/terminal differentiation programs and enable full phenotypic diversity of postinfection CD8$^+$ T cells. (See pp. E8865–E8874.)

Systems-level identification of PKA-dependent signaling in epithelial cells

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Maintenance of homeostasis is dependent on intercellular communication via secreted hormones that bind G protein-coupled receptors. Many of these receptors activate an enzyme called protein kinase A (PKA) that modifies cell function by covalently attaching phosphate groups to proteins. To comprehensively identify PKA substrates, we used genome editing (CRISPR-Cas9) to delete PKA from kidney epithelial cells followed by large-scale mass spectrometry to measure phosphorylation changes throughout the proteome; 229 PKA target sites were identified, many previously unrecognized. Surprisingly, PKA deletion caused seemingly paradoxical phosphorylation increases at many sites, indicating secondary activation of one or more mitogen-activated kinases. The data, coupled with transcriptomics and standard proteomics, identified a signaling network that explains the effects of PKA that regulate cellular functions. (See pp. E8875–E8884.)

Genome-wide engineering of an infectious clone of herpes simplex virus type 1 using synthetic genomics assembly methods

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Viruses with large DNA genomes, such as herpesviruses, are difficult to manipulate with existing genetic tools. We describe an application of synthetic genomics assembly tools that enables rapid and efficient generation of combinatorial mutations in herpesvirus genomes. The method provides the capacity to design, generate, and test numerous multiloci mutants in parallel, which can help us understand basic virus biology, facilitate vaccine development, and aid development of next-generation virus-based delivery systems. This class of viruses is being used as vectors for therapeutics and vaccines, with an oncolytic herpesvirus approved for the treatment of melanoma. Although such improvements in genome assembly and manipulation raise dual-use concerns, we believe the potential benefits substantially outweigh the risks. (See pp. E8885–E8894.)

Expanded subgenomic mRNA transcriptome and coding capacity of a nidovirus

Han Di, Joseph C. Madden Jr., Esther K. Morantz, Hsin-Yao Tang, Rachel L. Graham, Ralph S. Baric, and Margo A. Brinton

All members of the order Nidovirales, including *Simian hemorrhagic fever virus* (SHFV), produce subgenomic mRNAs (sg mRNAs) for their 3′ genes regulated by genomic transcription regulatory sequences (TRSs). We used a next-generation sequencing–facilitated approach to comprehensively analyze a nidovirus sg mRNA transcriptome. The discovery of high sg mRNA redundancy for individual genes and multiple previously unreported sg mRNAs encoding nonstructural proteins, alternative reading frame proteins, or C-terminal peptides of known proteins represents a paradigm shift in our understanding of SHFV genome-coding capacity and the complexity of transcription regulation that is expected to also be characteristic of other nidoviruses. High sg mRNA redundancy would ensure continued protein synthesis if a TRS is inactivated by random mutation. (See pp. E8895–E8904.)

Structures of human-infecting *Thogotovirus* fusogens support a common ancestor with insect baculovirus

Ruchao Peng, Shuijun Zhang, Yingzi Cui, Yi Shi, George F. Gao, and Jianxun Qi

Thogotoviruses belong to *Orthomyxoviridae* family and infect a variety of mammalian hosts, including humans. The emergence of these viruses poses great threats to public health and the economy. In this work, we performed structural and phylogenic analyses on the fusogenic glycoproteins of Thogoto and Dhori viruses, two representatives of the *Thogotovirus* genus that cause severe human infections. Previous studies have shown that thogotovirus glycoproteins share ~28% sequence identity with baculovirus Gp64s. Our structural analysis confirmed their homology in evolution and identified them as class III viral fusogens, in contrast to class I members of influenza viruses. Our studies provide structural evidence to help us to understand the evolution of these viruses and indicate a potential target for antiviral drug design. (See pp. E8905–E8912.)

Motor origin of temporal predictions in auditory attention

Benjamin Morillon and Sylvain Baillet

How the motor system participates in auditory perception is unknown. In a magnetoencephalography experiment involving auditory temporal attention, we show that the left sensorimotor cortex encodes temporal predictions, which drive the precise temporal anticipation of forthcoming sensory inputs. This encoding is associated with bursts of beta (18–24 Hz) neural oscillations that are directed toward auditory regions. Our data also show that the production of overt movements improves the quality of temporal predictions and augments auditory task performance. These behavioral changes are associated with increased signaling of temporal predictions in right-lateralized frontoparietal associative regions. This study points at a covert form of auditory active sensing, and emphasizes the fundamental role of motor brain areas and actual motor behavior in sensory processing. (See pp. E8913–E8921.)

Framework for gradual progression of cell ontogeny in the *Arabidopsis* root meristem

Jos R. Wendrich, Barbara K. Möller, Song Li, Shunsuke Saiga, Rosangela Sozzani, Philip N. Benfey, Bert De Rybel, and Dolf Weijers

Plants have the ability to live and grow for many thousands of years due to the activity of groups of cells called meristems. Meristems contain stem cells that can survive the entire life of the plant and ensure the continuous supply of new cells. Stem cells are thought to be qualitatively different compared with their neighboring daughter cells. Here we show that in the case of the proximal root meristem, there does not seem to be such an on-off type of organization. We show that the majority of transcripts, together with other cellular properties, gradually transition from stem cell activity to differentiation, by opposing gradients. This impacts our understanding of meristem organization and will determine the direction of future research. (See pp. E8922–E8929.)

Mediator subunit MED25 links the jasmonate receptor to transcriptionally active chromatin

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Sensing of the plant hormone jasmonate (JA) by the F-box protein CORONATINE INSENSITIVE 1 (COI1) triggers profound transcriptional changes that are regulated by the master regulator MYC2. However, it remains unclear how COI1 communicates with the general transcription machinery and chromatin. Here, we show that MED25, a subunit of the Mediator coactivator complex, physically and functionally interacts with COI1 on the promoters of MYC2 targets. MED25 also physically and functionally interacts with HISTONE ACETYLTRANSFERASE1 (HAC1), which selectively regulates histone (H) 3 lysine (K) 9 acetylation of MYC2 targets. Therefore, MED25 integrates regulatory signals that converge on the promoters of MYC2 targets. Our results reveal a fundamental mechanism by which Mediator coordinates the actions of both genetic and epigenetic regulators into a concerted transcriptional program. (See pp. E8930–E8939.)