PNAS Plus Significance Statements

An RNA motif advances transcription by preventing Rho-dependent termination

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Transcription factors typically bind to more sites than are functionally affected upon transcription factor inactivation. What, then, determines whether transcription factor binding impacts gene expression? Here we address this question by investigating Rho, the essential transcription termination factor that associates with most newly transcribed RNAs in bacteria, but promotes transcription termination only in a fraction of these transcripts. We uncover a novel RNA element that sequesters Rho in an inactive complex, thereby advancing transcription without affecting Rho binding. Our results indicate that the site of action of transcription factors is defined not only by sequences that mediate their recruitment but also by sequences that antagonize their activity. (See pp. E6835–E6843.)

Structural and functional analysis of two di-domain aromatase/cyclases from type II polyketide synthases

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Polyketides are a class of diverse natural products with well-documented bioactivity and medicinal importance. Enzymes known as aromatase/cyclases (ARO/CYCs) catalyze regiospecific cyclization and aromatization during type II polyketide biosynthesis. Understanding how ARO/CYCs catalyze cyclization and aromatization is critical for developing strategies for engineering biosynthetic pathways. This is the first study, to our knowledge, to use X-ray crystallography, bioinformatic and structural analysis, and in vitro functional assays to critically compare a reducing di-domain ARO/CYC (BexL) and a nonreducing di-domain ARO/CYC (StfQ). Together, these results fill in a missing link in the structural enzymology of polyketide biosynthesis and will have a direct effect on future biosynthetic engineering efforts and bioinformatic analysis of type II PKS gene clusters. (See pp. E6844–E6851.)

Single-molecule visualization of RecQ helicase reveals DNA melting, nucleation, and assembly are required for processive DNA unwinding

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DNA helicases are essential enzymes that unwind dsDNA to ssDNA to access the information encoded within those strands. We describe a new single-molecule imaging procedure to follow DNA unwinding in real-time and apply it to *Escherichia coli* RecQ helicase. Using a fluorescent sensor to detect ssDNA and fluorescence microscopy, we observe that DNA unwinding occurs by initial nucleation of RecQ at random DNA sites and concomitant local melting of duplex DNA. Subsequently, RecQ assembles into a distribution of multimeric species that processively unwind DNA at rates proportional to their assembled state. Thus, RecQ helicase acts by a mechanism that is distinctive in that the active form is a variable and heterogeneous ensemble of loosely coupled monomeric entities. (See pp. E6852–E6861.)

DksA regulates RNA polymerase in *Escherichia coli* through a network of interactions in the secondary channel that includes Sequence Insertion 1

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The transcription factor DksA is a critical determinant of the stringent response and is essential for virulence in many pathogenic proteobacteria. This ubiquitous transcription factor is also a model system for transcription regulation, making it essential to understand how DksA interacts with RNA polymerase (RNAP) at the molecular level. High-resolution structural information of the DksA–RNAP interaction is currently unavailable. Using genetic, biochemical, and computational approaches, we have generated a new high-quality model of the DksA–RNAP interaction that advances our understanding of DksA binding and activity and will serve as a springboard for future mechanistic investigations into DksA regulation. (See pp. E6862–E6871.)

ClpB N-terminal domain plays a regulatory role in protein disaggregation

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ClpB/Hsp100 chaperones protect cells from the devastating effects of protein inactivation and aggregation arising from extreme stress. This function is accomplished first by binding to the aggregates and then forcibly unraveling individual proteins by passing them through the central channel in the hexameric chaperones. Here, we investigate the role of the ClpB/Hsp100 N-terminal domain (NTD) in protein disaggregation. Our results demonstrate that ClpB recognizes exposed hydrophobic stretches in unfolded or aggregated client proteins via a substrate-binding groove in its NTD. We further show that the NTD has regulatory roles that include blocking the translocation channel in the absence of substrate and destabilizing client proteins upon binding, thus priming them for subsequent unfolding and disaggregation. (See pp. E6872–E6881.)

Identifying an ovarian cancer cell hierarchy regulated by bone morphogenetic protein 2

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Significant controversy persists regarding a hierarchical vs. stochastic model of cancer. Using a microfluidic single-cell culture device, we define for the first time, to our knowledge, the differentiation capacity of primary human ovarian cancer cells. We demonstrate that ovarian cancer follows a hierarchical model with rare stochastic events. Defining the differentiation capacity allowed us to explain apparently paradoxical actions of bone morphologenetic protein 2 (BMP2); BMP2 suppresses growth in vitro by suppressing bulk cell proliferation, but promotes growth in vivo by promoting cancer stem-like cell (CSC) expansion. This work supports BMP2 signaling as a critical therapeutic target regulating ovarian CSC growth. (See pp. E6882–E6888.)

Tracking the origins of Yakutian horses and the genetic basis for their fast adaptation to subarctic environments

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Yakutia is among the coldest regions in the Northern Hemisphere, showing ~40% of its territory above the Arctic Circle. Native horses are particularly adapted to this environment, with body sizes and thick winter coats minimizing heat loss. We sequenced complete genomes of two ancient and nine present-day Yakutian horses to elucidate their evolutionary origins. We find that the contemporary population descends from domestic livestock, likely brought by early horse-riders who settled in the region a few centuries ago. The metabolic, anatomical, and physiological adaptations of these horses therefore emerged on very short evolutionary time scales. We show the relative importance of regulatory changes in the adaptive process and identify genes independently selected in cold-adapted human populations and woolly mammoths. (See pp. E6889–E6897.)

Single-molecule motions and interactions in live cells reveal target search dynamics in mismatch repair

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We integrated single-molecule superresolution imaging with biochemical and genomic approaches to understand how the mismatch repair protein MutS efficiently identifies DNA mismatches during real time in living cells. We show that MutS molecules move fast, exploring the entire nucleoid, but can transition to a slow-moving population that is localized at the replisome even before a mismatch is produced. We show that bacterial MutS must initiate mismatch binding in very close proximity to the replisome. We also show that mismatch detection increases MutS speed, supporting the model for MutS sliding clamp formation after mismatch recognition. Our results provide fundamental insight into the searching behavior of single MutS molecules during DNA replication in live cells. (See pp. E6898–E6906.)

DNA polymerases δ and λ cooperate in repairing double-strand breaks by microhomology-mediated end-joining in *Saccharomyces cerevisiae*

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The human genome contains many segments of short repetitive DNA, known as microhomologies, which are potential sites for the types of rearrangements found in many different types of cancer. Therefore, understanding how microhomologies within DNA can initiate chromosome rearrangements and the genes involved in promoting this process is critical in understanding cancer development. We examined the repair efficiency of DNA containing microhomologies and found a positive correlation between microhomology length and quality with repair efficiency. Furthermore, our work directly implicates the major DNA replication and repair polymerase, DNA polymerase δ, in repair of DNA damage using microhomologies, which act in conjunction with DNA polymerase λ. Our data suggest a two-polymerase model for microhomology-mediated end-joining. (See pp. E6907–E6916.)

Temporal fate mapping reveals age-linked heterogeneity in naive T lymphocytes in mice

Thea Hogan, Graeme Gossel, Andrew J. Yates, and Benedict Seddon

T cells are essential components of vertebrate immune systems, but the mechanisms by which they are maintained are still poorly defined. Existing methods infer cell lifetimes and division rates using DNA labeling of dividing cells, but do not resolve heterogeneity in population dynamics well. We present a novel experimental system that, when combined with mathematical models, yields kinetic parameters and allows us to measure the effect of a cell's age on its ability to survive and divide. Our approach quantifies lymphocyte dynamics over a year of a mouse's life and reveals a first-in, last-out structure in which subpopulations of naive T cells generated early in life persist with slower kinetics and resist displacement by newer specificities. (See pp. E6917–E6926.)

Nrf2 in ischemic neurons promotes retinal vascular regeneration through regulation of semaphorin 6A

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Delayed revascularization of ischemic neural tissue is a major impediment to preservation of function in central nervous system (CNS) diseases including stroke and ischemic retinopathies. The key mechanisms

governing vascular recovery in ischemic CNS, including regulatory molecules governing transition from tissue injury to repair, are largely unknown. We report here on NF-E2-related factor 2 (Nrf2), a major stress-response transcription factor known for its cell-intrinsic cytoprotective function, in a novel capacity coordinating tissue repair and remodeling, including regulation of cell–cell crosstalk. Nrf2 activity in ischemic neurons reduces their resistance to reparative angiogenesis by suppressing expression of neuronal semaphorin 6A (Sema6A) and its antiangiogenic effects. Pharmacologic activation of Nrf2 or inhibition of Sema6A promote reparative angiogenesis in this ischemic setting, suggesting therapeutic avenues for ischemic retinopathies and other ischemic diseases. (See pp. E6927–E6936.)

HSV targeting of the host phosphatase PP1α is required for disseminated disease in the neonate and contributes to pathogenesis in the brain

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The increased severity of herpes simplex virus (HSV) disease in the newborn compared with the adult is a result of complex interactions between the virus and the host response. We studied viral targeting of host-mediated shutoff of protein synthesis during HSV disease in a murine model and found that HSV-1 targeting of the host phosphatase PP1α was required for disseminated disease in the newborn and contributed to HSV encephalitis. Additionally, this report demonstrates that the host response resulting in translational arrest is tissue-specific and also reveals a tissue-specific reliance on the type I IFN response for control of HSV-1. These results provide important insight into the mechanisms of severe HSV disease in the newborn compared with the adult. (See pp. E6937–E6944.)

Production of functional small interfering RNAs by an amino-terminal deletion mutant of human Dicer

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Although RNA interference (RNAi) is an important antiviral innate-immune response in plants and invertebrates, whether mammals mount effective RNAi responses remains controversial. Using human cells lacking functional Dicer and protein kinase RNA-activated genes, we examined whether wild-type or a deletion mutant of Dicer, lacking the helicase domain, could induce RNAi when presented with double-stranded RNAs derived from plasmids or generated during viral infections. Overexpression of the truncated Dicer mutant resulted in the production of siRNAs in both cases, and these were sufficient to inhibit the expression of cognate mRNAs. Whether the latent ability of human Dicer to induce RNAi will ever be unmasked in vivo remains unclear. (See pp. E6945–E6954.)

Tissue mechanics govern the rapidly adapting and symmetrical response to touch

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Recordings from Pacinian corpuscles in the 1960s showed that touch elicits symmetric activation followed by rapid adaptation. Sinusoidal stimulation resulted in frequency doubling within a sensitive frequency band, suggesting that these receptors function as frequency-tuned vibration sensors. At the time, the surrounding lamellar capsule was proposed to generate these response dynamics by acting as a mechanical filter. However, similar response dynamics have since been seen in many other mechanoreceptors, leading to controversy over the specificity of this hypothesis. Using a combination of in vivo electrophysiology, feedback-controlled mechanical stimulation, and simulation, we resolve this controversy in favor of a systems-level mechanical filter that is independent of specific anatomical features or specific mechanoelectrical transduction channels. (See pp. E6955–E6963.)

Synaptic P-Rex1 signaling regulates hippocampal long-term depression and autism-like social behavior

Jun Li, Anping Chai, Lifang Wang, Yuanlin Ma, Zhiliu Wu, Hao Yu, Liwei Mei, Lin Lu, Chen Zhang, Weihua Yue, Lin Xu, Yi Rao, and Dai Zhang

Impairments in social behavior and behavioral flexibility have been found in autistic people. However, the genetic mechanism that may contribute to these symptoms is unknown. Here we identified a previously unreported autism-associated gene that regulates synaptic plasticity. The mice lacking this gene exhibit deficits in AMPA receptor endocytosis and synaptic depression because of the blockade of a postsynaptic signaling pathway, leading to autism-like social recognition deficit and behavioral inflexibility. These findings provide new insights into the mechanisms underlying social recognition behavior and suggest that the synaptic depression-related signaling pathway might represent a new therapeutic target for treatment of social recognition deficit disorders such as autism spectrum disorders. (See pp. E6964–E6972.)

Origin of information-limiting noise correlations

Ingmar Kanitscheider, Ruben Coen-Cagli, and Alexandre Pouget

Populations of neurons encode information in activity patterns that vary across repeated presentation of the same input and are correlated across neurons (noise correlations). Such noise correlations can limit information about sensory stimuli and therefore limit behavioral performance in tasks such as discrimination between two similar stimuli. Therefore it is important to understand where and how noise correlations are generated. Most previous accounts focused on sources of variability inside the brain. Here we focus instead on noise that is injected at the sensory periphery and propagated to the cortex: We show that this simple framework accounts for many known properties of noise correlations and explains behavioral performance in discrimination tasks, without the need to assume further sources of information loss. (See pp. E6973–E6982.)

PSD-95 family MAGUKs are essential for anchoring AMPA and NMDA receptor complexes at the postsynaptic density

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The postsynaptic density (PSD) at the glutamatergic excitatory synapse is a macromolecular machine that underlies synaptic transmission and information storage. Membrane-associated guanylate

kinases (MAGUKs), the major scaffolding proteins at the PSD, are positively correlated with synaptic maturation and strength, but how MAGUKs sustain the strength of synaptic transmission remains unclear. Here, we remove three MAGUK proteins from neurons and find significant reductions in synaptic transmission by AMPARs and NMDARs with a concomitant reduction in PSD sizes and core scaffold and transmembrane structures. Our results show how MAGUKs anchor and organize both types of glutamate receptors, thereby regulating the strength of excitatory synapses. (See pp. E6983–E6992.)

Translational profiling identifies a cascade of damage initiated in motor neurons and spreading to glia in mutant SOD1-mediated ALS

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Amyotrophic lateral sclerosis can be caused by a mutation in superoxide dismutase. Ubiquitously expressed, disease mechanism involves damage within motor neurons (whose degeneration is responsible for progressive paralysis) and glia. By combining ribosome affinity purification from each of three cell types, a temporal cascade of damage is identified that initiates within motor neurons, with subsequent damage within glia driving disease propagation. Mutant-dependent damage to motor neurons, which are shown to express very low levels of endoplasmic reticulum chaperones, includes synapse and metabolic abnormalities and selective activation of the PERK arm of the unfolded protein response. Early changes in astrocytes are to genes involved in inflammation and metabolism, while dysregulation of myelination and lipid signaling pathways in oligodendrocytes occurs only after disease initiation. (See pp. E6993–E7002.)

ADP-stimulated contraction: A predictor of thin-filament activation in cardiac disease

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Diastolic dysfunction is characteristic of patients with cardiomyopathy. Evidence indicates that diseased hearts show basal sarcomeric activation capable of impairing diastolic performance. By activating human cardiomyopathy muscle in ADP-containing solutions without Ca^{2+}, we showed that actin–myosin blockade is disrupted. This may be caused by the presence of mutations and/or the reduced phosphorylation of myofilament proteins. Our mechanistic study supports the novel idea that protein kinase A-target phosphorylation and myosin-binding protein C regulate the OFF–ON transition of the thin filaments. ADP increased myofilament force and stiffness in the presence of Ca^{2+} in cardiomyopathy samples, suggesting this condition limits muscle relaxation through increased actin–myosin interactions. We conclude that ADP-stimulated contraction can be used to reveal conformational changes in the three-state model of thin-filament activation. (See pp. E7003–E7012.)

Designer and natural peptide toxin blockers of the KcsA potassium channel identified by phage display

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Peptide neurotoxins that inhibit specific ion channels are valuable for research and clinical care but unknown for most targets. Here we consider KcsA, an orphan potassium channel with no known toxin. We build a phage-display library expressing natural toxins related to the sea anemone toxin ShK and 1.5 million novel combinatorial variants. Peptides that bind tightly to KcsA are isolated and two are described: Hui1 is novel and specific for KcsA, and HmK is natural and promiscuous. The 3D structure and action of Hui1 validate our strategy and reveal an unexpected basis for channel inhibition wherein an arginine side chain, too large to enter the conduction pathway, interacts with potassium ions traversing the pore from the other side of the membrane. (See pp. E7013–E7021.)

Autotetraploid rice methylome analysis reveals methylation variation of transposable elements and their effects on gene expression

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Whole genome duplication (WGD) has long been recognized as a major force in angiosperm evolution. DNA methylation variation is known to be involved in polyploidization events. We synthesized autotetraploid rice that may rule out disturbances from hybridization and investigated the scope and scale of DNA methylation variation in response to WGD in neopolyploid rice. We found that WGD prompts increased methylation in class II transposable elements, which then suppress the genome-wide expression level of nearby genes. Hypermethylation of these transposable elements inhibits their transposition, stabilizing the integrity of chromosomes, and decreases nearby gene expression, potentially reducing deleterious genome-dosage effects. Our results indicate that DNA methylation plays an important role in assisting plant neopolyploids rapidly adapt to WGD. (See pp. E7022–E7029.)