PNAS Plus Significance Statements

Unique amalgamation of primary and secondary structural elements transform peptaibols into potent bioactive cell-penetrating peptides

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Using a combined approach relying on mass spectrometric analysis and molecular phylogeny, a fungus was identified that produced the gichigamins, which are peptaibols that contain a remarkable combination of structural features. The gichigamins possess a repeating α-residue/α-residue/β-residue motif creating a 3_{11}-*P*-helix secondary structure. These structural elements confer upon the gichigamins the unique ability among peptaibols to enter into cells whereupon they disrupt mitochondrial function. Semisynthetic modifications further enhanced gichigamin mitochondrial depolarization and cytotoxicity, while removing virtually all plasma-membrane pore-forming capabilities. These discoveries open vistas for engineering peptaibols into potent cytotoxins and intracellular delivery tools that are devoid of ion leakage effects. (See pp. E8957–E8966.)

Topological phenomena in classical optical networks

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We introduce a unique scheme to investigate topological behavior, using optical-passive elements and Kerr nonlinearities. Compared with previous proposals, the topological band gaps are dramatically broadened, leading to very robust edge modes. Our setup displays intriguing phenomena in the nonlinear regime, including instabilities and the production of squeezed light in the edge modes. This proposal promises unique avenues for engineering phases of light with topological character. (See pp. E8967–E8976.)

Mechanistic principles underlying regulation of the actin cytoskeleton by phosphoinositides

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Membrane phosphoinositides have emerged as key regulators of the actin cytoskeleton in cell migration, morphogenesis, cytokinesis, and endocytosis. However, the molecular mechanisms by which actin-binding proteins (ABPs) interact with phosphoinositide-rich membranes remain remarkably poorly understood. By applying a combination of biochemical, biophysical, and atomistic molecular dynamics simulation approaches on six central ABPs, we discovered that they employ multivalent electrostatic interactions for membrane binding. Strikingly, our experiments revealed that these proteins display enormous differences in their membrane interaction dynamics and in the ranges of phosphoinositide densities that they sense. These differences precisely correlate with the specific functions of these proteins in cytoskeletal dynamics. These findings uncover molecular principles by which membrane phosphoinositides regulate dynamics and architecture of the actin cytoskeleton in cells. (See pp. E8977–E8986.)

Broad role for YBX1 in defining the small noncoding RNA composition of exosomes

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Cells release vesicles containing selectively packaged cargo, including RNA, into the extracellular environment. Prior studies have identified RNA inside extracellular vesicles (EVs), but due to limitations of conventional sequencing methods, highly structured and posttranscriptionally modified RNA species were not effectively captured. Using an alternative sequencing approach (thermostable group II intron reverse transcriptase sequencing, TGIRT-seq), we found that EVs contain abundant small noncoding RNA species, including full-length transfer RNAs and Y RNAs. Using a knockout cell line, we obtained evidence that the RNA-binding protein YBX1 plays a role in sorting small noncoding RNAs into a subpopulation of EVs termed exosomes. These experiments expand our understanding of EV–RNA composition and provide insights into how RNA is sorted into EVs for cellular export. (See pp. E8987–E8995.)

Staufen1 inhibits MyoD translation to actively maintain muscle stem cell quiescence

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This work addresses a fundamental mechanism for the translational control of a master regulator of myogenic differentiation, MyoD, by the RNA binding protein Staufen1. We show that muscle stem cells express the MyoD transcript in the quiescent state in vivo but block its translation through direct repression by Staufen1. Loss of this translational repression leads to MyoD translation and cell cycle entry, highlighting

a novel role for MyoD in regulating the exit from quiescence. This mechanism of direct translational repression enables the cells to exist poised for activation and cell cycle entry. These data provide insight in the translational control of muscle stem cell quiescence. (See pp. E8996–E9005.)

Content of mitochondrial calcium uniporter (MCU) in cardiomyocytes is regulated by microRNA-1 in physiologic and pathologic hypertrophy

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Mitochondrial calcium uniporter (MCU) is the core channel subunit of the mitochondrial Ca^{2+} uniporter complex (MCUC) and contributes to the regulation of ATP production. Here, we identify that the adaptive and maladaptive remodeling occurring in rodent and human cardiomyocytes is associated with changes in MCU content, which are inversely correlated with those of its regulator microRNA-1 (miR-1). The present study thus defines the molecular mechanism by which MCU content and, consequently, mitochondrial Ca^{2+} uptake are directly modified by alteration of miR-1, a key regulator affecting physiological and pathological heart growth. These findings provide evidence for the miR-1/MCU axis as a potential target for the development of novel therapeutic approaches. (See pp. E9006–E9015.)

Modulation of apoptotic response by LAR family phosphatases–cIAP1 signaling during urinary tract morphogenesis

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Apoptotic morphogenesis requires strict regulation as both excessive and insufficient cell death is detrimental to tissue function. Here we use the process of ureter maturation, wherein the ureter connects to the bladder by the apoptosis-mediated removal of the intervening common nephric duct (CND), to investigate this paradigm. We find that LAR family phosphatases antagonize cIAP1 activity by decreasing its stability, thereby releasing the brake on CND elimination. In addition, we demonstrate that *Birc2* (cIAP1) mutant embryos exhibit increased CND apoptosis, leading to accelerated ureter maturation and vesicoureteral reflux. Together this highlights the importance of modulating the rate of apoptosis during morphogenesis, which may act as a morphogenetic timer to allow for appropriate tissue rearrangements during embryonic development. (See pp. E9016–E9025.)

Mutator genomes decay, despite sustained fitness gains, in a long-term experiment with bacteria

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Bacterial genomes are extremely diverse in size and composition. Biologists have long sought to explain such variability based on present-day selective and mutational forces. However, mutation rates can change dramatically over time, and experiments with hypermutable bacteria show that their genomes rapidly decay when propagated under the near absence of selection. Whether selection can prevent this decay is unclear. Here, we document the rapid genome decay of hypermutable bacteria even during tens of thousands of generations of sustained adaptation to a laboratory environment. These findings suggest the need to reexamine current ideas about the evolution of bacterial genomes, and they have implications for other hypermutable systems such as viruses and cancer cells. (See pp. E9026–E9035.)

Kinship and familiarity mitigate costs of social conflict between Seychelles warbler neighbors

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In nature, animals must compete with their neighbors for access to limited resources. Since conflict over resources can be extremely costly in terms of time, energy, and reproductive success, investigating how individuals resolve conflict is crucial to understanding the evolution of social behaviors. In the Seychelles warbler, we demonstrate two mechanisms by which individuals minimize costs of conflict and show the benefits individuals gain from doing so. Birds that live near relatives or familiar individuals invest less energy in defending and maintaining territory borders and also show less aging-related signs of physiological damage. Our results suggest that conflict between neighbors can be mitigated by kin-selected benefits of sharing resources with relatives but also through direct mutual benefits of cooperation. (See pp. E9036–E9045.)

UTX-guided neural crest function underlies craniofacial features of Kabuki syndrome

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Several chromatin-modifying enzymes are mutated in human craniofacial disorders. These factors function genome-wide to regulate accessibility and expression of extensive gene sets. Therefore, understanding chromatin-modifier function requires identification of responsible cellular origins and genomic characterization of phenotypes in primary cells. We now combine reporter flow cytometry with low cell number genomics to identify neural crest stem-cell factors as molecular targets of UTX in Kabuki syndrome. UTX demethylates histones to regulate some gene expression, but many UTX-bound regions are subject to novel mechanisms of transcriptional regulation. This study identifies UTX cellular and molecular targets in craniofacial development and this methodology is broadly adaptable to study genome distribution of other chromatin factors in neural crest disorders. (See pp. E9046–E9055.)

Human papillomavirus oncoproteins induce a reorganization of epithelial-associated γδ T cells promoting tumor formation

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Of all tumor-infiltrating leukocytes, T cells bearing γδ T-cell receptors have been associated with the most favorable prognosis. However, we show here, in a mouse model of carcinogenesis induced by human papillomavirus (HPV) oncoproteins, that γδ T cells promoted the development of HPV-induced lesions. Indeed, HPV-oncoprotein expression induced an infiltration of γδ T cells producing IL-17A, a proangiogenic cytokine, and

decreased density of antitumor $V\gamma5^+$ γδ T subsets. Supporting the clinical relevance of our observations, IL-$17A^+$ γδ T cells were detected in human cervical cancer, where HPV oncoproteins are highly expressed, but not in less advanced cervical lesions. These results support the notion that viral oncoproteins can induce a switch from antitumoral to protumoral γδ T subsets in solid tumors. (See pp. E9056–E9065.)

Packaging and transfer of mitochondrial DNA via exosomes regulate escape from dormancy in hormonal therapy-resistant breast cancer

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Increasing evidence suggests that extracellular vesicles (EVs) can transfer genetic material to recipient cells. However, the mechanism and role of this phenomenon are largely unknown. Here we have made a remarkable discovery: EVs can harbor the full mitochondrial genome. These extracellular vesicles can in turn transfer their mtDNA to cells with impaired metabolism, leading to restoration of metabolic activity. We determined that hormonal therapy induces oxidative phosphorylation-deficient breast cancer cells, which can be rescued via the transfer of mtDNA-laden extracellular vesicles. Horizontal transfer of mtDNA occurred in cancer stem-like cells and was associated with increased self-renewal potential of these cells, leading to resistance to hormonal therapy. We propose that mtDNA transfer occurs in human cancer via EVs. (See pp. E9066–E9075.)

Human papillomavirus oncogenes reprogram the cervical cancer microenvironment independently of and synergistically with estrogen

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A subset of human papillomaviruses (HPVs) causes 5% of human cancers, including virtually all cancers of the cervix. In a mouse model of cervical cancer, estrogen is a necessary cofactor that contributes to disease by signaling through the underlying tumor microenvironment. In this study, we discovered that epithelial expression of the HPV oncoproteins reprograms the cervical tumor microenvironment and its response to estrogen. These changes involve the elicitation of paracrine-acting factors implicated in carcinogenesis, and the expression of a subset of these factors was also induced in cocultures of human cervical cancer cells and stromal fibroblasts. We hypothesize that HPV oncogenes cause cancer in part by creating a unique tumor microenvironment that synergizes with estrogen in the cervix. (See pp. E9076–E9085.)

PAF promotes stemness and radioresistance of glioma stem cells

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Glioblastoma multiforme (GBM) is uniformly lethal and shows resistance to all forms of therapy. Glioma stem cells (GSCs) have been shown to support GBM maintenance and exhibit enhanced resistance to ionizing radiation, a cornerstone of GBM therapy. This study establishes that proliferating cell nuclear antigen-associated factor (PAF) depletion profoundly reduces GSC frequency and tumorigenicity, in part, by down-regulating DNA replication and pyrimidine metabolism. Moreover, PAF depletion impairs error-prone DNA translesion synthesis (TLS) and enhances sensitivity of GSCs to radiation treatment. Pharmacological impairment of DNA replication and TLS diminished GSC maintenance and radioresistance, illuminating a potential GBM treatment strategy of combined TLS inhibition and radiation therapy. (See pp. E9086–E9095.)

Cardiovascular homeostasis dependence on MICU2, a regulatory subunit of the mitochondrial calcium uniporter

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Hypertension increases the risk for development of abdominal aortic aneurysms, a silent pathology that is prone to rupture and cause sudden cardiac death. Male gender, smoking, and hypertension appear to increase risk for development of abdominal aortic aneurysms by provoking oxidative stress responses in cardiovascular tissues. Here we uncovered unexpected linkages between the calcium-sensing regulatory subunit MICU2 of the mitochondrial calcium uniporter and stress responses. We show that naive *Micu2*$^{-/-}$ mice had abnormalities of cardiac relaxation but, with modest blood pressure elevation, developed abdominal aortic aneurysms with spontaneous rupture. These findings implicate mitochondrial calcium homeostasis as a critical pathway involved in protecting cardiovascular tissues from oxidative stress. (See pp. E9096–E9104.)

Spatial organization of a model 15-member human gut microbiota established in gnotobiotic mice

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Spatial structure is postulated to have a powerful influence on establishing and sustaining the signaling and metabolic exchanges that define relationships among members of the gut microbiota and host. However, information about gut community spatial structure is limited. Simultaneous imaging of components of a 15-member model human gut bacterial community over a range of spatial scales in gnotobiotic mice revealed that the colon is better conceptualized as an incompletely mixed bioreactor, rather than having sharply stratified luminal and mucosal compartments. Identifying host and microbial factors that constrain the ability of community members to establish sizeable single or oligotaxon agglomerations should yield new insights about how "micro"-scale mixing defines community function. (See pp. E9105–E9114.)

Visual perception as retrospective Bayesian decoding from high- to low-level features

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The nature of perceptual decoding remains an open, fundamental question. Many studies assume that decoding follows the same low- to high-level hierarchy of encoding, yet this assumption was never rigorously tested. We performed such a test, which refutes the assumption to the extent that absolute and relative/ordinal orientations are features of different levels. Additionally, the backward aftereffect we discovered cannot be explained by the efficient-coding theories of adaptation. Finally, we proposed a new theory that explains our data as retrospective Bayesian decoding from high

to low levels in working memory. This decoding hierarchy is justified by considering memory stability/distortion of high/low-level features. Thus, our work rejects the currently dominant decoding scheme and offers a framework that integrates perceptual decoding and working memory. (See pp. E9115–E9124.)

Opioid and orexin hedonic hotspots in rat orbitofrontal cortex and insula

Daniel C. Castro and Kent C. Berridge

Orbitofrontal cortex, insula, and related cortical regions are implicated in pleasure and motivation. However, determining whether cortical sites help cause hedonic reactions or instead merely encode signals generated elsewhere to facilitate other functions such as cognition remains unresolved. By mapping hedonic effects of individual drug microinjections, we generate detailed anatomical maps for potential gain-of-function affective sites in rat limbic cortex. Here, we show that opioid or orexin stimulations in orbitofrontal cortex and insula causally enhance hedonic "liking" reactions to sweetness and find a third cortical site where the same neurochemical stimulations reduce positive hedonic impact. For comparison, we also map overlapping but separable regions where stimulations increase the motivation to eat. (See pp. E9125–E9134.)

M344 promotes nonamyloidogenic amyloid precursor protein processing while normalizing Alzheimer's disease genes and improving memory

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Hundreds of failed clinical trials with Alzheimer's disease (AD) patients over the last fifteen years demonstrate that the one-target–one-disease approach is not effective in AD. In silico, structure-based, multitarget drug design approaches to treat multifactorial diseases have not been successful in the context of AD either. Here, we show that M344, an inhibitor of class I and IIB histone deacetylases, affects multiple AD-related genes, including those related to both early- and late-onset AD. We also show that M344 improves memory in the 3xTg AD mouse model. This work endorses a shift to a multitargeted approach to the treatment of AD, supporting the therapeutic potential of a single small molecule with an epigenetic mechanism of action. (See pp. E9135–E9144.)

Perceiving social interactions in the posterior superior temporal sulcus

Leyla Isik, Kami Koldewyn, David Beeler, and Nancy Kanwisher

Humans spend a large percentage of their time perceiving the appearance, actions, and intentions of others, and extensive previous research has identified multiple brain regions engaged in these functions. However, social life depends on the ability to understand not just individuals, but also groups and their interactions. Here we show that a specific region of the posterior superior temporal sulcus responds strongly and selectively when viewing social interactions between two other agents. This region also contains information about whether the interaction is positive (helping) or negative (hindering), and may underlie our ability to perceive, understand, and navigate within our social world. (See pp. E9145–E9152.)

Dynein/dynactin is necessary for anterograde transport of *Mbp* mRNA in oligodendrocytes and for myelination in vivo

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Oligodendrocytes in the brain insulate neuronal axons in layers of fatty myelin to facilitate fast electrical signaling. Myelin basic protein (MBP), an important myelin component, is transported as mRNA away from the cell body before being translated into protein. In zebrafish, the anterograde motor kinesin transports *mbp* mRNA away from the cell body. We now identify myelination defects in zebrafish caused by a mutation in the retrograde motor complex dynein/dynactin, which normally transports cargos back toward the cell body. However, this mutant displays defects in anterograde *mbp* mRNA transport. We confirm in mammalian oligodendrocyte cultures that drug inhibition of dynein arrests transport in both directions and decreases MBP protein levels. Thus, dynein/dynactin is paradoxically required for anterograde *mbp* mRNA transport. (See pp. E9153–E9162.)

β_2-Adrenoceptor signaling in airway epithelial cells promotes eosinophilic inflammation, mucous metaplasia, and airway contractility

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Activation of β_2-adrenoreceptors (β_2ARs) on airway smooth muscle cells produces airway relaxation, and β_2AR agonists are the most widely used bronchodilators for treating asthma. Paradoxically, murine models show β_2AR activation is also required for expression of cardinal features of the asthma phenotype, including airway hyperresponsiveness (AHR), inflammation, and mucous metaplasia. However β_2ARs are expressed on all the cell types implicated in the pathogenesis and maintenance of asthma, and which cell type(s) control these asthmatic effects is unknown. Here we show activation of β_2AR signaling solely on airway epithelium is sufficient to restore/promote the cardinal features of asthma, including inflammation, mucous metaplasia, and AHR. These studies support the role of the airway epithelium as a master regulator of key features of asthma. (See pp. E9163–E9171.)

Pathogenesis of hypothyroidism-induced NAFLD is driven by intra- and extrahepatic mechanisms

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Cross-sectional studies have demonstrated that hypothyroidism is an independent risk factor for nonalcoholic fatty liver disease (NAFLD). However, the pathogenesis of hypothyroidism-induced NAFLD has yet to be characterized. Here we found that hypothyroidism induces NAFLD through a pleiotropic effect of thyroid hormones (THs) on insulin secretion and adrenergic stimulation of lipolysis in adipose tissue. A mild reduction in serum TH levels impairs insulin secretion, leading to impaired suppression of lipolysis and increased shuttling of fatty acids to the liver, where they induce NAFLD. Surprisingly, a severe reduction in serum TH levels protects against the development of NAFLD through a constitutive suppression of lipolysis. These results shed light on mechanisms that either induce or protect against NAFLD in hypothyroidism. (See pp. E9172–E9180.)