PNAS Plus Significance Statements

Phevamine A, a small molecule that suppresses plant immune responses

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Bacterial pathogens cause plant diseases that threaten the global food supply. To control diseases, it is important to understand how pathogenic bacteria evade plant defense and promote infection. We identify from the phytopathogen *Pseudomonas syringae* a small-molecule virulence factor—phevamine A. Both the chemical structure and mode of action of phevamine A are different from known bacterial phytotoxins. Phevamine A promotes bacterial growth by suppressing plant immune responses, including both early (the generation of reactive oxygen species) and late (the deposition of cell wall reinforcing callose in leaves and leaf cell death) markers. This work uncovers a widely distributed, small-molecule virulence factor and shows the power of a multidisciplinary approach to identify small molecules important for plant infection. (See pp. E9514–E9522.)

Energy-dependent quenching adjusts the excitation diffusion length to regulate photosynthetic light harvesting

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Plants' photosynthetic mechanism adjusts to fluctuations in light intensity. Intermittent bright sunlight can damage light-harvesting proteins; to preempt this, plants dissipate excess absorbed excitation energy as heat. Energy-dependent quenching (qE) of excitations occurs on the seconds to minutes timescale through conformational changes in antenna proteins. Using a multiscale model of photosystem II, we show that changes in light harvesting due to qE can be explained using a single parameter, the excitation diffusion length, which decreases as qE activates. These findings have implications for the interpretation of pulse amplitude-modulated fluorescence, a common noninvasive measurement of photosynthetic activity in leaves. (See pp. E9523–E9531.)

Use of scenario ensembles for deriving seismic risk

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High death tolls from recent earthquakes have highlighted the need to better identify ways to effectively reduce seismic risk. We address this need by developing a new earthquake scenario ensemble approach. We model impacts from multiple different earthquake scenarios, identifying impacts that are common to multiple scenarios. This method allows us to estimate whether particular impacts are specific to certain earthquakes or occur irrespective of the location or magnitude of the next earthquake. Our method provides contingency planners with critical information on the likelihood, and probable scale, of impacts in future earthquakes, especially in situations where robust information on the likelihood of future earthquakes is incomplete, allowing disaster risk-reduction efforts to focus on minimizing such effects and reducing seismic risk. (See pp. E9532–E9541.)

Conductively coupled flexible silicon electronic systems for chronic neural electrophysiology

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A critical challenge for flexible biomedical implants is in the development of materials and structures that enable intimate coupling to biotissues with long-term stability. The results presented here address this problem through a materials and integration strategy that combines highly doped silicon nanomembranes chemically bonded to thin films of thermal silicon dioxide in a construct that simultaneously serves as a biofluid barrier and a conductively coupled biointerface. Use of this approach with various flexible electronic systems, including passive and active electrodes for electrophysiological sensing and electrical stimulation, illustrate capabilities in high-fidelity operation. Systematic accelerated lifetime studies in artificial biofluids highlight the stability of these systems for chronic operation, without electrical leakage or other forms of degradation. (See pp. E9542–E9549.)

ZFAND5/ZNF216 is an activator of the 26S proteasome that stimulates overall protein degradation

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ZFAND5/ZNF216, a member of the ubiquitous ZFAND protein family, contains two zinc finger domains. It is induced in skeletal muscle during atrophy and was shown to be essential for the resulting large

www.pnas.org/cgi/doi/10.1073/pnas.ss11541

loss of muscle mass. We show here that purified ZFAND5 stimulates the 26S proteasomes' capacity to degrade peptides and ubiquitinated proteins. In cells, it promotes the degradation of endogenous cell proteins through its ubiquitin-binding zinc finger domain (A20). Unlike several ZFAND proteins, it is not induced in proteotoxic stresses. Unlike other proteasome activators, ZFAND5 actually stimulates overall protein breakdown by the ubiquitin proteasome pathway. (See pp. E9550–E9559.)

ATP hydrolysis-coupled peptide translocation mechanism of *Mycobacterium tuberculosis* ClpB

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The *Mycobacterium tuberculosis* (*Mtb*) ClpB is a ring-shaped, ATP-driven disaggregase. The ability to rescue aggregated proteins is crucial for *Mtb* to grow and persist in the host. Despite extensive studies in the past two decades, it is still not well understood how a bacterial disaggregase couples ATP binding and hydrolysis to peptide translocation. Our cryo-EM study of the *Mtb* ClpB in the presence of a peptide substrate and the slowly hydrolysable adenosine 5′-[γ-thio]triphosphate revealed two active conformations in the midst of the substrate-threading process. This, together with the resolved nucleotide state in each of the 12 nucleotide-binding domains of the ClpB hexamer, helps define a detailed atomic trajectory that couples ATP binding and hydrolysis to mechanical protein translocation. (See pp. E9560–E9569.)

Role for ERK1/2-dependent activation of FCHSD2 in cancer cell-selective regulation of clathrin-mediated endocytosis

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Clathrin-mediated endocytosis (CME) determines the internalization of receptors and their downstream signaling. We discovered that CME is differentially regulated by specific signaling kinases in cancer cells. In particular, ERK1/2-mediated phosphorylation of the FCH/F-BAR and double SH3 domains-containing protein 2 (FCHSD2) regulates CME and the trafficking and signaling activities of EGF receptors. This reciprocal interaction negatively regulates cancer proliferation and migration. The expression level of FCHSD2 is positively correlated with higher lung cancer patient survival rates. This study identifies signaling pathways that impinge on the endocytic machinery and reveals a molecular nexus for crosstalk between intracellular signaling and CME. Cancer cells specifically adapt this crosstalk as a determinant for tumor progression, which has implications for novel therapeutics against cancers. (See pp. E9570–E9579.)

ERβ-mediated induction of cystatins results in suppression of TGFβ signaling and inhibition of triple-negative breast cancer metastasis

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Triple-negative breast cancer (TNBC) is the most aggressive form of breast cancer and patients exhibit high rates of recurrence and mortality in part due to lack of treatment options beyond standard-of-care chemotherapy regimens. In the subset of TNBCs that express estrogen receptor beta (ERβ), ligand-mediated activation of ERβ elicits potent anticancer effects. We report here the elucidation of the ERβ cistrome and transcriptome in TNBC and identify a mechanism whereby ERβ induces cystatin gene expression resulting in inhibition of canonical TGFβ signaling and a blockade of metastatic phenotypes. These findings suggest that ERβ-targeted therapies represent a treatment option for the subset of women with ERβ-expressing TNBC. (See pp. E9580–E9589.)

Mechanism of drug extrusion by brain endothelial cells via lysosomal drug trapping and disposal by neutrophils

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Located at the apical (blood-facing) site of brain capillary endothelial cells that form the blood–brain barrier (BBB), the efflux transporter P-glycoprotein (Pgp) restricts the brain entry of various lipophilic xenobiotics, which contributes to BBB function. Pgp may become saturated if exposed to too-high drug concentrations. Here, we demonstrate a second-line defense mechanism in human brain capillary endothelial cells—that is, Pgp-mediated intracellular lysosomal drug trapping. Furthermore, we describe a mechanism of drug disposal at the BBB, which is shedding of lysosomal Pgp/substrate complexes at the apical membrane of human and porcine BBB endothelial cells and subsequent phagocytosis by neutrophils. Thus, we have discovered a fascinating mechanism of how Pgp might contribute to brain protection. (See pp. E9590–E9599.)

BRCA1-IRIS promotes human tumor progression through PTEN blockade and HIF-1α activation

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Spontaneous overexpression of endogenous IRIS, an alternatively spliced product of the tumor suppressor gene *BRCA1*, allows it to function as an oncoprotein that stimulates a potentially lethal outcome, i.e. metastasis of human cancer cells to tissues served, in part, by the arterial circulation. It does so by suppressing phosphatase and tensin homolog (PTEN) mRNA synthesis, thereby stabilizing and activating HIF-1α in normoxic cells. Thus, this study provides a strong rationale for exploring the therapeutic value of interfering with spontaneously overexpressed IRIS function in multiple types of tumors that can naturally overexpress it. (See pp. E9600–E9609.)

Versatility of multivalent orientation, inverted meiosis, and rescued fitness in holocentric chromosomal hybrids

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Changes in the number and/or structure of chromosomes (i.e., chromosomal rearrangements) have the potential to drive speciation. However, their accumulation in a population is considered both difficult and unpredictable, because the greatly reduced reproductive fitness of chromosomal hybrids prevents fixation of novel karyotypes. Here, we provide evidence for a

mechanism that rescues fertility of chromosomal hybrids in species with holocentric chromosomes. We demonstrate that chromosomal heterozygotes of *Leptidea* Wood White butterflies have a reverse order of main meiotic events in which the first and most critical stage of the chromosome number reduction is replaced by the less risky stage of sister chromatid separation. This may facilitate long-term persistence of chromosomal rearrangements, which is a major prerequisite for chromosomal speciation. (See pp. E9610–E9619.)

Mutations of mitochondrial DNA are not major contributors to aging of fruit flies

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Mutations of mtDNA accumulate in aging humans and other mammals to cause mitochondrial dysfunction in a subset of cells in various tissues. Furthermore, experimental induction of mtDNA mutations causes a premature aging syndrome in the mouse. To study if mitochondrial dysfunction is universally involved in shortening life span in metazoans, we generated a series of fruit fly lines with varying levels of mtDNA mutations. Unexpectedly, we report that fruit flies are remarkably tolerant to mtDNA mutations, as exemplified by their lack of effect on physiology and lifespan. Only an artificially induced, very drastic increase of the mtDNA mutation load will lead to reduced lifespan, showing that mtDNA mutations are unlikely to limit lifespan in natural fruit fly populations. (See pp. E9620–E9629.)

Cochaperone Mzb1 is a key effector of Blimp1 in plasma cell differentiation and β1-integrin function

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Antibody-secreting plasma cells are effectors of the humoral immune response. Transcription factor Blimp1 (Prdm1) is essential for the generation and function of plasma cells, and it regulates many genes, including *Mzb1* (*pERp1*). Mzb1 protein is localized in the endoplasmic reticulum and acts as a cochaperone for the substrate-specific chaperone Grp94 (gp96). By the analysis of $Mzb1^{-/-}Prdm1^{+/gfp}$ mice, we find that Mzb1 is required for T cell-independent immune responses and differentiation of plasma cells. In $Mzb1^{-/-}Prdm1^{+/gfp}$ mice, we also observe impaired β1-integrin activation and trafficking of plasma cells to the bone marrow. Notably, we show that Mzb1 accounts for many of the Blimp1-associated downstream functions, suggesting that Mzb1 is a key effector of the Blimp1 regulatory network in plasma cells. (See pp. E9630–E9639.)

Hypoxia-inducible factor 1-dependent expression of adenosine receptor 2B promotes breast cancer stem cell enrichment

Jie Lan, Haiquan Lu, Debangshu Samanta, Shaima Salman, You Lu, and Gregg L. Semenza

In order for a single breast cancer cell to form a recurrent tumor after therapy or a metastasis at a distant site such as the lung, it must have the properties of a breast cancer stem cell. In this paper, we show that adenosine receptor 2B (A2BR) plays a critical role in breast cancer stem cell specification. Adenosine receptor 2A (A2AR) signaling has been shown to play an important role in enabling cancer cells to evade antitumor immunity and A2AR-selective inhibitors are in clinical trials. Our results suggest that inhibiting A2BR may also provide therapeutic benefit to breast cancer patients. (See pp. E9640–E9648.)

Commercial AHAS-inhibiting herbicides are promising drug leads for the treatment of human fungal pathogenic infections

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Human fungal pathogens resistant to conventional therapeutics pose a major threat to global human health. Thus, there is an urgent need to discover new antifungal drugs that act via novel mechanisms of action. Here, we show that commercial herbicides that inhibit acetohydroxyacid synthase (AHAS) have potent and broad-spectrum antifungal activity in vitro and that chlorimuron ethyl, a member of the sulfonylurea herbicide family, has antifungal activity in a mouse model. Thus, this study shows that AHAS inhibitors have strong potential to be developed into potent antifungal therapeutic agents. (See pp. E9649–E9658.)

IL-15 regulates susceptibility of $CD4^+$ T cells to HIV infection

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HIV creates a persistent reservoir, which is largely resistant to current antiretroviral treatment aimed at inhibiting HIV replication. $CD4^+$ T memory lymphocytes, key components of this reservoir, are generally refractory to infection, but stimulation with γc-chain cytokines, such as IL-15, renders these cells more susceptible to HIV. We found that, by inducing cell cycle entry of $CD4^+$ T cells, JAK1 is a key mediator responsible for counteracting the antiviral activity of SAM domain and HD domain-containing protein 1 (SAMHD1). Pharmacological inhibition of these kinases resulted in restoration of SAMHD1 in $CD4^+$ T cells. Protecting these cells during the critical IL-15 surge observed during primary infection has the potential to limit reservoir establishment. (See pp. E9659–E9667.)

Let-7i inhibition enhances progesterone-induced functional recovery in a mouse model of ischemia

Trinh Nguyen, Chang Su, and Meharvan Singh

Pgrmc1 plays an important role in mediating progesterone's protective effects in that it is a critical mediator of progesterone-induced BDNF release. Here, we identified the microRNA *let-7i*, which increased in stroke, as a negative regulator of Pgrmc1 and BDNF expression. Conversely, inhibition of *let-7i* enhanced progesterone's protective effects against stroke. In addition to enhancing progesterone's neuroprotective effects, the fact that *let-7i* also diminishes the expression of BDNF suggests that inhibition of *let-7i* may also be useful to any intervention that targets the enhancement of BDNF signaling and, as such, may be relevant to the treatment of a variety of brain disorders where BDNF is diminished, to include depression, traumatic brain injury, and Alzheimer's disease. (See pp. E9668–E9677.)

FUS interacts with ATP synthase beta subunit and induces mitochondrial unfolded protein response in cellular and animal models

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In this study, we used an inducible cellular model for FUS proteinopathy to demonstrate that mitochondrial dysfunction occurs as the earliest detectable change induced by FUS. In cellular and fly models, FUS interacts with the mitochondrial ATP synthase β-subunit (ATP5B), disrupts ATP synthase complex assembly, suppresses the activity of mitochondrial ATP synthase, and activates the mitochondrial unfolded protein response (UPR^{mt}). ATP5B expression is increased in cells and flies expressing FUS. Down-regulating expression of ATP5B or UPR^{mt} genes ameliorates FUS-induced neurodegeneration. Our data uncover a previously unknown role of FUS in targeting mitochondrial ATP synthesis and activating UPR^{mt}. (See pp. E9678–E9686.)

Blood-derived plasminogen drives brain inflammation and plaque deposition in a mouse model of Alzheimer's disease

Sarah K. Baker, Zu-Lin Chen, Erin H. Norris, Alexey S. Revenko, A. Robert MacLeod, and Sidney Strickland

We demonstrate that depletion of blood plasminogen is sufficient to protect against both innate immune cell activation in the brain and Alzheimer's disease (AD) pathology in a mouse model of AD. This work provides a molecular mechanism for initiation of AD-related brain inflammation and for regulation of β-amyloid deposition, and could lead to therapeutic strategies in human AD patients, including the targeting of systemic molecules. (See pp. E9687–E9696.)

Single-molecule analysis of endogenous β-actin mRNA trafficking reveals a mechanism for compartmentalized mRNA localization in axons

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De novo protein synthesis in neuronal axons plays important roles in neural circuit formation, maintenance, and disease. Key to the selectivity of axonal protein synthesis is whether an mRNA is present at the right place to be translated, but the mechanisms behind axonal mRNA localization remain poorly understood. In this work, we quantitatively analyze the link between axonal β-actin mRNA trafficking and its localization patterns. By developing a single-molecule approach to live-image β-actin mRNAs in axons, we explore the biophysical drivers behind β-actin mRNA motion and uncover a mechanism for generating increased density at the axon tip by differences in motor protein-driven transport speeds. These results provide mechanistic insight into the control of local translation through mRNA trafficking. (See pp. E9697–E9706.)

Activation of autophagy rescues synaptic and cognitive deficits in fragile X mice

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Fragile X syndrome (FXS) is the most common form of inherited intellectual disability and is a leading genetic cause of autism. The mammalian target of rapamycin (mTOR) complex 1 cascade is a central regulator of protein translation, cell growth, proliferation, survival, and autophagy. Findings in the present study demonstrate that autophagy and protein degradation via the autophagy/lysosomal pathway are reduced in hippocampal neurons of *Fmr1*-KO mice, a model of human FXS. We show that excess mTOR activity is causally related to decreased autophagy, which induces spine defects, exaggerated synaptic plasticity, and impaired cognition in *Fmr1*-KO mice. These findings increase our understanding of the etiology of FXS and suggest components of the autophagy pathway as promising targets for amelioration of FXS in humans. (See pp. E9707–E9716.)

ASTN2 modulates synaptic strength by trafficking and degradation of surface proteins

Hourinaz Behesti, Taylor R. Fore, Peter Wu, Zachi Horn, Mary Leppert, Court Hull, and Mary E. Hatten

Neurogenetic studies demonstrate that copy number variations (CNVs) in the *ASTN2* gene occur in patients with neurodevelopmental disorders (NDDs), including autism spectrum. Here, we show that ASTN2 associates with recycling and degradative vesicles in cerebellar neurons, and binds to and promotes the endocytic trafficking and degradation of synaptic proteins. Overexpression of ASTN2 in neurons increases synaptic activity and reduces the levels of ASTN2 binding partners, an effect dependent on its FNIII domain, which is recurrently perturbed by CNVs in patients with NDDs. These findings suggest that ASTN2 is a key regulator of dynamic trafficking of synaptic proteins and lend support to the idea that aberrant regulation of protein homeostasis in neurons is a contributing cause of complex NDDs. (See pp. E9717–E9726.)

Genetically defined cellular correlates of the baseline brain MRI signal

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Understanding the structure and function of the human brain at a cellular level is a fundamental aim of neuroscience. Tremendous progress has been made in recent years based on different in vivo and ex vivo approaches, including major advances in brain MRI. However, uncertainties remain in determining how brain MRI measurements relate to the brain's underlying cellular composition. In this paper we use a recently developed MRI technique, quantitative gradient recalled echo (qGRE), and information on gene profiles in the human brain available from the Allen Human Brain Atlas. We demonstrate that qGRE and related MRI techniques can be used to probe the underlying cellular composition of the human brain in vivo. (See pp. E9727–E9736.)

Cold exposure causes cell death by depolarization-mediated Ca^{2+} overload in a chill-susceptible insect

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Insects comprise the largest class of animals and include numerous species of direct importance to humans, including pests and pollinators. Cold tolerance is arguably among the most important traits determining the distribution of insects. Hypothermia has been proposed to induce cell injury directly by promoting membrane phase transitions resulting in cell leak. Additionally, hypothermia induces hemolymph hyperkalemia, and it has been proposed that hypothermia induces injury indirectly through the resulting depolarization by inducing Ca^{2+}

influx promoting apoptosis/necrosis. Here we show that depolarization is a principal mechanism for hypothermic cell injury. Furthermore, we show that intracellular Ca^{2+} increases upon depolarization and that this increase must flux through Ca^{2+} channels in the membrane to accumulate and induce injury. (See pp. E9737–E9744.)

Nanoscale remodeling of ryanodine receptor cluster size underlies cerebral microvascular dysfunction in Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a hereditary neuromuscular disease that results from mutations in the gene encoding dystrophin. The effects of the disease on cardiac and skeletal muscle have been intensely investigated, but much less is known about how DMD impacts vascular smooth muscle cells (SMCs). Using superresolution nanoscopy, we demonstrate that clusters of ryanodine receptors (RyR2s) on the sarcoplasmic reticulum (SR) of cerebral artery SMCs from the *mdx* mouse model of DMD are larger compared with controls. Increased RyR2 cluster size is associated with augmented SR Ca^{2+} release and Ca^{2+}-activated K^+ channel activity, resulting in impaired vasoconstriction of cerebral microvessels. Our findings demonstrate that remodeling of RyR2 clusters at the molecular level results in cerebral microvascular dysfunction during DMD. (See pp. E9745–E9752.)