PNAS Plus Significance Statements

Iron oxides stimulate sulfate-driven anaerobic methane oxidation in seeps

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Anaerobic oxidation of methane (AOM) coupled to sulfate reduction has been shown to consume up to 90% of the greenhouse gas methane produced within the subseafloor environment; however, the mechanism of this process has remained enigmatic. Here, we provide geochemical evidence based on sulfur, oxygen, and carbon isotopes for the involvement of iron oxides in sulfate-driven AOM in methane seeps. Our results (pp. E4139–E4147) suggest that, beyond the function of iron as nutrient, the presence of iron oxides stimulates sulfate-driven AOM to a greater extent than in sediments with low concentrations of iron oxides. The isotope analyses further indicate that sulfate reduction in methane seep habitats differs than sulfate reduction in diffusive profiles in and above the sulfate–methane transition zone.

Basis for substrate recognition and distinction by matrix metalloproteinases

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Specificity-determining positions (SDPs) account for distinctions in function across a protein family. Many theories on the evolution of functional specificity have led to approaches for predicting SDPs in silico, but large experimental datasets allowing a statistical assignment are lacking. Here (pp. E4148–E4155), the SDPs of matrix metalloproteinases are elucidated by querying the proteolytic efficiency of eight matrix metalloproteinases, representing three phylogenetic branches, in an extended and diverse substrate space. More than 10,000 measures of cleavage efficiency reveal a near-perfect correlation between similarity in proteolytic function and sequence identity at 50–57 positions on the front face of the catalytic domain. These positions are assigned as SDPs. Transmutation of proteolytic function is possible by swapping SDPs nearest to bound substrate.

Structural insights into the role of iron–histidine bond cleavage in nitric oxide-induced activation of H-NOX gas sensor proteins

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Nitric oxide (NO) influences diverse biological processes, ranging from vasodilation in mammals to communal behavior in bacteria. Heme-nitric oxide/oxygen (H-NOX) binding domains, a recently discovered family of heme-based gas sensor proteins (pp. E4156–E4164), have been implicated as regulators of these processes. Crucial to NO-dependent activation of H-NOX proteins is rupture of the heme–histidine bond and formation of a five-coordinate NO complex. To delineate the molecular details of NO binding, high-resolution crystal structures of a bacterial H-NOX protein in the unligated and intermediate six- and five-coordinate NO-bound states are reported. From these structures, it is evident that NO-induced scission of the heme–histidine bond elicits a pronounced conformational change in the protein as a result of structural rearrangements in the heme pocket.

Modeling putative therapeutic implications of exosome exchange between tumor and immune cells

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A better understanding of mechanisms of immune evasion by cancer cells and the role of the tumor microenvironment is crucial for developing new effective cancer therapeutic strategies. The challenge is posed by the enormous complexity of both the immune system and the tumor microenvironment, and the intricate cancer–immunity signaling network. Here (pp. E4165–E4174), we develop a tractable theoretical framework to study the putative role of exosome communication in the cancer–immunity interplay. Exosomes are small (30–200 nm) vesicles that transfer proteins, mRNAs, and microRNAs to nearby and faraway cells. Guided by this model, we compare the effectiveness of administering radiation therapy alone or in combination with immunotherapy, illustrating how the model can shed light on the design and assessments of combination therapies.

Structural and energetic determinants of adhesive binding specificity in type I cadherins

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Type I cadherins comprise a family of cell–cell adhesion proteins that dimerize in a highly specific fashion. There are small differences in dimerization affinities among family members that are evolutionarily conserved and that have profound effects on cell-patterning behavior. There are few examples where the molecular origins of small affinity differences between closely related proteins have been explored in depth. We have brought an unusually broad range of technologies to bear on the problem in a unique integrated approach. Our results (pp. E4175–E4184) reveal how a subtle combination of physical interactions combine to tune binding affinities and, in the course of our analysis, we discover a new conformational entropy-based mechanism that can also be exploited by other multidomain proteins.

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Bimodal activation of BubR1 by Bub3 sustains mitotic checkpoint signaling

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The mitotic checkpoint (or the spindle assembly checkpoint) ensures genome integrity by preventing premature chromosome segregation. The pathway is triggered locally by kinetochores, multiprotein complexes assembled onto centromeres. Unattached kinetochores produce Mad2 bound to Cdc20, the mitotic activator of the E3 ubiquitin ligase APC/C. The initial Mad2–Cdc20 complex is then converted into the final mitotic checkpoint inhibitor Bub3–BubR1–Cdc20 that blocks APC/C (anaphase promoting complex or cyclosome)-dependent ubiquitination of cyclin B and securin, thereby stabilizing them and preventing an advance to anaphase. In this study (pp. E4185–E4193), we identify dual mechanisms by which Bub3 promotes mitotic checkpoint signaling. Bub3 binding to BubR1 promotes two distinct BubR1–Cdc20 interactions, one acting at unattached kinetochores and the other cytoplasmically to facilitate production of the mitotic checkpoint inhibitor.

Beclin-1 deficiency in the murine ovary results in the reduction of progesterone production to promote preterm labor

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The success of mammalian reproduction is contingent upon the production of hormones within the female to not only promote germ cell development, but to establish and maintain pregnancy. We demonstrate (pp. E4194–E4203) that abrogating autophagy, a cellular process to maintain energy stores, can lead to reproductive defects that prevent a successful pregnancy in mice. Females that lack the crucial autophagy gene *Beclin1* (*Becn1*) in the progesterone-producing cells of the ovary demonstrate reduced circulating progesterone and a preterm birth phenotype concurrent with the loss of litters, which is rescued by the administration of exogenous progesterone. Because progesterone is a necessary hormone for mammalian pregnancy, these data suggest that autophagy may play a role in steroidogenesis and, thus, in successful human reproduction.

Reforestation as a novel abatement and compliance measure for ground-level ozone

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Despite often decadeslong control efforts, in many regions of the world ambient concentrations of ground-level ozone threaten human and ecosystem health. Furthermore, in many places the effects of continuing land use and climate change are expected to counteract ongoing efforts to reduce ozone concentrations. Combined with the rising cost of more stringent conventional technological ozone controls, this creates a need to explore novel approaches to reducing tropospheric ozone pollution. Reforestation of peri-urban areas, which removes ozone and one of its precursors, may be a cost-effective approach to ozone control and can produce important ancillary benefits. We identify (pp. E4204–E4213) key criteria for maximizing the ozone abatement and cost effectiveness of such reforestation and the substantial potential for its application in the United States.

Transformation of quiescent adult oligodendrocyte precursor cells into malignant glioma through a multistep reactivation process

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How malignant gliomas arise in a mature brain remains a mystery, which hinders the development of effective treatments. Which cell types can escape their quiescent, adult state and how they do so is unknown. Additionally, because gliomas are only detected at advanced stages, the full course of transformation remains uncharacterized. Here (pp. E4214–E4223) we report that adult oligodendrocyte precursor cells, despite their relatively quiescent properties, can be reactivated to a highly proliferative state by *p53* and *NF1* mutations and give rise to malignant gliomas. Furthermore, we describe the early phase of gliomagenesis for the first time, revealing a multistep process of reactivation, dormancy, and final transformation in which mammalian target of rapamycin signaling plays a critical role at both early and late steps.

An extended Shine–Dalgarno sequence in mRNA functionally bypasses a vital defect in initiator tRNA

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Initiation, a regulatory step in mRNA translation is characterized by initiator tRNA ($tRNA^{fMet}$) binding to 30S ribosome together with initiation factors (IFs) to form 30S pre-initiation complex (PIC). This step is followed by binding of 50S ribosome and release of IFs to form elongation competent 70S complex. $tRNA^{fMet}$ is special in possessing a vital feature of three consecutive G-C (3GC) base pairs in its anticodon stem. However, the role of this feature has remained unclear. We show (pp. E4224–E4233) that the 3GC base pairs facilitate $tRNA^{fMet}$ retention in the ribosome during the transitions that mark conversion of 30S PIC into 70S complex. Furthermore, we show that translation of mRNAs having an extended Shine–Dalgarno sequence bypasses the requirement of the 3GC pairs in $tRNA^{fMet}$.

Chromatin reader L(3)mbt requires the Myb–MuvB/DREAM transcriptional regulatory complex for chromosomal recruitment

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Histone binding proteins are critical for chromosome function, and mechanisms targeting them to nucleosomes are crucial. The *Drosophila* tumor suppressor L(3)mbt binds to methylated lysines of nucleosomal histones repressing gene transcription. We show that L(3)mbt chromosomal targeting requires proteins of a site-specific DNA binding complex [Myb–MuvB (MMB)/DREAM] and

Mip120, an MMB/DREAM core component, is critical for recruitment. Surprisingly, chromosome association of L(3)mbt is insufficient for repression, as other MMB/DREAM members are required for L(3)mbt-mediated repression but not its chromosome targeting. Loss of *l(3)mbt* leads to lethal malignant brain tumors. We discuss our findings (pp. E4234–E4243) in the context of complex mechanisms where specific genes activated by loss of *l(3)mbt* may help tumor progression, whereas deletion of MMB genes may suppress this phenotype.

Involvement of the host DNA-repair enzyme TDP2 in formation of the covalently closed circular DNA persistence reservoir of hepatitis B viruses

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Chronic hepatitis B virus (HBV) infection puts >250 million humans at risk for developing liver cirrhosis and liver cancer. Current therapies are not curative because they do not target HBV´s persistence reservoir, the plasmid-like covalently closed circular DNA (cccDNA). RNA production from cccDNA initiates the generation of progeny virus via protein-primed reverse transcription, yielding viral polymerase-linked relaxed-circular DNA (RC-DNA). Its conversion, upon infection, into cccDNA requires multiple poorly understood steps, including polymerase removal. We found (pp. E4244–E4253) that the host enzyme tyrosyl-DNA-phosphodiesterase 2 (TDP2), important for repair of cellular protein–DNA adducts, performed this step in vitro and that TDP2 depletion impaired the conversion of RC-DNA to cccDNA in cells. These data establish a functional link between HBV and cellular DNA repair and pave the way for targeting HBV persistence.

Shigella IpaH7.8 E3 ubiquitin ligase targets glomulin and activates inflammasomes to demolish macrophages

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Shigella modulates macrophage cell death by activating nucleotide-binding oligomerization domain–like receptor (NLR) inflammasome to secure its own dissemination. Here (pp. E4254–E4263) we report that *Shigella* invasion plasmid antigen H7.8 (IpaH7.8) plays a central role in inducing macrophage cell death via activation of NLR family pyrin domain-containing 3 and NLR family CARD domain-containing 4 inflammasomes in an IpaH7.8 enzyme 3 (E3) ligase-dependent manner. Importantly, an IpaH7.8-deficient mutant was unable to egress from macrophages efficiently, resulting in delayed bacterial multiplication. We identified glomulin—a member of the S-phase kinase-associated protein 1–F-box–like complex that originally was identified as a protein required for normal vascular development—as a target for IpaH7.8 E3 ligase-mediated polyubiquitination, which leads to NLR inflammasome activation. In vitro and in vivo studies confirmed that IpaH7.8-mediated glomulin degradation during *Shigella* infection activated NLR inflammasomes and promoted cell death.

Object and spatial mnemonic interference differentially engage lateral and medial entorhinal cortex in humans

Zachariah M. Reagh and Michael A. Yassa

Episodic memories are complex records of experience, consisting of "what" happened as well as "where" and "when" it happened. Animal studies have demonstrated distinct brain networks supporting memory for information about what experience occurred and information about where the experience occurred. However, such dissociations have been elusive in humans. Using a memory interference task that pits object (i.e., what) vs. spatial (i.e., where) memories against each other and high-resolution fMRI, we report evidence for two parallel but interacting networks in the human hippocampus and its input regions, supporting prior work in animals. We propose (pp. E4264–E4273) a conceptual model of how object and spatial interference are reduced in the regions providing input to the hippocampus, allowing rich, distinct memories to be built.

α-Synuclein assembles into higher-order multimers upon membrane binding to promote SNARE complex formation

Jacqueline Burré, Manu Sharma, and Thomas C. Südhof

Physiologically, α-synuclein promotes soluble NSF attachment protein receptor (SNARE) complex assembly during synaptic exocytosis. Pathologically, however, α-synuclein forms neurotoxic aggregates that promote neurodegeneration and represent hallmarks of Parkinson's disease and other synucleinopathies. α-Synuclein exists in a monomeric unfolded state in solution and in an α-helical folded state upon binding to membranes. Yet the relation between these conformational states and their physiological and pathological roles remain unknown. Here, we demonstrate that α-synuclein multimerizes during membrane binding and that the membrane-bound, multimeric form of α-synuclein mediates SNARE complex assembly in presynaptic terminals. Our data (pp. E4274–E4283) delineate a folding pathway for α-synuclein that ranges from a monomeric unfolded form in cytosol to a physiologically functional multimeric form that is membrane bound and chaperones SNARE complex assembly, and that may protect against neurodegeneration.