PNAS Plus Significance Statements

The Holocene temperature conundrum

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Marine and terrestrial proxy records suggest global cooling during the Late Holocene, following the peak warming of the Holocene Thermal Maximum (~10 to 6 ka) until the rapid warming induced by increasing anthropogenic greenhouses gases. However, the physical mechanism responsible for this global cooling has remained elusive. Here (pp. E3501–E3505), we show that climate models simulate a robust global annual mean warming in the Holocene, mainly in response to rising CO_2 and the retreat of ice sheets. This model-data inconsistency demands a critical reexamination of both proxy data and models.

A sequence-specific transcription activator motif and powerful synthetic variants that bind Mediator using a fuzzy protein interface

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How transcription activators recognize their coactivator targets is a longstanding question and is important for understanding activator specificity and synergy. Most activators are not obviously related in sequence, but they recognize a common set of coactivators, raising the question of whether these interactions are sequence-specific. We show (pp. E3506–E3513) that the yeast transcription factor Gcn4 central activation domain works via a short sequence-specific motif that can be optimized to generate powerful synthetic activators. Like many natural activators, the synthetic derivatives have redundant sequence and bind the Mediator subunit Gal11 with high affinity using a "fuzzy" protein interface. Our results suggest a mechanism to explain how a subset of natural activators use redundant sequence motifs and great flexibility in the binding interface to target unrelated coactivators.

Structural basis for the recruitment and activation of the *Legionella* phospholipase VipD by the host GTPase Rab5

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A long-standing question in the field of microbial pathogenesis is how virulence factors are regulated within host cells and how their activity is specifically directed toward a particular host cell compartment. *Legionella pneumophila* resolves this dilemma by tightly coupling the phospholipase A1 activity of one of its effectors, vacuolar protein sorting inhibitor protein D (VipD), to this protein's interaction with endosomal host GTPases. We now present the crystal structure of VipD in complex with host cell Rab5c, providing a detailed look into the ingenious molecular mechanisms underlying the allosteric activation of a virulence factor by a host protein and its spatiotemporal regulation. These results (pp. E3514–E3523) open the path for the development of novel therapeutics aimed at blocking the VipD activation process rather than the enzyme's active site.

Electron spin changes during general anesthesia in *Drosophila*

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One hundred sixty years after its discovery, the molecular mechanism of general anesthesia remains a notable mystery. A very wide range of agents ranging from the element xenon to steroids can act as general anesthetics on all animals from protozoa to man, suggesting that a basic cellular mechanism is involved. In this paper (pp. E3524–E3533), we show that volatile general anesthetics cause large changes in electron spin in *Drosophila* fruit flies and that the spin responses are different in anesthesia-resistant mutants. We propose that anesthetics perturb electron currents in cells and describe electronic structure calculations on anesthetic–protein interactions that are consistent with this mechanism and account for hitherto unexplained features of general anesthetic pharmacology.

Distinct isoform of FABP7 revealed by screening for retroelement-activated genes in diffuse large B-cell lymphoma

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Sequences derived from transposable elements (TEs) are abundant in the human genome and can influence gene expression. In normal cells, most TEs are silenced by epigenetic mechanisms such as DNA methylation but, in cancer, normally dormant TEs can become active. We hypothesized (pp. E3534–E3543) that cancer-specific release of epigenetic suppression of TEs could result in gene expression perturbations, which could promote oncogenesis. Using a bioinformatics method, we identified many genes expressed in diffuse large B-cell lymphoma (DLBCL) via activation of TE promoters. Further analysis of one, *FABP7*, showed it was expressed in some DLBCL samples through use of a TE promoter. The TE-driven *FABP7* transcript encodes a novel isoform of the protein, which is required for optimal DLBCL cell line proliferation.

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Calcineurin determines toxic versus beneficial responses to α-synuclein

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Ca^{2+} homeostasis is indispensable for the well being of all living organisms. Ca^{2+} homeostasis is disrupted by α-synuclein (α-syn), whose misfolding plays a major role in neurodegenerative diseases termed synucleinopathies, such as Parkinson disease. We report (pp. E3544–E3552) that α-syn can induce sustained and highly elevated levels of cytoplasmic Ca^{2+}, thereby activating a calcineurin (CN) cascade that results in toxicity. CN is a highly conserved Ca^{2+}–calmodulin (CaM)-dependent phosphatase critical for sensing Ca^{2+} concentrations and transducing that information into cellular responses. Limiting, but not eliminating, the availability of CaM, CN and/or CN substrates directly with genetic or pharmacological tools shifts the α-syn–induced CN cascade to a protective mode. This has mechanistic implications for CN's activity and provides a therapeutic venue for the treatment of synucleinopathies.

Small RNA combination therapy for lung cancer

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Small RNAs can potently and precisely regulate gene expression; as a result, they have tremendous clinical potential. However, effective delivery of small RNAs to solid tumors has remained challenging. Here we report that a lipid/polymer nanoparticle can deliver small RNAs to treat autochthonous tumors in the so-called "KP" mouse model of lung cancer. Nanoparticles formulated with mimics of the p53-regulated miRNA miR-34a downregulated target genes and delayed tumor progression, while nanoparticles formulated with siRNA targeting Kirsten rat sarcoma viral oncogene homolog (siKras) slowed tumor growth and increased apoptosis. Notably, concurrent delivery of miR-34a and siKras increased anti-tumor effects, and led to tumor regression. These results (pp. E3553–E3561) demonstrate that small RNA therapies can impact solid lung tumor growth, and that targeted RNA combination therapies may be used to improve therapeutic response.

Murine $CD27^{(-)}$ $V\gamma6^{(+)}$ γδ T cells producing IL-17A promote ovarian cancer growth via mobilization of protumor small peritoneal macrophages

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Tumor development is impacted by a set of diverse infiltrating leukocyte populations that can either inhibit or, paradoxically, enhance tumor cell growth. This study (pp. E3562–E3570) characterizes a cellular cross-talk between γδ T lymphocytes and small peritoneal macrophages (SPMs) that is mediated by the proinflammatory cytokine, IL-17, and promotes ovarian cancer growth. IL-17 is preferentially produced by a population of γδ T cells, displaying a distinctive $CD27^{(-)}$ $V\gamma6^{(+)}$ phenotype, that strongly proliferate in response to tumor challenge. This associates with the mobilization of SPMs that express protumor and proangiogenic molecular mediators upregulated by IL-17. Critically, these SPMs can directly enhance ovarian cancer cell growth. Our work identifies an IL-17–dependent γδ T cell/SPM axis that promotes tumor development and thus counteracts cancer immunosurveillance.

DELAY OF GERMINATION 1 mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination

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Mechanisms of plant seed dormancy evolved to delay germination to a season favorable for seedling growth. Germination timing is an important adaptive early-life history trait which determines plant fitness in natural and agricultural ecosystems. The *DELAY OF GERMINATION 1* (*DOG1*) gene provides natural genetic variation in dormancy, was the first dormancy-specific gene cloned, and encodes a protein of unknown function. We show here (pp. E3571–E3580) that DOG1 controls dormancy of different species by setting the optimal ambient temperature window for germination. This timing is achieved by temperature-dependent alteration of the gibberellin hormone metabolism, which in turn leads to altered expression of genes required for the biomechanical weakening of the coat encasing the embryo. The conserved DOG1-mediated coat-dormancy mechanism controls the timing of seed germination in a temperature-dependent manner.