PNAS Plus Significance Statements

Noncanonical DNA-binding mode of repressor and its disassembly by antirepressor

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The canonical method of inactivating DNA-binding repressors is through the competitive binding of an antirepressor to the operator-binding site of the repressor. Here, structural and functional studies of a homotetrameric repressor (Rep 92–198) and a hetero-octameric complex between the repressor and its antirepressor (Ant) from the temperate *Salmonella* phage SPC32H revealed a noncanonical mechanism of repressor-operator disassembly. Notably, Ant does not compete for the DNA-binding region of Rep. Instead, the tetrameric Ant binds to the N-terminal and C-terminal domains of two asymmetric Rep dimers, causing the stably bound Rep to detach from the DNA. These studies also suggested that the dimer pairs of the N-terminal DNA-binding domains of Rep originate from different dimers of a Rep tetramer. (See pp. E2480–E2488.)

Slide-and-exchange mechanism for rapid and selective transport through the nuclear pore complex

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The nuclear pore complex (NPC) mediates the trafficking of macromolecules in and out of the nucleus of eukaryotic cells. Here, we characterize how transport factors diffuse rapidly through multiple layers of disordered phenylalanine-glycine (FG) repeat domains lining the NPC. Transport factors interact with FG repeats through a dynamic sliding motion, enabling faster translocation through the NPC than that attainable by a two-state binding mechanism as well as effectively blocking the passage of large macromolecules that do not bind to transport factors. Thus, the NPC exemplifies a dynamic system in living cells, the function of which depends on protein–protein interactions that are transient on the one hand, and highly specific on the other. (See pp. E2489–E2497.)

Antibiotic treatment enhances the genome-wide mutation rate of target cells

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The evolution of antibiotic resistance by pathogenic bacteria poses a major challenge for human health. Whereas it is clear that natural selection promotes resistance-conferring mutations, our understanding of the response of the mutation rate to antibiotics is limited. With hundreds of *Escherichia coli* cell lines evolving in a near-neutral scenario under exposure to the fluoroquinolone norfloxacin, this study reveals a significant linear relationship between the mutation rate and antibiotic concentration, while also demonstrating that antibiotic treatment compromises the efficiency of DNA oxidative-damage repair and postreplicative mismatch repair. Thus, antibiotics not only impose a selective challenge to target and off-target bacteria but also accelerate the rate of adaptation by magnifying the rate at which advantageous mutations arise. (See pp. E2498–E2505.)

Rational elicitation of cold-sensitive phenotypes

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Temperature-sensitive (ts) and cold-sensitive mutants (cs) provide rapid and reversible means to lower the level of a specific gene product at any stage in the life cycle of an organism. cs mutants are rare, and the molecular determinants of cs phenotypes are poorly understood. We present and validate a method for the rational elicitation of cold-sensitive phenotypes that involves the design of partial loss-of-function mutants based solely on amino acid sequence, and the coupling of such mutants to a heat responsive promoter. This study provides insight into the molecular determinants of cold sensitivity. Such designed cs mutants provide insight into gene function and also can be coupled with ts mutants to order genes in a pathway. (See pp. E2506–E2515.)

Comparative systems pharmacology of HIF stabilization in the prevention of retinopathy of prematurity

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In all premature births, oxygen supplementation is a necessary life-sustaining measure, but unfortunately for these high-risk babies, oxygen toxicity may adversely and permanently affect the retina. Pharmacological activation of the hypoxia-inducible factor (HIF) pathway can prevent experimental oxygen-induced retinopathy and thus has the potential to prevent blindness in 100,000 children annually. Comprehensive analysis of liver and retinal transcriptomes after HIF stabilization demonstrates that select small molecules,

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given systemically, protect the retina by two pathways: stimulating the liver to secrete angiogenic hepatokines or locally stimulating retinal protection. These findings support a low dose, intermittent, systemic approach for preventing oxygen induced injury to premature infants. (See pp. E2516–E2525.)

NF-κB–driven suppression of FOXO3a contributes to EGFR mutation-independent gefitinib resistance

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Gefitinib is a small molecular inhibitor that targets EGFR tyrosine kinases (EGFR-TKI) and has been used as a first-line treatment for advanced lung cancer. However, not all lung cancer patients respond to gefitinib treatment, and resistance to gefitinib has been apparent for lung cancer patients who have undergone treatment for a few months. We observed that FOXO3a expression is inversely correlated with lung cancer patients who responded poorly to EGFR-TKI treatment and identified an underlying mechanism of FOXO3a in EGFR mutation-independent cancer stemness and gefitinib resistance through the epigenetic regulation of NF-κB/miR-155. This finding highlights the potential of targeting the NF-κB/miR-155/FOXO3a pathway as a novel therapeutic strategy for lung cancer with the acquisition of resistance to EGFR-TKIs. (See pp. E2526–E2535.)

Fine processes of Nestin-GFP–positive radial glia-like stem cells in the adult dentate gyrus ensheathe local synapses and vasculature

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A population of adult neural stem cells supplies the dentate gyrus with new neurons that play a role in mechanisms of learning and memory. Radial glia-like stem cells have a unique morphology, including a dense arbor of fine processes that infiltrate the neurogenic niche. Here, we provide what is, to our knowledge, the first detailed ultrastructural description of these processes, and we reveal that these cells establish a variety of contacts with local blood vessels, synapses, and astrocytes. Given that signals derived from neurons, astrocytes, and blood vessels regulate the process of adult neurogenesis, the identification of these contacts provides a structural framework for elucidating the mechanisms by which this regulation occurs. These results contribute to a greater understanding of the adult hippocampal neurogenic niche. (See pp. E2536–E2545.)