PNAS Plus Significance Statements

Secondary interaction between MDMX and p53 core domain inhibits p53 DNA binding

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MDMX is a critical regulator of p53 and a potential drug target. The mechanisms by which MDMX inhibit p53 are not fully understood. Results in this report suggest that MDMX inhibits p53 DNA-binding function. Using a protein fragment release assay, MDMX and p53 were found to engage in multiple strong secondary interactions following initial binding through the canonical binding domains. These secondary interactions are involved in blocking p53 DNA binding and stabilizing the MDMX–p53 complex. The results suggest that secondary interactions play important roles in regulating the function of multidomain protein complexes. (See pp. E2558–E2563.)

biGBac enables rapid gene assembly for the expression of large multisubunit protein complexes

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At the molecular level, most processes in living systems are mediated by multisubunit protein complexes. Recombinant forms of these complexes are essential for analyzing their structure and function. Multigene expression constructs greatly improve recombinant protein complex preparations, but the generation of such constructs can be a rate-limiting step. To overcome this limitation, we have adapted Gibson assembly reactions for the rapid, efficient, and fast generation of numerous expression constructs in parallel and used the resulting biGBac method for expression of different cell-cycle complexes, composed of up to 17 different subunits. The biGBac technique enables the analyses of large protein complexes by systematic mutagenesis approaches that were not feasible before. (See pp. E2564–E2569.)

Mechanism of APC/C^{CDC20} activation by mitotic phosphorylation

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The ability of eukaryotic cells to pass their genomes properly from one cell generation to the next depends on the 1.2-MDa ubiquitin ligase complex APC/C (anaphase-promoting complex/cyclosome) and on the correct timing of its activation by the substrate adaptor CDC20 (cell division cycle 20). Although it has been known for two decades that mitotic APC/C phosphorylation is required for its activation by CDC20, the mechanistic basis of this process remained unknown, in part because the existence of numerous phospho-sites on APC/C made systematic mutagenesis approaches difficult. Here we have used the biGBac technique for the rapid assembly of multigene expression constructs to overcome this limitation and discovered that APC/C contains an autoinhibitory loop region that prevents CDC20 binding until it becomes phosphorylated in mitosis. (See pp. E2570–E2578.)

Programmable RNA-binding protein composed of repeats of a single modular unit

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The ability to monitor and perturb RNAs in living cells would benefit greatly from a protein architecture that targets RNA sequences in a programmable way. We report four protein building blocks, which we call Pumby modules, each of which targets one RNA base and can be concatenated in chains of varying composition and length. The Pumby building blocks will open up many frontiers in the measurement, manipulation, and biotechnological utilization of unmodified RNAs in intact cells and systems. (See pp. E2579–E2588.)

Canonical and noncanonical intraflagellar transport regulates craniofacial skeletal development

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Intraflagellar transport (IFT) plays a critical role in assembling primary cilia that mediate growth factor signaling. The disruption or dysfunction of IFT components can generate multiple diseases, including skeletal dysplasia. However, the mechanism by which IFT regulates skeletogenesis remains elusive. In the present study, we show that neural crest-specific deletion of the gene that encodes intraflagellar transport 20 (IFT20) in mice compromises ciliogenesis and the intracellular transport of collagen, leading to osteopenia in the face. Our findings highlight a unique function of IFT beyond its role in cilium assembly during craniofacial development, suggesting that IFT20 is indispensable for the regulation of not only ciliogenesis but also the intracellular

trafficking of collagen in the unique multipotent stem cell population of cranial neural crests. (See pp. E2589–E2597.)

Comparison of syncytiotrophoblast generated from human embryonic stem cells and from term placentas

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Syncytiotrophoblast (STB) is responsible for nutrient and gas exchange in the human placenta. STB also forms when human embryonic stem cells (ESCs) differentiate to trophoblast. Here we compare ESC-derived STB with cytotrophoblasts isolated from term placentas before and after such cells had fused to form STB. Although both types of STB expressed all common trophoblast marker genes, there were dissimilarities indicative of altered function and ontology. We propose that STB derived from ESCs represents syncytial tissue encountered at the initiation of placental development. These cells may provide the first in vitro model for studying origins of diseases of placentation ranging from implantation failure and early pregnancy loss to intrauterine growth retardation and preeclampsia. (See pp. E2598–E2607.)

Aridity and plant uptake interact to make dryland soils hotspots for nitric oxide (NO) emissions

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Nitric oxide (NO) controls the atmosphere's oxidative capacity. In soils, NO emissions are thought to be controlled by a tradeoff that develops in response to changes in soil moisture: dry soils limit substrate diffusion, whereas wet soils limit gas diffusivity, such that moist soils favor NO emissions. In drylands, however, NO emissions can be highest when soils are dry and immediately following rewetting. Aridity and vegetation interact to generate unexpected NO emission patterns. The shutdown in plant N uptake during the dry season causes NO emissions to increase, whereas arid conditions concentrate resources in dry soils, stimulating NO pulses upon rewetting. Chemistry governs the rapid initial NO pulse, whereas biological processes control later emissions as microbes recover from drought stress. (See pp. E2608–E2616.)

Origin and evolution of developmental enhancers in the mammalian neocortex

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The neocortex mediates complex cognitive and motor tasks in all mammals. A long-debated question is how this complex structure evolved in primitive mammals. Here we investigate the role of novel mammalian gene regulatory sequences in the emergence of the neocortex and the mechanisms by which these sequences emerged. We find that ~20% of elements active during human and mouse neocortical development were born in early mammals. These novel mammalian elements enrich for cell migration, cell signaling, and axon guidance functions, implicating these processes in neocortical origins. In contrast to recent studies, we propose a model in which novel regulatory elements emerge as short sequences of minimal biological significance. Many disappear, but those that survive become increasingly complex over time. (See pp. E2617–E2626.)

Targeting IL-17A attenuates neonatal sepsis mortality induced by IL-18

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Infants born prematurely suffer the greatest incidence of and impact from sepsis among all age groups. Therapeutic interventions aimed at reducing morbidity and mortality in this vulnerable population have been unsuccessful. Interleukin (IL)-18 is a proinflammatory member of the IL-1 superfamily. Serum IL-18 concentrations in uninfected premature infants are increased as compared with healthy adults. We show that IL-18 in the setting of sepsis results in gut injury, a potentiation of the host's inflammatory response, increased bacteremia, and mortality mediated by IL-1 receptor 1 (IL-1R1)–dependent IL-17A produced by γδT and myeloid cells. The discovery of this novel IL-18/IL-1R1/IL-17A axis brings new hope for therapeutic interventions that target downstream IL-17A and ultimately reduce the increased mortality from sepsis in this understudied population. (See pp. E2627–E2635.)

Large-scale sequence and structural comparisons of human naive and antigen-experienced antibody repertoires

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We applied a very recently developed experimental strategy for high-throughput sequencing of paired antibody heavy and light chains along with large-scale computational structural modeling to delineate features of the human antibody repertoire at unprecedented scale. Comparison of antibody repertoires encoded by peripheral naive and memory B cells revealed (*i*) preferential enrichment or depletion of specific germline gene combinations for heavy- and light-chain variable regions and (*ii*) enhanced positive charges, higher solvent-accessible surface area, and greater hydrophobicity at antigen-binding regions of mature antibodies. The data presented in this report provide fundamental new insights regarding the biological features of antibody selection and maturation and establish a benchmark for future studies of antibody responses to disease or to vaccination. (See pp. E2636–E2645.)

Durable antitumor responses to CD47 blockade require adaptive immune stimulation

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Therapeutic antitumor antibodies are widely used clinically. CD47 is an antiphagocytic ligand expressed by tumors that binds the inhibitory receptor signal regulatory protein alpha (SIRPα) on phagocytic cells. Interruption of CD47–SIRPα interactions in immunodeficient mice bearing human tumors enhances therapeutic antitumor antibody responses by promoting phagocytosis of antibody-bound tumor cells. Here, we use a novel anti-CD47 single domain antibody, derived from an alpaca, in an immunocompetent mouse model of melanoma and find that, in contrast to immunodeficient models, CD47 blockade alone is insufficient to enhance the effects of antimelanoma antibodies. However, when combined with blockade of programmed death-ligand 1 (PD-L1), an immune receptor that inhibits antitumor T cell responses, we find

synergistic activity, suggesting a role for both innate and adaptive inhibitory pathways in the response to therapeutic antibodies. (See pp. E2646–E2654.)

Retinal neurodegeneration may precede microvascular changes characteristic of diabetic retinopathy in diabetes mellitus

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Diabetic retinopathy (DR), a primary cause of blindness, is characterized by microvascular abnormalities. Recent evidence suggests that retinal diabetic neuropathy (RDN) also occurs in people with diabetes, but little is known about the temporal relationship between DR and RDN. This longitudinal study in people with diabetes with no or minimal DR shows that RDN precedes signs of microvasculopathy and that RDN is progressive and independent of glycated hemoglobin, age, and sex. This finding was further confirmed in human donor eyes and in two experimental mouse models of diabetes. The results suggest that RDN is not ischemic in origin and represent a shift in our understanding of the pathophysiology of this complication of diabetes that potentially affects vision in all people with diabetes mellitus. (See pp. E2655–E2664.)

Polarized localization of voltage-gated Na^+ channels is regulated by concerted FGF13 and FGF14 action

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Neurons concentrate voltage-gated sodium channels (VGSCs) in axons while limiting VGSCs in the somatodendritic compartment. This axonal concentration is important for efficient generation of the action potential, but it remains unclear what limits the concentration of VGSCs in the somatodendritic compartment. Here, we report that two highly similar molecules, FGF13 and FGF14, collaborate to maintain proper VGSC localization. FGF13 is a critical mediator of the process that keeps VGSCs low in the somatodendritic compartment, whereas FGF14 is an important regulator of VGSC localization to the axon. (See pp. E2665–E2674.)

Astrocytes regulate cortical state switching in vivo

Kira E. Poskanzer and Rafael Yuste

Astrocytes—a type of glial cell—and neurons function together in neural circuits, but how astrocytes affect circuit function remains poorly understood. By measuring the fluorescent calcium activity of astrocytes while recording the electrophysiological oscillations in the mouse cortex, we find that astrocytes, through regulation of extracellular glutamate, are involved in triggering a slow neuronal rhythm in the brain that has been shown to be important in sleep and memory formation. (See pp. E2675–E2684.)

Astrocytes regulate heterogeneity of presynaptic strengths in hippocampal networks

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We addressed the basic mechanisms underlying synapse heterogeneity, and we identified a form of regulation that serves to increase the variations in the efficacy with which neurons communicate with each other through synapses. We demonstrate that this process requires astrocytes, which, previously, have been thought to play mostly a passive role in maintaining neuronal functions. The cellular mechanism that regulates synaptic efficacy requires astrocyte membrane depolarization, activation of astrocyte NMDA receptors, and astrocyte calcium signaling. The fundamental nature of the regulation is underscored by the preservation of the mechanism from acute brain slices down to dissociated cultures that lack the native topology of brain networks. (See pp. E2685–E2694.)

α/β-Hydrolase domain-containing 6 (ABHD6) negatively regulates the surface delivery and synaptic function of AMPA receptors

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AMPA receptors (AMPARs) are major postsynaptic receptors that mediate fast excitatory neurotransmission and synaptic plasticity. The proper functioning of AMPARs is essential for brain function; AMPAR dysfunction can cause multiple neurologic disorders, including autism. Native AMPARs are macromolecular complexes associated with a variety of auxiliary proteins, including α/β-hydrolase domain-containing 6 (ABHD6), which was recently identified. However, the physiological significance of the ABHD6–AMPAR association has not been investigated. Here, using both loss-of-function and gain-of-function approaches, we show that ABHD6 negatively regulates the surface delivery and synaptic function of AMPARs in neurons. The cytoplasmic tail of GluA1, but not the hydrolase activity of ABHD6, is essential for this functional interaction. Thus, these new findings expand our understanding of the molecular mechanisms governing AMPAR trafficking in the brain. (See pp. E2695–E2704.)

IL-33 ameliorates Alzheimer's disease-like pathology and cognitive decline

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Dysfunction of the innate immune system is involved in the pathogenesis of Alzheimer's disease (AD); however, the pathophysiological mechanisms underlying these dysfunctions are unclear. Here we report that stimulation of IL-33/ST2 signaling rescues memory deficits and reduces the accumulation of β-amyloid in APP/PS1 mice that exhibit select pathologies associated with AD. Although impaired IL-33/ST2 signaling is associated with early progression of AD, IL-33 injection rescues contextual memory deficits and reduces the accumulation of β-amyloid in APP/PS1 mice. IL-33 skews the microglia toward an alternative activation state with enhanced Aβ phagocytic capacity and elevated antiinflammatory gene expression, which results in a decreased proinflammatory response in the brain. Thus, this study suggests that IL-33 can be developed as a new therapeutic intervention for AD. (See pp. E2705–E2713.)

Memory retrieval of inhibitory avoidance requires histamine H_1 receptor activation in the hippocampus

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Several neurotransmitters contribute to memory formation by modulating selectively acquisition, consolidation, and/or retrieval. Integrity of the brain histamine system is necessary for the consolidation of inhibitory avoidance (IA) memory. Here, we report that cerebral histamine depletion also impairs retrieval of IA

in rats and blunts retrieval-induced c-Fos activation and cAMP-responsive element binding protein phosphorylation in the CA1 region of the hippocampus. Histamine infusion into the CA1 restores IA retrieval in histamine-depleted rats by targeting brain histamine H_1 receptors. Our study uncovers previously unidentified mechanisms involved in memory retrieval and may offer possible targets for eventual pharmacotherapies to treat dysfunctional aversive memories, including phobias, panic attacks, and posttraumatic stress disorders, as well as improve the efficacy of exposure psychotherapies. (See pp. E2714–E2720.)

$GABA_B$ receptor-mediated, layer-specific synaptic plasticity reorganizes gamma-frequency neocortical response to stimulation

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How the brain deals with the barrage of sensory information during wakefulness determines cognitive performance. Strategies include bias toward attending to novel sensory information and an ability to enhance or habituate cortical responses to repeated inputs. Here we show both enhancement and habituation occur simultaneously in different layers of cortex and that the plastic processes involved require activation of the $GABA_B$ subtype of neuronal inhibition. The work demonstrates that the brain can change the way it routes repeatedly presented sensory information in two complementary ways: It optimizes the local cortical representation, including more information as the stimulus is repeated; and it minimizes the output to other areas, preserving only outputs most closely correlated with the local cortical representation. (See pp. E2721–E2729.)

Sex differences in the circadian regulation of sleep and waking cognition in humans

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Circadian rhythms affect our physiology and psychology, in health and disease. Most of our knowledge about the human circadian timing system is based on research in men. Some circadian characteristics, such as the intrinsic frequency of the circadian clock and the amplitude of the melatonin rhythm, have been shown to differ between men and women. Whether the circadian regulation of mental functions differs between men and women is unknown. Here we show that circadian rhythmicity in mental functions exhibits sex differences so that the night-time impairment in cognitive performance is greater in women than in men. These findings are significant in view of shift-work–related cognitive deficits and disturbances of mood, which are more prevalent in women. (See pp. E2730–E2739.)

Tobacco mosaic virus-directed reprogramming of auxin/indole acetic acid protein transcriptional responses enhances virus phloem loading

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For plant viruses a successful infection correlates with the ability to access the vascular phloem and move systemically into distal tissues. However, how viruses gain access to and usurp vascular tissues is poorly understood. Here we show how tobacco mosaic virus (TMV) enhances its access to the phloem of mature plant tissues through the targeted disruption of auxin/indole acetic acid (Aux/IAA) transcriptional regulators that control expression of host genes involved in virus cell-to-cell movement, plasmodesmata gating, and defense. TMV's ability to disrupt Aux/IAA function successfully confers a significant advantage in the systemic spread of this virus, allowing it to outcompete nondisrupting viruses. In summary, TMV interacts with Aux/IAA proteins to reprogram the vascular phloem, making it more conducive to systemic movement. (See pp. E2740–E2749.)

Gelada vocal sequences follow Menzerath's linguistic law

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Human language follows a variety of structural principles, known as linguistic laws. One of these, Menzerath's law, states that, the larger the size of the construct (e.g., the size of a word in terms of syllable number), the smaller the size of the individual constituent parts (e.g., syllables). We show for the first time (to our knowledge) that Menzerath's law also holds in the vocal communication of a nonhuman species. In the gelada (*Theropithecus gelada*), a primate living in the highlands of Ethiopia, longer vocal sequences produced by adult males were associated with shorter individual calls. This result suggests that general—perhaps universal—principles underpin the structure of vocal communication in our own species and others. (See pp. E2750–E2758.)